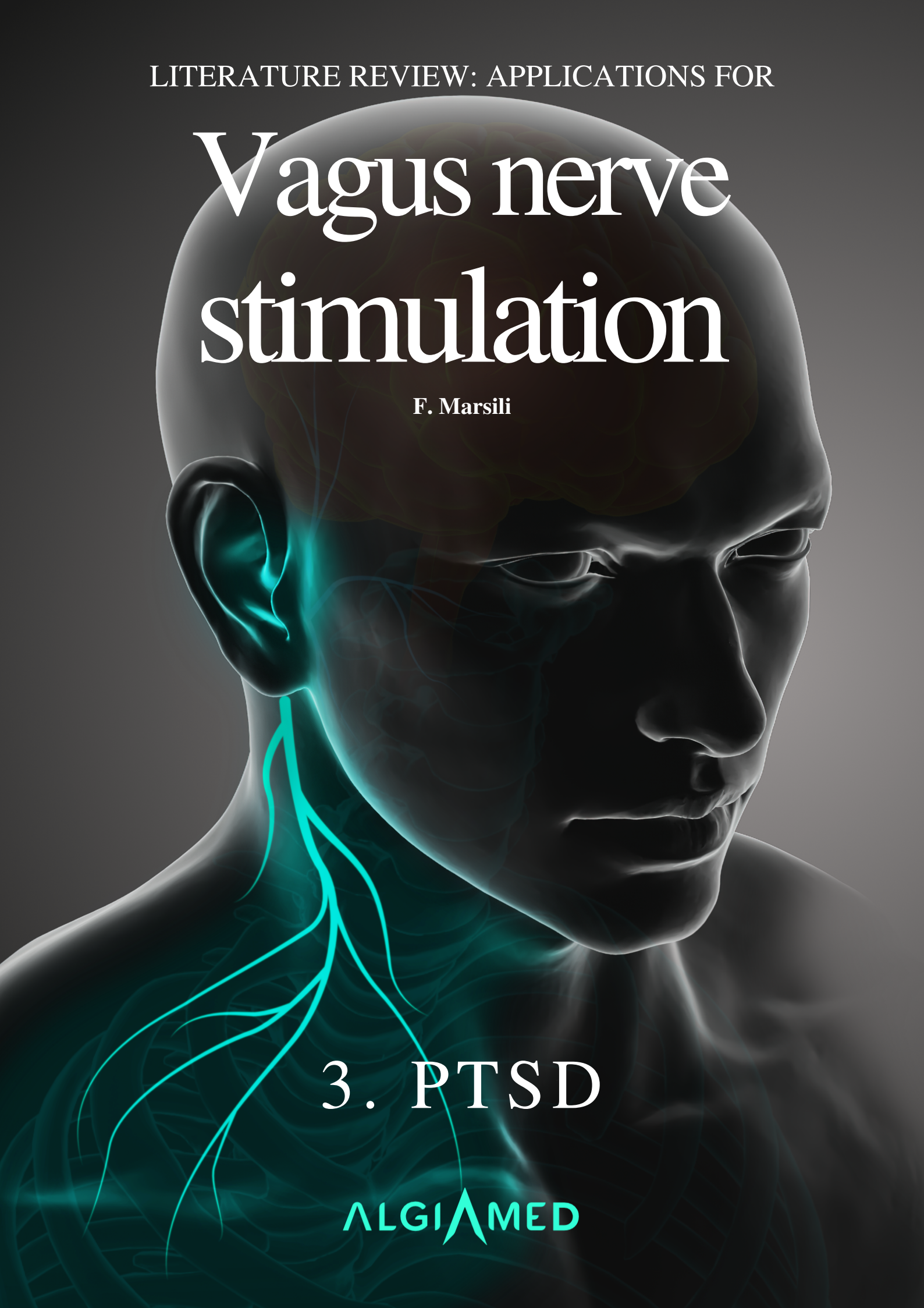
LITERATURE REVIEW: APPLICATIONS FOR

Vagus nerve stimulation

F. Marsili



3. PTSD

ALGIAMED

Author's choice

The papers in this collection focus on the application of Vagus Nerve Stimulation (VNS) as established therapeutic solution for difficult-to-treat conditions.

The vagus nerve is the longest cranial nerve and is widely distributed throughout the body, traversing the neck, thorax and abdomen. It is composed by motor fibres and sensory fibres from sympathetic and parasympathetic branches. [1], [2]. Afferent branches of the vagus nerve innervate brain behavioural areas involved in depressive states, and it desynchronises cortical activity with anti epileptic effects [3], [4]. Efferent branches of the vagus nerve regulate gastrointestinal secretory and motor function [5]. Recent advances in the field, have unraveled an anti-inflammatory role of the efferent vagus nerve via the Cholinergic Anti-inflammatory Pathway (CAP), a known mechanism for neural inhibition of inflammation linked to the activation of the autonomic nervous system (ANS) [6], [7].

Electrical stimulation of the VN modulates the nervous system at central, peripheral, and autonomic levels without the need for pharmacological interventions. For decades, invasive techniques of VNS have demonstrated their clinical efficacy in VN-related diseases and, to these days, efforts have been made to create a more safe, effective, and non-invasive solution to VNS.

The auricular branch is the only peripheral branch of the VN on the human body, it is part of the afferent portion of the VN that directly connects to the brainstem. Thus, auricular VN has become the most favourable access point for non-invasive VNS. Neuroimaging studies on animal models and humans have confirmed the modulatory efficacy of auricular VNS (aVNS). For examples, fMRI studies show identical activation patterns in the brain between invasive and aVNS, with significant inhibitory and anti-inflammatory effects. Due to the existence of different control systems, the anti-inflammatory effects of aVNS (i.e., release of norepinephrine and noradrenaline, and neurotrophic factors) seem to occur immediately after intervention, while neuroplastic changes only occur as a consequence of sustained regenerative efforts [7].

Colleciton 1 and collection 2 are the most extensive selections, since VNS has been standard-of-care for epilepsy and depression for decades. Collection 3 explores the possibility of using VNS for the treatment of posttraumatic stress disorders. Collection 4 focuses on fibromyalgia and collection 5 on multiple sclerosis. Collection 6 and 7 corroborates the hypothesis that VNS can be used to activate the cholinergic anti-inflammatory pathway to treat inflammatory diseases, such as inflammatory bowel disease or rheumatoid arthritis. Collection 8 and 9 focus on the use of VNS for ameliorating pain sensitivity in chronic pain conditions and for rehabilitating upper limb motor fibres after ischemic strokes, respectively. In conclusion, collection 10 opens up other possibilities for clinical applications of VNS, ranging from cardiovascular diseases, through ADHD disorders, to tinnitus.

To summarise, VNS is a novel technology and its non-invasive configuration is still under investigation. Further clinical examinations are mandatory in order to understand the underlying mechanism of VNS and to open the door to new possible therapeutic applications. However, being a non-invasive, safe, and efficient therapeutic solution, VNS is an attractive tool for further implementation and new creative clinical applications.

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3. VNS and PTSD

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ORIGINAL ARTICLE

Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats

LJ Noble, IJ Gonzalez, VB Meruva, KA Callahan, BD Belfort, KR Ramanathan, E Meyers, MP Kilgard, RL Rennaker and CK McIntyre

Exposure-based therapies help patients with post-traumatic stress disorder (PTSD) to extinguish conditioned fear of trauma reminders. However, controlled laboratory studies indicate that PTSD patients do not extinguish conditioned fear as well as healthy controls, and exposure therapy has high failure and dropout rates. The present study examined whether vagus nerve stimulation (VNS) augments extinction of conditioned fear and attenuates PTSD-like symptoms in an animal model of PTSD. To model PTSD, rats were subjected to a single prolonged stress (SPS) protocol, which consisted of restraint, forced swim, loss of consciousness, and 1 week of social isolation. Like PTSD patients, rats subjected to SPS show impaired extinction of conditioned fear. The SPS procedure was followed, 1 week later, by auditory fear conditioning (AFC) and extinction. VNS or sham stimulation was administered during half of the extinction days, and was paired with presentations of the conditioned stimulus. One week after completion of extinction training, rats were given a battery of behavioral tests to assess anxiety, arousal and avoidance. Results indicated that rats given SPS 1 week prior to AFC (PTSD model) failed to extinguish the freezing response after eleven consecutive days of extinction. Administration of VNS reversed the extinction impairment and attenuated reinstatement of the conditioned fear response. Delivery of VNS during extinction also eliminated the PTSD-like symptoms, such as anxiety, hyperarousal and social avoidance for more than 1 week after VNS treatment. These results provide evidence that extinction paired with VNS treatment can lead to remission of fear and improvements in PTSD-like symptoms. Taken together, these findings suggest that VNS may be an effective adjunct to exposure therapy for the treatment of PTSD.

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INTRODUCTION

Post-traumatic stress disorder (PTSD) affects 22.4 million Americans and can develop following highly stressful experiences, such as combat or sexual assault.[1] Although most individuals who have traumatic experiences exhibit transient symptoms of stress, ~ 30% of these individuals suffer from symptoms for longer than 1 month and meet the criteria for diagnosis of PTSD.[1] According to the fifth edition of the *Diagnostic and Statistical Manual*, an individual may be diagnosed with PTSD after experiencing or witnessing trauma in addition to presenting the following symptoms: re-experiencing the trauma (that is, experiencing emotional or physical distress in response to reminders of the trauma); avoidance of trauma-related stimuli; negative affect, including loss of interest in enjoyable activities; and heightened startle and arousal. Symptoms must persist for more than 1 month and cause significant social or occupational dysfunction.[2] The prevalence of PTSD is greater in individuals who have experienced multiple traumatic events, suggesting that earlier stressors predispose individuals to the development of PTSD following a traumatic event later in life.[3–5]

Exposure-based therapies are considered the gold standard of treatment for PTSD.[6] The goal of exposure-based therapies is to replace conditioned associations of the trauma with new, more appropriate associations. These therapies are based on Pavlov's observations that learned associations can be modified with extinction training.[7] Despite their demonstrated therapeutic efficacy, exposure-based therapies for PTSD have high nonresponse and dropout rates.[8–10] PTSD patients appear to be resistant to exposure-based therapies because of a generalized extinction deficit.[11–14] Further, PTSD patients are impaired in their ability to extinguish conditioned fears that are acquired in controlled laboratory studies.[12,15,16] Adjuvant treatments that improve the consolidation of extinction learning may improve the effectiveness of exposure-based therapies.

Vagus nerve stimulation (VNS) was approved by the Federal Food and Drug Administration for the prevention of seizures in patients with drug-resistant epilepsy in 1997. Considering that VNS can enhance memory consolidation in rats and humans,[17,18] we hypothesized that administration of VNS during extinction training could enhance consolidation of an extinction memory. We recently reported that VNS enhanced consolidation of fear extinction following auditory fear conditioning (AFC) and promoted synaptic plasticity in the brain circuitry underlying extinction memory.[19,20] These findings suggest that VNS might also be effective in enhancing extinction memory in a rat model of PTSD. To investigate this possibility, we used the single prolonged stress (SPS) procedure in rats, which models successive traumatic events and increases susceptibility for PTSD-like symptoms following fear conditioning.[21] Like PTSD patients, rats subjected to SPS and fear conditioning exhibit impaired extinction of conditioned fear;[21,22] this impairment is specifically seen during consolidation of extinction.[23] Rats subjected to SPS show behaviors that can be compared to PTSD symptoms, including re-experiencing the trauma, elevated anxiety, arousal and avoidance.[21–24] Here we investigated the effects of pairing VNS with exposure to the conditioned stimulus (CS) during extinction

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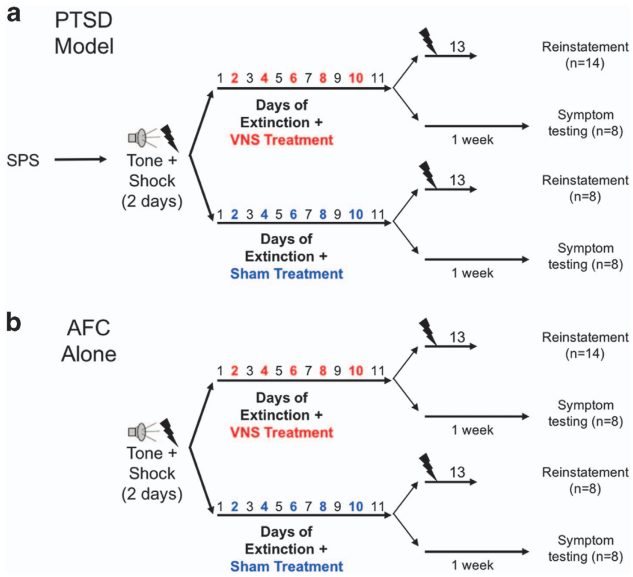

Figure 1. Timeline for extinction, reinstatement and behavioral assays. (**a**) Protocol for PTSD model rats. Rats underwent the SPS procedure followed by 2 days of AFC and 11 days of extinction, five were paired with VNS (red) or sham stimulation (blue) on alternate (even) days. Freezing in the presence of the CS was used as a measure of conditioned fear. Conditioned fear was measured on the odd days that fell between VNS or sham stimulation days. Following extinction, some rats underwent a reinstatement trial where they received one unsignaled footshock. The day after the footshock, these rats were given another day of extinction to measure conditioned fear. The remaining rats were tested on a battery of behaviors to measure PTSD symptoms 1 week after the end of extinction. The order of the tests was counterbalanced. (**b**) Protocol for AFC alone rats. Rats in the AFC alone group were treated exactly like PTSD model rats; however, the AFC alone group did not undergo the SPS procedure. AFC, auditory fear conditioning; PTSD, post-traumatic stress disorder; SPS, single prolonged stress; VNS, vagus nerve stimulation.

on the conditioned fear response and on other PTSD-like symptoms in rats subjected to SPS. The findings suggest that VNS enhances extinction and attenuates reinstatement of fear. Furthermore, VNS administration during extinction is associated with a reduction in PTSD-like symptoms 1 week later.

MATERIALS AND METHODS

Animals

All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Dallas. Male Sprague-Dawley rats (Taconic, Hudson, NY, USA) weighing 225–250 g on arrival were housed on a 12-h light/dark cycle (lights on at 0700 hours) with access to food and water *ad libitum*. Only male rats were used, as previous results indicate that female rats are not susceptible to the extinction impairment produced by SPS.[24] Criteria for exclusion of rats from the analysis was performance $\geqslant 2$ s.d. away from the mean on any task.

To investigate the effects of VNS on extinction and reinstatement of conditioned fear, 22 rats were subjected to the SPS procedure (see 'Rat model of PTSD' section below),[21] followed 1 week later by AFC. Rats subjected to the SPS procedure and AFC were referred to as 'PTSD model' rats. Extinction training began 24 h after AFC. The full course of extinction consisted of 11 days of exposure to the CS without reinforcement. During extinction, odd numbered days (extinction days 1, 3, 5, 7, 9 and 11) were used as tests of conditioned fear; four presentations of the CS were administered and conditioned fear to the CS was measured. On even numbered days (extinction days 2, 4, 6, 8 and 10), VNS or sham stimulation was administered and temporally paired with the four CS presentations. Fourteen of the PTSD model rats were given VNS during extinction, and eight were given sham stimulation (Figure 1a).

Twenty-four rats were given the same AFC and extinction without the SPS procedure. These rats were referred to as 'AFC alone' rats. Of these, 14 were given VNS during extinction, and 8 were given sham stimulation during extinction (Figure 1b). Twenty-four hours after extinction day 11, rats were given a single reminder footshock. Reinstatement was tested the following day by measuring freezing in response to the conditioned cue.

To test the effects of VNS on PTSD-like symptoms, separate rats were given AFC with SPS (PTSD model rats, $n = 16$) or without SPS (AFC alone rats, $n = 16$) and exposed to the same 11 days of extinction. Eight rats from each group were given VNS during extinction and eight were given sham stimulation. Seven to ten days after completion of all 11 extinction days, rats were tested on a battery of behavioral tests to examine generalized anxiety, arousal, avoidance and social interaction (Figure 1). The order of test administration was counterbalanced to control for potential interactions. These rats were not given a reminder shock or reinstatement test.

Cuff electrode preparation

Cuff electrodes were prepared as previously described.[25] In brief, platinum–iridium wire electrodes were affixed to biocompatible micro-renathane cuffs (1.25 mm inner diameter, 2.5 mm outer diameter, 4.0 mm long). Omnetics four-pin connectors were used to connect the VNS cuff to an AM systems stimulator. Two of the connector pins made contact with the platinum–iridium wires in the cuff in order to deliver stimulation to the vagus nerve.

Surgical implantation of cuff electrode

Surgery protocols are described in detail elsewhere.[19,20,25] In brief, rats were anesthetized with isoflurane (2% at an oxygen flow rate of 600–800 ml/min). The left vagus nerve was located at the cervical level and isolated from other tissue. The left vagus nerve was selected to avoid stimulation effects on the sinoatrial node. Central activation from the left vagus nerve is bilateral.[26] The cuff was placed around the nerve and secured in place with a suture. The platinum–iridium wires were tunneled subcutaneously behind the ear to the top of the head and connected to the omnetics connector, which was affixed to the skull using acrylic, to make the headcap. Cessation of breathing was used to test for correct implantation and effectiveness of the VNS cuff; following implantation, while under anesthesia, current (0.8 mA, 1 s) was applied through the cuff to test for cessation of breathing. If cessation of breathing was not observed, the cuff was adjusted or replaced. For sham rats, surgery was conducted in the same manner to isolate the vagus nerve, but the rats were not implanted with a cuff. Animals were given 1 week to recover following surgery.

Rat model of PTSD

Procedures for SPS were adapted from methods developed by Liberzon *et al*.[21] In brief, rats were restrained for 2 h in a plastic cone. Immediately after restraint, rats were forced to swim in a tank of water (22.0-inch diameter, 20 °C) for 20 min. Following a 15-min recuperation period, rats were placed in a desiccator and exposed to diethyl ether vapor (Sigma, St. Louis, MO, USA) until they became anesthetized and unresponsive. They were immediately returned to their home cages and left socially isolated for 1 week.

AFC

On the first day of AFC, rats were exposed to four pretones (four 9 kHz tones, lasting 30 s, at an intensity of 70 dB, administered without any reinforcement) to assess baseline freezing to the tone. Immediately after the pretones, rats received eight tones coupled with a footshock (1 s, 0.4 mA). The tones were presented at a random inter-stimulus interval of between 120 and 240 s. Each shock was administered at a randomized time during the last 25 s of the 30 s tone presentation. Twenty-four hours later, rats underwent a second day of AFC consisting of eight more tone-shock pairings administered in the same way as the previous day, excluding the pretones. All AFC took place in context A (electric grid floor, no olfactory cue). To compare acquisition of conditioned fear between AFC alone rats and PTSD model rats, sessions were recorded and scored by two researchers blind to the treatment conditions. Freezing during the tone, defined as the cessation of movement aside from breathing,[27] was used as a measure of conditioned fear. We chose this AFC protocol to compare

findings in the PTSD model to what we have observed in normal rats using a similar protocol. In this previous study, we found that eight tone-shock pairings per day for 2 days, with unpredictable shock timing during the 30 s tone produced conditioned fear that was not fully extinguished after 11 days of extinction in sham-treated controls.[19]

Extinction days

Twenty-four hours after both days of AFC, rats underwent 11 days of extinction in context B. Context B consisted of the same conditioning chamber, but contained a distinct plexiglas insert to change the texture of the floor and the addition of an odor (peppermint oil). Each day of extinction consisted of four presentations of the CS (tone) in the absence of any reinforcement (shock). Based on evidence that VNS can enhance memory consolidation, extinction was carried out over several days to allow for consolidation of the extinction memory. This extended protocol for extinction more closely resembles a clinical timeline for individuals with PTSD who would undergo multiple days of exposure-based therapies. Two observers who were blind to the treatment groups measured the percent of time spent freezing during each 30-s tone, which was recorded as the measure of conditioned fear. Freezing times below 10% of the total 120 s of tone exposure was considered remission of fear.[19] During extinction, rats initially respond to being connected to the stimulator, this occurs in both sham-treated and VNS-treated rats. This response is typically present only during the first extinction session. To avoid potential performance effects of VNS or sham stimulation, conditioned fear responses were measured only on alternate, non-stimulation days (extinction days 1, 3, 5, 7, 9 and 11) when the rats were not connected to the stimulator (Figure 1). Although we have not systematically measured unintended effects of VNS, we have noticed that both sham- and VNS-treated rats occasionally attend to the connector after it is attached to the headcap. This variable behavior could interfere with the conditioned response. In addition, measuring conditioned fear on days when rats were not receiving VNS or sham stimulation made it less likely that interoceptive state-dependent effects of VNS could serve as a safety signal, and provided an opportunity to observe the effect of VNS on consolidation of extinction memory.

VNS and sham stimulation

Treatment with VNS or sham stimulation was given during extinction on even numbered days (extinction days 2, 4, 6, 8 and 10). To administer stimulation, an AM systems stimulator was connected to the cuff connector on the headcap via a 25.0 cm long PVS multiconductor cable (Cooner Wire, Los Angeles, CA, USA). Stimulation started 150.0 ms before the onset of each tone and then continued for the duration of the tone. VNS was given at a frequency of 20Hz, an intensity of 0.4 mA for 30 s with a 100 μs pulse width. Sham-treated rats were connected to the stimulator in the same way as VNS-treated rats, but did not receive stimulation.

Reinstatement

Following the completion of extinction, reinstatement of conditioned fear was tested. Twenty-four hours after the 11th day of extinction, rats ($N = 44$) were placed in context A and given one unsignaled footshock (unconditioned stimulus) delivered for 1 s at 0.4 mA intensity, in the absence of the tone (CS). Rats remained in context A for 5 min after the footshock. To observe the reaction to the reinstatement shock, sessions were recorded and scored by two researchers blind to the treatment conditions. Freezing was recorded during the entire 5-min observation period. Twenty-four hours after administration of the unconditioned stimulus, rats were exposed to the CS in context B, to test for reinstatement of fear.

Elevated plus maze

To test generalized anxiety, rats were placed on the central part of an elevated plus-shaped maze (10.0 cm wide, 50.0 cm long, 55.0 cm off the floor) with walls (30.0 cm tall) on two opposing arms and no walls on the other opposing arms. During a 10-min test, time spent in the open arms, time spent in the closed arms and time spent in the center of the maze were recorded. Rats were considered to be in an arm when all four paws were in that arm at one time. All behavior was recorded and scored by two blind researchers. Time spent in the open arms and entries into the open arms were taken as a measure of risk taking.[28] Percent of total time spent moving was taken as a control measure of general locomotion.

Acoustic startle response

Before startle behaviors were measured, rats were placed into the apparatus for 5 min to habituate them to the cage. On the following day, rats were placed into the same $20 \times 20 \times 20$ cm^3 wire-mesh cage centered on a startle platform (Lafayette Instrument, Lafayette, IN, USA) that used a piezoelectric transducer to generate a continuous record of the rat's activity. Startle responses were elicited by 50.0 ms bursts of white noise at 95 dB sound pressure level. Each rat was subjected to 20 presentations of the startle stimulus with an inter-stimulus interval of 180 s. The waveform of each response served as the measure of the startle response.

Marble burying

To test novel object avoidance, rats underwent a marble burying task. Rats exposed to a noxious object in their homecage will vigorously bury that object, a phenomenon known as defensive burying.[29] This avoidance behavior can be seen in rats following fear conditioning with non-salient, novel objects.[30] This defensive burying of novel objects is sensitive to anxiolytic treatments and is used to measure anxiety and avoidance behavior.[31] Following habituation to the novel bedding, rats were individually placed into a cage that was identical to their homecage with BioFresh nitrocellulose comfort bedding (3.0 cm deep). Fifteen identical, shiny marbles were placed in three rows in the rear third of the cage. After 10 min, the number of marbles buried was counted and recorded as a measure of novel stimulus avoidance. Only marbles that were more than 2/3 covered by bedding were considered buried. Percent of marbles buried = (number marbles buried/number of marbles present) × 100.

Social interaction

A three-chamber social interaction task was used to assess social behaviors. The apparatus consisted of three equal-sized chambers: the nonsocial zone, the social zone and the center. The nonsocial zone contained a small wire cage that was empty and sealed; the social zone contained an identical wire cage with a stimulus rat inside. The stimulus rat was matched in size and sex to the experimental rat. The experimental rat was placed into the center of the apparatus, facing the nonsocial zone, and allowed to explore for 10 min. Interactions of the experimental rat with the stimulus rat, time spent in the nonsocial zone, time spent in the social zone and time spent in the center were recorded. A rat was considered to be in a zone of the apparatus only when all four paws were in that zone at once. All behavior was recorded and scored by two experimenters who were blind to treatment conditions. The sociability index (time spent in the social zone − time spent in the nonsocial zone)/(time spent in the social zone + time spent in the nonsocial zone) was used to indicate a preference to interact with or avoid the stimulus rat.

Data analysis

Data were analyzed using a two-way repeated measures ANOVA or a one-way ANOVA, with a Greenhouse-Geisser correction followed by a Tukey's *post hoc* test for multiple comparisons or a Holm–Bonferroni sequential correction test for non-independent samples. Statistically significant effects were defined as those with *P*-values that were < 0.05. All error bars represent standard error of the mean.

RESULTS

VNS administration during exposure to the conditioned stimulus enhanced extinction and reduced reinstatement of conditioned fear

We modeled PTSD by combining SPS with AFC 1 week later. A two-factor repeated measures ANOVA indicated a significant effect of group across days ($F(18\,246) = 4.764$, $P < 0.0001$). Although animals with and without SPS exposure demonstrated comparable levels of conditioned fear following AFC, SPS treatment resulted in significantly higher levels of freezing in response to the CS after 11 consecutive days of extinction (Figure 2a, sham). Without VNS, the PTSD model rats did not reach remission of conditioned fear ($< 10\%$ freezing to the CS). Administration of VNS treatment during five out of the eleven extinction days led to remission of CS-evoked freezing behavior in

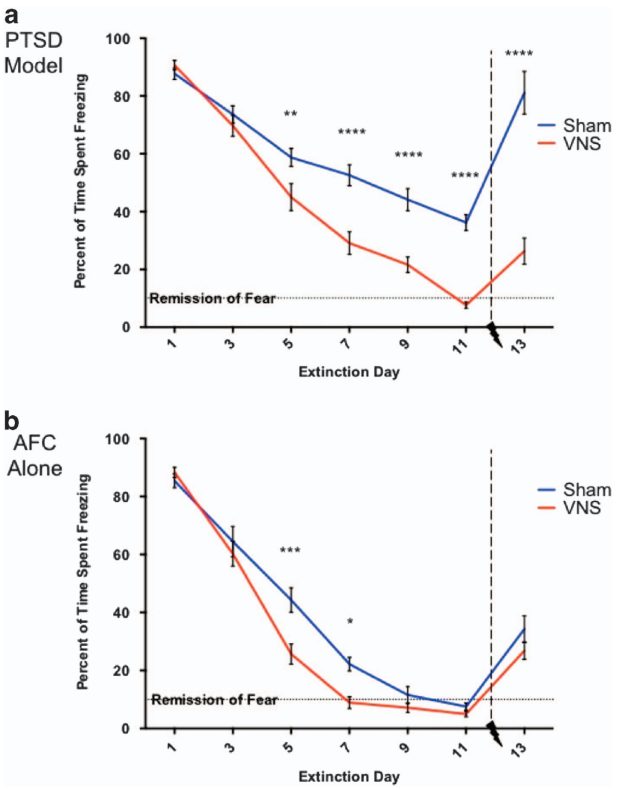

Figure 2. Conditioned fear responding across extinction days. (**a**) VNS treatment reverses maladaptive fear seen in PTSD model rats. Following 11 consecutive days of extinction, rats exposed to the SPS procedure 1 week before AFC (PTSD model rats) did not reach remission of fear. A single, unpaired reminder of the unconditioned stimulus was sufficient to reinstate conditioned fear to the level of freezing measured on the first day of extinction in the PTSD model. PTSD model rats given VNS reached remission of fear by the end of treatment. VNS also attenuated the reinstatement of conditioned fear. Freezing in rats given VNS was significantly reduced compared with freezing on the first day of extinction. PTSD model rats given VNS showed significantly less fear than sham-treated rats on extinction day 5 ($^{**}P < 0.01$) and extinction days 7, 9 and 11 ($^{****}P < 0.0001$). PTSD model rats given VNS showed reduced reinstatement of conditioned fear versus sham-treated rats ($^{****}P < 0.0001$). (**b**) VNS treatment accelerates extinction in control rats subjected to AFC alone**.** All rats that underwent AFC alone reached remission of fear by the end of extinction. VNS led to more rapid remission of fear than sham stimulation. VNS-treated rats showed reduced freezing versus sham-treated rats on extinction day 5 ($^{***}P < 0.001$) and extinction day 7 ($^{*}P < 0.05$). AFC alone rats alone did not show complete reinstatement of conditioned fear following a reminder of the unconditioned stimulus. AFC, auditory fear conditioning; PTSD, post-traumatic stress disorder; SPS, single prolonged stress; VNS, vagus nerve stimulation.

PTSD model rats (Figure 2a, VNS). By extinction day 5, PTSD model rats given VNS showed decreased freezing versus rats given sham stimulation ($P < 0.01$). This effect continued until the completion of treatment: extinction day 7 ($P < 0.0001$); extinction day 9 ($P < 0.0001$); and extinction day 11 ($P < 0.0001$). This supports the hypothesis that VNS treatment can enhance extinction of conditioned fear in an animal model of PTSD.

A single reminder of the unconditioned stimulus (reinstatement footshock on day 12) was sufficient to increase freezing to the CS when presented 24 h later. In PTSD model rats given sham stimulation, the level of conditioned fear returned to that observed before any extinction; freezing behavior on extinction day 13 was not significantly different from extinction day 1 ($P > 0.05$). This result indicates that a single stressor is sufficient to

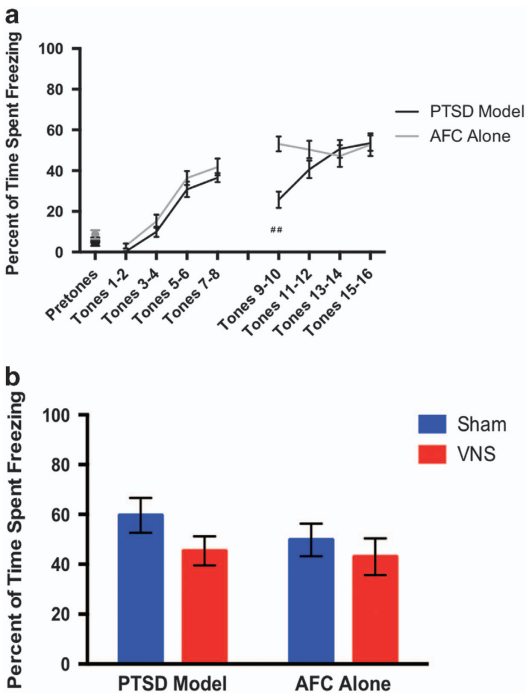

Figure 3. AFC alone rats and PTSD model rats respond similarly to footshock. (**a**) Acquisition of conditioned fear is similar between groups. Prior to VNS- or sham-paired extinction, rats underwent 2 days of AFC. On the first day of AFC, rats were exposed to four pretones to assess baseline freezing to the tone, pretone freezing was similar between groups. Acquisition of conditioned fear was similar between all rats on these days; however, PTSD model rats show a reduction in freezing at tones 9 and 10 versus AFC alone rats ($^{\#\#}P < 0.01$). This effect is no longer present on subsequent tones. (**b**) Following reinstatement shocks, rats show similar levels of freezing. Following a reinstatement shock in context A, rats from all groups show comparable levels of freezing ($P > 0.05$). AFC, auditory fear conditioning; PTSD, post-traumatic stress disorder; VNS, vagus nerve stimulation.

restore strong fear behavior in PTSD model rats despite a long period of extinction. The addition of VNS during extinction prevented the reinstatement of conditioned fear observed in PTSD model rats. Freezing behavior in PTSD model rats given VNS was dramatically reduced on extinction day 13 compared to extinction day 1 ($P < 0.00001$) (Figure 2a). This result indicates that VNS during extinction makes PTSD model rats resilient to stress-induced relapse.

To compare the fear demonstrated by the PTSD model rats, we examined fear in rats that underwent AFC in the absence of SPS (AFC alone rats). These rats exhibited remission of fear behavior (⩽10% freezing) and resistance to reinstatement (Figure 2b, sham); freezing behavior in sham-treated rats was dramatically reduced on extinction day 13 compared to extinction day 1 ($P < 0.00001$). These results indicate that the stability and degree of fear extinction is substantially different between PTSD model rats and AFC alone rats, as previously reported.[21–23]

Still, in rats that received AFC alone, VNS during extinction accelerated extinction of conditioned fear (Figure 2b). On average, VNS-treated rats reached remission of fear 2 days earlier than sham-treated rats ($P < 0.01$). In rats given AFC alone, VNS treatment reduced freezing versus sham on extinction day 5 ($P < 0.001$) and extinction day 7 ($P < 0.05$). Freezing following reinstatement was not different between the sham- and VNS-treated rats ($P > 0.05$).

Prior to VNS- or sham-paired extinction, rates of acquisition of conditioned fear between AFC alone rats and PTSD model rats

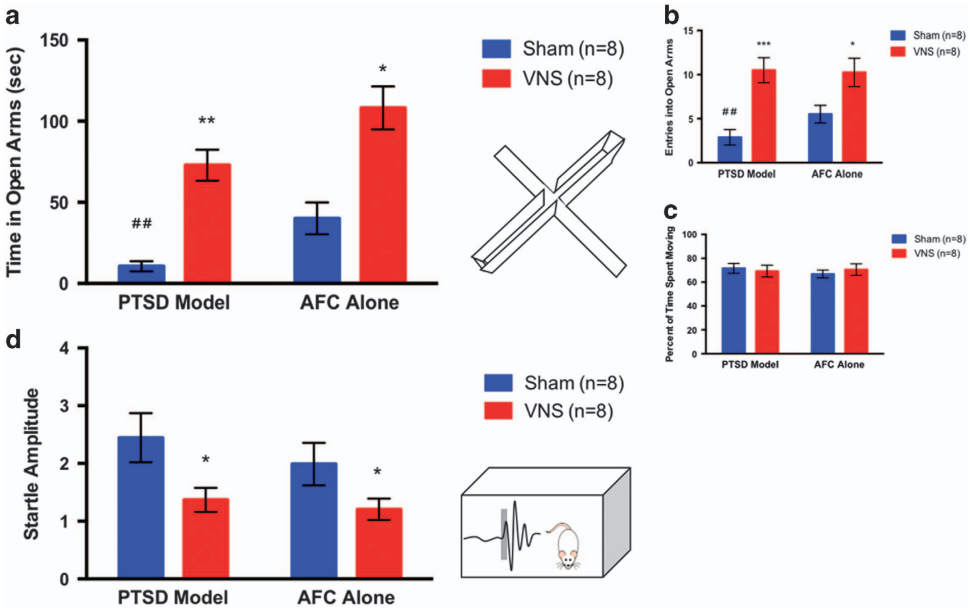

Figure 4. VNS treatment reversed PTSD-like symptoms of anxiety and exaggerated startle. (**a**) VNS administration during extinction reduced anxiety. One week after the completion of extinction, PTSD model rats spent less time in the open arms of the maze than AFC alone rats ($^{\#\#}P < 0.01$), indicating heightened anxiety. VNS during extinction reversed this effect in PTSD model rats and also decreased anxiety in AFC alone rats. VNS-treated rats spent more time in the open arms versus sham-treated rats in the PTSD model ($^{**}P < 0.01$) and in the control group that underwent AFC alone ($^{*}P < 0.05$). (**b**) VNS treatment increased entries into the open arms. Similar to time spent in the open arms, PTSD model rats showed a reduced number of entries into the open arms versus AFC alone rats ($^{\#\#}P < 0.01$). Administration of VNS during extinction reversed this effect and increased entries into the open arms in PTSD model rats ($^{***}P < 0.001$) and in AFC alone rats ($^{*}P < 0.05$). (**c**) Total locomotion was not affected. Total time spent moving in the EPM was similar between groups ($P > 0.05$). (**d**) VNS treatment reduced startle responses. Following extinction, there was no difference in PTSD model rats versus AFC alone rats. Administration of VNS during extinction reduced startle amplitudes in PTSD model rats versus sham ($^{*}P < 0.05$) and in rats that underwent AFC alone versus sham ($^{*}P < 0.05$). AFC, auditory fear conditioning; EPM, elevated plus maze; PTSD, post-traumatic stress disorder; VNS, vagus nerve stimulation.

were examined (Figure 3a). A two-factor repeated measures ANOVA indicated a significant effect of group across tones for acquisition of conditioned fear ($F(7, 168) = 2.473$, $P < 0.05$). All rats show a significant effect of time, as levels of freezing increase as the number of tone-shock pairings increases ($F(7, 168) = 41.36$, $P < 0.0001$). A Holm–Bonferroni sequential correction revealed a significant difference between PTSD model rats and AFC alone rats only at tones 9 and 10 ($P < 0.01$). PTSD model rats showed a deficit in fear retention versus AFC alone rats ($P < 0.01$) at the start of the second day of fear conditioning. This could be explained by evidence that SPS impairs consolidation, but has no learning effect within a session.[23] This effect was not present during other AFC tones or at the start of extinction training (extinction day 1).

A one-way ANOVA revealed no significant effect of freezing following the reinstatement shock ($F(2.582, 8.94) = 0.522$, $P > 0.05$). All rats showed similar levels of conditioned fear immediately following reinstatement shock (Figure 3b), indicating the shock was equally aversive to all rats. However, 24 h later, there was a significant difference between PTSD model rats given sham stimulation and all other rats ($P < 0.0001$).

A total of two AFC alone rats (1 sham and 1 VNS) met the exclusion criteria. These rats were not included in analysis because they did not exhibit conditioned fear following AFC (freezing behavior was < 2 s.d. away from the mean).

VNS administration during extinction sessions reduced PTSD-like symptoms

EPM. To test the effect of VNS on general anxiety, rats were tested on the elevated plus maze (EPM) (Figures 4a and b). A one-way ANOVA revealed a significant effect across groups ($F(2.430, 17.01) = 17.26$, $P < 0.0001$). PTSD model rats given sham stimulation spent less time in the open arms than AFC alone rats given sham stimulation ($P < 0.05$), and made fewer entries into the open arms ($P < 0.01$), indicating that anxiety was elevated in the PTSD model rats. VNS treatment reversed this effect, in that PTSD model rats given VNS during the extinction sessions spent more time in the open arms ($P < 0.01$), and made more entries into the open arms versus PTSD model rats given sham stimulation ($P < 0.001$). PTSD model rats given VNS during extinction sessions spent a similar amount of time in the open arms as AFC alone rats ($P > 0.05$). VNS treatment also increased time spent in the open arms ($P < 0.05$) and entries into the open arms in AFC alone rats ($P < 0.05$). These results demonstrate that VNS treatment during extinction reduced general anxiety 1 week after treatment. Total locomotion was not different in PTSD model rats versus AFC alone rats, and administration of VNS did not affect total locomotion (Figure 4c).

Acoustic startle response. To test for hyperarousal, rats underwent an acoustic startle response test. VNS treatment during extinction reduced startle responses in PTSD model rats and in AFC alone rats (Figure 4d). A one-way ANOVA indicated a significant effect across groups ($F(1.40, 9.80) = 6.980$, $P < 0.05$). Startle amplitudes prior to habituation (the first 15 startle bursts) were similar in PTSD model rats given sham stimulation and sham-treated AFC alone rats ($P > 0.05$). PTSD model rats that received VNS during extinction showed a reduction in startle amplitude versus sham-treated rats ($P < 0.05$). VNS also decreased startle amplitude versus sham-treated AFC alone rats ($P < 0.05$). These results demonstrate that although SPS did not increase startle responses, VNS was still effective in reducing startle amplitude.

Marble burying. To test for avoidance of novel objects, rats were tested on a marble burying task. VNS treatment during extinction reduced avoidance in PTSD model rats (Figure 5a). A one-way

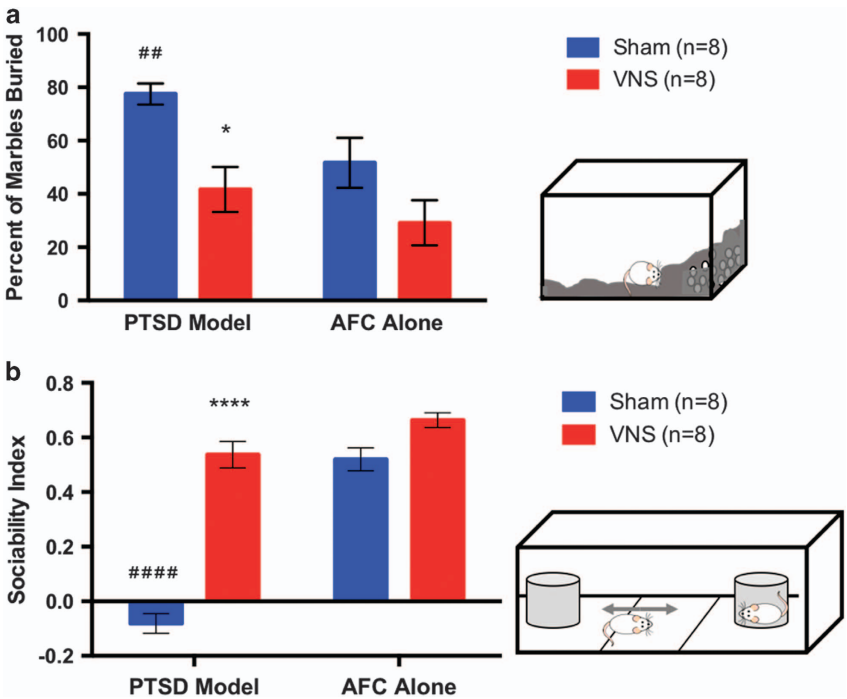

Figure 5. VNS during extinction decreases novel avoidance and social withdrawal behavior. (**a**) VNS reduced novel object avoidance in PTSD model rats. One week following extinction, PTSD model rats showed increased marble burying versus AFC alone rats ($^{\#\#}P < 0.01$). VNS during extinction reversed this effect PTSD model rats ($^{*}P < 0.05$ versus sham). There was no difference in AFC alone rats. (**b**) VNS increased social interaction. PTSD model rats given sham treatment showed diminished social interaction versus AFC alone rats ($^{\#\#\#\#}P < 0.0001$). Administration of VNS during extinction reversed this effect and increased social interaction ($^{****}P < 0.0001$ versus sham). There was no difference between VNS and sham stimulation in AFC alone rats. AFC, auditory fear conditioning; PTSD, post-traumatic stress disorder; VNS, vagus nerve stimulation.

ANOVA indicated a significant effect across groups $F(2.694, 18.86) = 6.622$, $P < 0.01$. PTSD model rats given sham stimulation buried more marbles than AFC alone rats ($P < 0.01$). PTSD model rats given VNS during extinction buried fewer marbles than those given sham stimulation ($P < 0.05$), and buried a similar number of marbles as AFC alone rats ($P > 0.05$). This indicates that VNS treatment during extinction reduced novel avoidance behavior in PTSD model rats.

In AFC alone rats, there was no difference between VNS and sham stimulation ($P > 0.05$).

Social interaction. To test whether VNS during extinction could reverse abnormal social interactions characteristic of PTSD, rats were evaluated using a social interaction test. PTSD model rats showed social withdrawal. VNS during extinction reversed the social withdrawal and restored normal social behavior (Figure 5b). A one-way ANOVA indicated a significant effect across groups $F(2.632, 18.42) = 59.44$, $P < 0.0001$. PTSD model rats given sham stimulation had a negative sociability index, indicating the typical preference to interact with the stimulus rat was deficient. The sociability index for the PTSD model rats was significantly lower than that of AFC alone rats ($P < 0.00001$). This was reversed by VNS; PTSD model rats given VNS had a higher sociability index than those given sham stimulation ($P < 0.0001$), and the sociability index was not significantly different from that of AFC alone rats. These results show that VNS treatment during extinction improved social interaction in PTSD model rats. There was no significant difference between social interaction indexes of VNS- versus sham stimulation-treated AFC alone rats.

Taken together, these results demonstrate that anxiety-related behavior in PTSD model rats is qualitatively and quantitatively distinct from that of AFC alone rats.

DISCUSSION

The SPS rat model of PTSD shares important characteristics with PTSD in human subjects. For clinical diagnosis of PTSD, patients must exhibit symptoms from each of the four criteria for more than 1 month.[2] PTSD patients show extinction impairments that may be responsible for the persistence of fear and anxiety symptoms. Here we observed that exposure to SPS (restraint, swim stress, loss of consciousness and social isolation) 7 days prior to fear conditioning makes rats susceptible to impaired extinction of the fear response. This is consistent with previous observations of extinction impairments in the SPS model.[22,23] However, we found that SPS combined with subsequent AFC lead to a fear response to the CS even after 11 days of extinction. PTSD model rats also showed significantly higher reinstatement, a measure of relapse, following a reminder of the unconditioned stimulus. One of the symptom clusters of PTSD is intrusion symptoms, such as distress and re-experiencing after exposure to traumatic reminders. The present findings indicate that the animal model of PTSD demonstrates resistance to extinction learning and re-experiencing the trauma (inferred from the freezing response) in the presence of reminders of the trauma.

Alterations in arousal and reactivity, such as exaggerated startle responses, make up the second symptom cluster. One week after completion of extinction, PTSD model rats showed heightened anxiety on an EPM, but the acoustic startle response was not significantly different in PTSD model rats and AFC alone rats. PTSD patients demonstrate hypervigilance and exaggerated startle responses. It is possible that auditory fear conditioning alone increases the acoustic startle response, obscuring the effect of multiple stressors. For example, the acoustic startle response is potentiated in fear-conditioned rats and humans when it is tested in the presence of conditioned cues.[32,33] Avoidance is another symptom cluster that is described in the *Diagnostic and Statistical*

Manual-5. PTSD model rats showed an increase in the novel avoidance task of marble burying.

The fourth symptom cluster of PTSD is negative alterations in cognition or mood, such as social withdrawal and persistent negative emotions. Social interaction scores were significantly lower for PTSD model rats than they were for AFC alone rats. In fact, AFC alone rats showed a strong preference for the social zone in the social interaction test, whereas PTSD model rats did not show a preference at all for the social zone over the nonsocial zone. These findings provide evidence of social withdrawal and less engagement in normal activities in the PTSD model. Taken together, these findings suggest that the SPS model of PTSD shows many behaviors that resemble PTSD symptoms, and may be useful in the study of the effects of traumatic events on the brain and behavior.

VNS administration during extinction reversed the extinction impairment observed in PTSD model rats, and VNS improved symptoms from each PTSD symptom cluster, including re-experiencing fear, elevated anxiety, arousal, avoidance and social withdrawal. PTSD model rats continued to exhibit a freezing response to the CS after 11 consecutive days of extinction. Others have shown enhancement in conditioned fear following contextual fear conditioning in the SPS model.[34] The extinction impairment in PTSD model rats reported here cannot be explained by a conditioning enhancement, as PTSD model rats do not show an enhancement in conditioning, in fact they show a temporary deficit in fear retention during tones 9 and 10. VNS administration during extinction reversed the extinction impairment in the rat PTSD model. Like AFC alone rats, PTSD model rats given VNS during extinction demonstrated remission of conditioned fear. This reduction in conditioned fear was also observed following reinstatement. A single reminder of the unconditioned stimulus was sufficient to fully reinstate conditioned fear in PTSD model rats, but VNS treatment during extinction prevented this relapse. These findings suggest that VNS may facilitate progress in exposure therapy by enhancing extinction of conditioned fear and reducing relapse.

Although persistent fear in the presence of reminders of the trauma is a well-recognized PTSD symptom, generalized anxiety, hyperarousal and avoidance behaviors can also be disabling. The administration of VNS during extinction reduced anxiety and avoidance behavior 1 week later on tasks that did not involve the CS. The observation that VNS treatment reduced avoidance of novel stimuli and startle responses, and increased exploratory and social behavior in PTSD model rats suggests that this adjuvant therapy can improve pathological behaviors that are not directly related to specific trauma cues.

Extinction is the goal of exposure-based therapies and VNS enhances extinction. However, the mechanisms by which VNS enhances extinction are not yet known. VNS enhances memory consolidation[17–19] and alters the release of neuromodulators into the brain that may promote experience-dependent plasticity.[20,35–39] Pairing VNS with an auditory stimulus alters auditory cortical maps while pairing VNS with motor learning modulates maps in the motor cortex, indicating that the nature of the plasticity is driven by the training that is paired with VNS.[40–44] We recently reported that VNS promotes plasticity in the pathway from the infralimbic area of the prefrontal cortex to the basolateral complex of the amygdala in rats.[20,45] Humans with PTSD exhibit reduced activation of the ventromedial prefrontal cortex and increased activation of the amygdala.[46,47] In addition, extinction impairments, like those observed in rats exposed to SPS, are associated with decreased prefrontal cortical control over amygdala activity.[48,49] VNS enhancement of consolidation of the extinction memory via facilitation of plasticity in this circuitry could be responsible for successful extinction following VNS in PTSD model rats.

VNS may also enhance extinction by inhibiting activity of the sympathetic nervous system.[50,51] The vagus nerve is sometimes referred to as the 'vagal brake' as activation of the vagus nerve activates the parasympathetic system and slows heart rate following the sympathetic stress response.[52] One study showed that chronic VNS reduced a measure of anxiety in rats,[53] and another suggested that chronic VNS improved scores on the Hamilton Anxiety Scale in human patients suffering from treatment-resistant depression.[54] It is possible that an immediate VNS-induced reduction in anxiety contributes to VNS-driven extinction by interfering with the sympathetic response to the CS, thus breaking the association of the CS with fear. In addition, a total of 20 trains of VNS administered over the course of 11 days may be sufficient to produce lasting anxiolytic effects, as has been observed following chronic VNS. Such a long-lasting anxiolytic effect would explain the reduction of general PTSD-like symptoms in VNS-treated rats. However, it is not likely that a general and lasting anxiolytic effect is responsible for VNS-driven remission of fear as unpaired administration of VNS did not enhance extinction of conditioned fear in a previously reported study.[19]

These findings demonstrate that VNS treatment can reverse extinction impairments and provide benefits across a variety of symptoms in a rat model of PTSD. Extinction of conditioned fear in nonhuman animals is frequently used as a preclinical model of exposure therapy.[13,55–57] The present findings suggest that VNS may be an effective adjunct to exposure therapy. Since VNS has been used in tens of thousands of patients with drug-resistant epilepsy[58] and delivery during exposure therapy requires considerably less stimulation, VNS may be safely used to enhance extinction in the treatment of PTSD and other disorders that show improvements with exposure-based therapies.

CONFLICT OF INTEREST

RLR is an owner of Vulintis Inc. and Optokinetics. RLR is a consultant for Konan Medical USA. None of these financial interests are related to this work. MPK is a paid consultant for and shareholder of MicroTransponder. MPK and CKM are authors of a patent entitled 'Enhancing Fear Extinction using Vagus Nerve Stimulation'. The remaining authors declare no conflict of interest.

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ORIGINAL RESEARCH
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Non-invasive Vagal Nerve Stimulation Effects on Hyperarousal and Autonomic State in Patients with Posttraumatic Stress Disorder and History of Mild Traumatic Brain Injury: Preliminary Evidence

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Posttraumatic stress disorder (PTSD) is a reaction to trauma that results in a chronic perception of threat, precipitating mobilization of the autonomic nervous system, and may be reflected by chronic disinhibition of limbic structures. A common injury preceding PTSD in veterans is mild traumatic brain injury (mTBI). This may be due to the vulnerability of white matter in these networks and such damage may affect treatment response. We evaluated transcutaneous vagal nerve stimulation (tVNS), a non-invasive, low-risk approach that may alter the functions of the limbo-cortical and peripheral networks underlying the hyperarousal component of PTSD and thus improve patient health and well-being. In this single visit pilot study evaluating the impact of tVNS in 22 combat veterans, we used a between-subjects design in people with either PTSD with preceding mTBI or healthy controls. Participants were randomized into stimulation or sham groups and completed a posturally modulated autonomic assessment and emotionally modulated startle paradigm. The primary measures used were respiratory sinus arrhythmia (high-frequency heart rate variability) during a tilt-table procedure derived from an electrocardiogram, and skin conductance changes in response to acoustic startle while viewing emotional images (International Affective Picture System). The stimulation was well tolerated and resulted in improvements in vagal tone and moderation of autonomic response to startle, consistent with modulation of autonomic state and response to stress in this population. Our results suggest that tVNS affects systems underlying emotional dysregulation in this population and, therefore, should be further evaluated and developed as a potential treatment tool for these patients.

Keywords: posttraumatic stress disorder, traumatic brain injury, hyperarousal, autonomic, transcutaneous vagal nerve stimulation, vagal, transcutaneous, sympathetic

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common mental illness affecting military veterans (1). Even in transient cases, recurrence can occur in older age. In Vietnam veterans, 10% experienced recurrence of PTSD symptoms nearly three decades after their worst trauma (2). Chronic emotional dysregulation is associated with impairment in quality of life as well as early onset of cognitive decline and serious health consequences (3–6). A common preceding comorbidity is mild traumatic brain injury (mTBI). The etiological contribution of mTBI to manifestation of symptoms of PTSD is not known. However, disruption of limbic white matter may play a role (3), as has been reported in neuroepidemiological studies of the phenomenology of white matter injury in mTBI (7, 8). These systems are directly germane to shifts in emotional and autonomic state to a vigilant and mobilized disposition.

A core deficit in PTSD is a bias toward a defensive strategy to environmental features along with an inability to shift away from a defensive state (3). This defensive strategy manifests in part as hyperarousal features including decreased respiratory sinus arrhythmia [RSA, high frequency heart rate variability (HRV)] and increased sympathetic nervous system response to stressors. In order to shift from defensive dispositions to socially engaging dispositions (e.g., interacting positively with other people), the individual needs to determine safety and inhibit limbic structures that control flight, flight, or freeze behaviors. PTSD is associated with chronic disinhibition of limbic structures such as the amygdala, which may explain symptoms such as exaggerated startle and autonomic nervous system (ANS) mobilization. Because mTBI may effect white matter and, in particular, limbic white matter inputs (7), there may be increased neurological vulnerability to development of symptoms of PTSD and of emotional dysregulation. Though PTSD is a transient condition in many patients, factors such as preceding mTBI may increase the likelihood of a chronic presentation.

The chronic stress associated with PTSD is a critical health issue as the physiological reaction to threat detection is clinically costly. Patients with PTSD have reduced HRV in response to trauma cues, require a longer recovery time (9), and have higher blood pressure (10) than their non-PTSD peers. Several features in resting autonomic behavior are correlated with mortality (11, 12). Reduced resting low frequency HRV is linked to coronary artery disease (13), and lower nighttime RSA is linked to increased stroke risk (14). Further, HRV in patients who recover from PTSD is indistinguishable from healthy controls (15). This suggests that negative health consequences of PTSD may be reversible if treatment success is achieved prior to cumulative damage from chronic stress.

Comorbidities are frequent in patients with PTSD, possibly due to issues with diagnostic clarity [see the recently revised DSM-V (16)], but also because trauma-induced responses are unpredictable and may represent a continuum of defensive state modulation. This produces behaviors consistent with fight, flight, or immobilization that fluctuate depending on internal state and interactions with perceived threat (17–19). Variations in these defensive response styles may, for example, present as depression (immobilization), intermittent explosive disorder (fight), or anxiety (flight).

Many currently available treatments target these states or associated disrupted emotional systems; however, the degree of effectiveness is variable. For example, a common first line pharmacotherapy approach is to use selective serotonin reuptake inhibitors. Unfortunately, clinical response rates, defined as a >30% reduction in symptoms, are rarely over 60%, and fewer than 20–30% of patients with PTSD taking these medications achieve full remission (20–23). Further, although a double-blinded placebo-controlled trial of venlafaxine, a serotonin–norepinephrine reuptake inhibitor, achieved a 78% clinical response rate, only 40% achieved remission (24). Several psychotherapies have been demonstrated to be helpful; however, the effect size varies significantly between reports, and combinations of therapy approaches were more effective in many, but not all, studies (23, 25–28), thus novel approaches are needed to treat those who do not achieve remission with current treatments.

Transcutaneous vagal nerve stimulation (tVNS) is a non-invasive nerve stimulation technique in which the auricular branch of the vagus is targeted. tVNS has been shown to have an impact on the neuronal systems that are involved in emotional regulation (29, 30), including the amygdala, and should be effective in the treatment and rehabilitation of PTSD. In addition, tVNS has been demonstrated to have a low risk profile, which is a significant departure from implanted vagal nerve stimulation (31, 32).

The putative mechanism of action of tVNS is through activation of the nucleus tractus solitarius, which has widespread projections throughout key brain networks involved in emotional regulation and PTSD, and the locus coeruleus. Published pilot fMRI studies have reported BOLD signal alterations in both the nucleus tractus solitarius and the locus coeruleus in the brain stem as well in amygdala activity in response to tVNS as contrasted to a sham stimulation (30). These fMRI changes were observed in healthy individuals who were given tVNS or active sham stimulation. The specific sham or contrasted stimulation varies between studies depending on the specific hypotheses tested; however, findings have generally been consistent. These data support the proposed mechanistic hypothesis, as a reduction in the BOLD signal in emotional (limbic) brain networks should correspond to diminished emotional reactivity and increased socially adaptive emotional regulation (inhibition of fight or flight behaviors), and these same regions and networks are abnormally active in response to emotional stimuli in individuals with PTSD.

Alteration of nucleus tractus solitarius activity should increase high frequency HRV, which has been demonstrated with tVNS in healthy controls (33). Increased high frequency (0.15–0.4 Hz) HRV (RSA) is associated with improved social function, better health outcomes, and better cognitive function. Conversely, lower RSA is associated with many psychiatric and psychological disorders including major depressive disorder, generalized anxiety disorder, high levels of aggression, and trauma history (34–36). tVNS has been shown in healthy people to induce an increase in high frequency HRV for at least 15 min after tVNS use without appreciably altering mean heart rate and other cardiovascular safety measures (32). The

latter is important, as it further speaks to the relative safety of tVNS when compared with its implanted counterpart (31). It is unknown whether tVNS has these same positive effects in patients with PTSD or those with disruptions in fronto-limbic function and further, whether tVNS changes emotionally modulated autonomic response.

Because of the high rates of PTSD, particularly subsequent to mTBI, combined with the large proportion of patients who do not achieve clinical response, let alone remission, there is a need for novel treatment approaches. Given the reported impact of tVNS on many brain regions implicated in the development and expression of PTSD, as well as autonomic state, it is a logical tool to develop to potentially treat PTSD. As an initial step toward that end, we have designed the current study to evaluate the impact of tVNS on indices of hyperarousal including vagal tone, as measured by high frequency HRV, and sympathetic nervous system activity in response to emotionally modulated startle as measured by electrodermal activity. We hypothesized that vagal tone would increase and that emotionally modulated sympathetic nervous system activity would attenuate in response to tVNS.

MATERIALS AND METHODS

Subjects

We recruited and received informed consent from 22 combat Veterans to participate in a U.S. Department of veterans affairs (VA) funded, University of Florida IRB approved pilot study designed to assess the effects of tVNS on autonomic symptoms of PTSD. Participants were recruited from a study designed to evaluate the impact of white matter damage in mTBI on the manifestation of PTSD cluster symptoms. Participants were contacted randomly, and three people declined participation due to logistical issues. Participants either had been diagnosed with both PTSD and closed-head mTBI injury ($n = 12$) or were healthy combat controls with no diagnosis of either ($n = 10$). At time of participation, average age was 29.7 years (SD 7) for the healthy combat control group and 30.4 years (SD 5.4) for the PTSD and mTBI group; minimum age was 22, maximum 43. Diagnosis status of mTBI and PTSD were verified *via* a clinical consensus conference using established criteria for each category using VA and DOD diagnostics guidelines. mTBI was defined as an injury to the head as a result of blunt or blast injury with any period of observed or self-reported transient confusion, disorientation or impaired consciousness, dysfunction of memory immediately before or after the time of injury, loss of consciousness less than 30 min, and signs of neurological or neuropsychological dysfunction identified soon after the injury. PTSD status was determined *via* a structured interview and electronic medical record review (VA PTSD clinical evaluations) and self report scales including the PCLD checklist—military (PCL-M), and Symptom Checklist 90—Revised (see **Table 1**). We also calculated symptom domains by aggregating PCL-M items, with re-experiencing (items 1–5), avoidance (6–7), dysphoria (8–15), and hyperarousal (16–17) as suggested by Pietrzak et al. (37). Mean PCL-M total scores were 24.6 ± 4.83 and 28.0 ± 2.94 ($p = 0.23$) for the healthy combat controls in the tVNS and sham groups, respectively, and 48.5 ± 4.43 and 42.0 ± 12.3 ($p = 0.31$) in the PTSD and mTBI group for the tVNS and sham groups, respectively. The average time since diagnosis of PTSD was 3.55 years, with a range of 1 month to 9 years, although symptom history typically began several years prior to diagnosis after the experienced trauma. One apparently healthy control was excluded upon *post hoc* review of their medical record for sickle cell anemia and one mTBI/PTSD subject was excluded for not having a DOD or VA reported history of mTBI.

Exclusion criteria were: premorbid severe psychiatric disorders, other neurological disorder, traumatic brain injury of greater

TABLE 1 | SCL-90-R, BDI-II, and PCLD checklist—military (PCL-M) symptoms per group, mean ± SD, and two sample *t*-test *p*-value.

	Healthy control	Posttraumatic stress disorder/ mild traumatic brain injury	*p*
SCL-90-R scale			
Somatization	0.43 ± 0.43	0.89 ± 0.74	0.101
Obsessive–compulsive	0.91 ± 0.58	1.71 ± 0.76	0.016
Interpersonal sensitivity	0.54 ± 0.45	1.07 ± 0.48	0.022
Depression	0.54 ± 0.32	1.22 ± 0.75	0.016
Anxiety	0.41 ± 0.29	1.05 ± 0.69	0.016
Hostility	0.61 ± 0.56	1.38 ± 0.8	0.021
Phobic anxiety	0.14 ± 0.23	0.82 ± 0.64	0.006
Paranoid ideation	0.67 ± 0.43	1.14 ± 0.48	0.032
Psychoticism	0.23 ± 0.24	0.7 ± 0.5	0.160
Global severity index	0.51 ± 0.28	1.12 ± 0.56	0.007
Positive symptom distress index	1.45 ± 0.31	1.72 ± 0.37	0.092
Positive symptom total	30.89 ± 15.59	55.91 ± 20.64	0.006
BDI_II	8.22 ± 4.44	18.18 ± 10.85	0.015
PCL-M			
Disturbing memories	1.33 ± 0.5	2.8 ± 0.79	<0.001
Disturbing dreams	1.11 ± 0.33	2.8 ± 1.03	<0.001
Re-experiencing	1.11 ± 0.33	1.7 ± 0.67	0.029
Upset when reminded of experience(s)	1.67 ± 0.71	2.4 ± 0.7	0.037
Physical reactions when reminded of experience(s)	1.22 ± 0.44	2.4 ± 0.97	0.004
Avoid thinking or talking about experience(s)	1.33 ± 0.71	3 ± 1.25	0.003
Avoid activities or talking about experience(s)	1.56 ± 0.73	2.8 ± 1.23	0.016
Trouble remembering experience(s)	1.33 ± 0.5	2.1 ± 1.1	0.069
Loss of interest	1.56 ± 0.73	2.8 ± 1.4	0.027
Feeling distant or cut off	1.56 ± 0.73	3 ± 1.49	0.017
Feeling emotionally numb	1.22 ± 0.67	2.7 ± 1.25	0.006
Feeling future will be cut short	1.11 ± 0.33	1.9 ± 1.2	0.072
Difficulty sleeping	1.89 ± 0.93	3.3 ± 0.82	0.003
Irritability or angry outbursts	1.89 ± 0.93	2.5 ± 1.27	0.245
Difficulty concentrating	2.11 ± 0.78	2.8 ± 1.23	0.161
Hyperarousal (alert or on guard)	2.11 ± 1.05	2.9 ± 0.88	0.097
Feeling jumpy or easily startled	2.11 ± 1.17	2.8 ± 1.14	0.210
PCL-M total	26.11 ± 4.26	44.6 ± 10.83	<0.001
PCL-M aggregate symptom domains			
Re-experiencing	1.29 ± 0.20	2.42 ± 0.53	<0.001
Avoidance	1.44 ± 0.68	2.90 ± 1.22	0.006
Dysphoria	1.58 ± 0.27	2.63 ± 0.80	0.002
Hyperarousal	2.11 ± 1.08	2.85 ± 0.91	0.13

severity than mild (e.g., open-head TBI; loss of consciousness greater than 30 min), medications, which affect ANS responses (e.g., β blockers such as propranolol), and current substance abuse. Assessment of medical exclusion/inclusion criteria was achieved *via* both consensus conference review of VA and DoD medical records and self-report during a structured interview as part of this study. In the healthy combat control group, some participants had recently (within 2 weeks) taken ibuprofen, omeprazole, hormonal birth control, clindamycin, and adalimumab (for colitis/Crohn's). In the participants with mTBI and PTSD, most (7) were not currently medicated; one was taking simvastatin, trazodone and gabapentin at night, and omeprazole; one venlafaxine, gemfibrozil, ibuprofen, docusate, and trazodone and prazosin at night; one each were taking mirtazapine and paroxitine. Trazadone and prazosin have α-adrenergic pharmacological impacts, but minimal/no β-adrenergic activity, and testing was conducted several half-life durations after most recent dose. Mirtazapine antagonizes α_{2A} and α_{2A} receptors, and to a much lesser extent α_1, but not β, so an impact on norepinephrine (NE)-mediated effects of tVNS is not expected. Paroxetine has NE transport inhibition and weak α1 receptor activity, but again not β, so an impact on NE-mediated effects of tVNS is not expected.

Study Design

Participants were randomized into either tVNS or sham (stimulus calibration only) subgroups and were then given a series of assessments of ANS function including emotionally modulated startle and postural HRV assessments. All participants were fitted with custom tVNS electrodes and had comfort threshold calibration, but stimulus amplitude was set to 0 (sham) or 80% of threshold (tVNS) for the remainder of each participant's session as pre-assigned by randomization.

Blinding

A researcher blinded to the stimulus condition conducted the self-report questionnaires, structured interview, and data processing. Stimulus grouping (stim versus sham) was un-blinded for interpretation of statistical analysis. Participants were not informed of their stimulus condition but were informed that, after calibration, the stimulus intensity would be set below the calibration level. The investigator who conducted the calibration and set the stimulus level according group (stim/sham) was a different researcher than those who interviewed participants and conducted data processing.

Vagal Nerve Stimulation

The tVNS stimulus was a 20 Hz, current controlled, 100 μS, alternating polarity pulse delivered *via* an earpiece custom molded for each participant's left ear with an Ag/AgCl disk electrode held at the interface of the poster wall of the left external auditory meatus and the posterior face of the left tragus, a convenient location to access the auricular branch of the vagus nerve (38). A return electrode was affixed just anterior to the tragus, minimizing stray currents and constraining stimulation. Prior to calibration, participants were informed that the stimulus would be slowly increased until they reported any discomfort, and that the stimulus intensity would then be reduced to a comfortable level for the remainder of the experiment. Individual sensitivity to tVNS stimulation was evaluated with a structured stepped-ramp protocol, with a brief pause at each step during which the participant was asked what the stimulus felt like to them and if they experienced any discomfort. The stimulation intensity was then set to 80% of threshold or 0%, for stimulus and sham groups, respectively; the mean threshold for comfort was 5.6 mA (range 3–11.3 mA). Discomfort was typically described as a mild buzzing or scratching sensation.

Postural HRV

Participants stood with their backs against a motorized tilting table/bed that can be slowly tilted (~2°/s) from 90° (standing) to prescribed angled supine positions (60° and 30°) with the soles of their feet supported at all times by a steel platform (see **Figure 1**). Continuous blood pressure and electrocardiograms were recorded in 3-min intervals at 90° (standing), 60°, and 30° static table angles. R waves were extracted with a data collection software package (AcqKnowledge 4.1, Biopac systems Inc.) and processed with custom Matlab scripts to correct for missed R-wave detections and apply appropriate filters to extract RSA high frequency HRV. RSA was then analyzed with a mixed effects model with fixed factors for angle and stimulus condition and a random effect of subject in R v3.2.3. One healthy control was dropped from RSA analysis due to having an abnormally low RSA (mean of 2.97, next lowest subject mean RSA was 4.06, overall mean across subjects was 4.99).

Startle-Blink Paradigm

Participants were given an emotionally modulated startle test while receiving tVNS (or sham stimulation). Participants viewed images from the international affective picture system (39, 40) and were asked to provide an evaluation of the valence (positive—negative) and arousal (neutral/none—high) of each image. During the viewing of these images, an acoustic startle probe (a 50 ms, 95 dB white noise pulse) was delivered during viewing for a predetermined subset of the images. Startle responses, particularly the electrodermal responses (EDA) were recorded and time-synched to the startle probe. Participant's perceptions of the valence and intensity of affective content were recorded. The resulting electrodermal response (EDA) data were processed with Ledalab V3.4.8, decomposing the signal into tonic

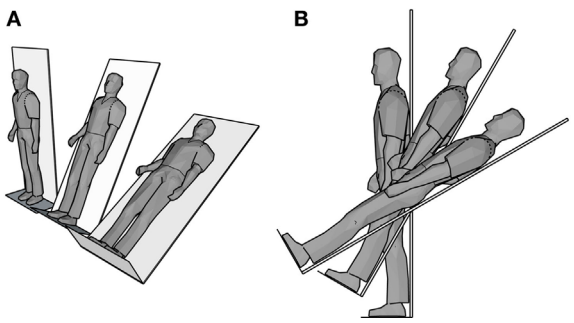

FIGURE 1 | Schematic of postural heart rate variability tilt table test. **(A)** Isometric perspective and **(B)** side view of the three positions (90°, 60°, 30°).

and phasic components (41), with analysis epochs triggered on the startle probe onset. Non-responding participants, one sham PTSD/TBI, one tVNS PTSD/TBI, and one tVNS control, identified by no or minimal changes in EDA signal throughout the task, were dropped from analysis; approximately 10% of participants are generally expected to be non-responders (42). We applied a mixed model in R v3.2.3 with fixed effects of group (PTSD/TBI or healthy control) and stimulus (tVNS or sham) and a random effect of subject, with dependent variables measured within each response window of maximum total deflection (phasic and tonic) continuous decomposition (CDA) measure of maximum phasic activity (Phasic Max) and amplitude sum as calculated by Ledalab (41).

RESULTS

Mean RSA was higher with tVNS than sham across all three postural positions (see **Figure 2**) indicative of increased parasympathetic activity [tVNS effect, $F(1, 17) = 3.33$, estimated Cohen's $d = 0.88$]. Diagnosis groups were pooled for this analysis due to insufficient sample size per cell for the full design and the primacy of the question of tVNS efficacy. This finding is consistent with prior reports of increased HRV with tVNS stimulation (32) in healthy populations.

Transcutaneous vagal nerve stimulation appeared to reduce sympathetic reactivity as measured with EDA and analyzed with continuous decomposition into tonic, or baseline, and phasic activity (41). Our primary concern is with phasic EDA measures maximum deflection within the response window (max deflection) and continuous decomposition analysis phasic (transient response to stimulus) response maximum (phasic max). The estimated effect sizes (Cohen's *d*) were 0.74, 0.56, and 0.43 for phasic max and max deflection and amplitude sum, respectively (see **Table 2** and **Figure 3**). This is consistent with our anticipated short-term impact of tVNS on emotional/behavioral measures and on RSA.

DISCUSSION

The primary findings of this preliminary study are that resting parasympathetic activity is increased and task-dependent emotionally modulated sympathetic nervous system activity is decreased with tVNS. The effect size estimates are promising and the estimated effects are consistent, though it is important to note that the sample sizes are small and full interaction models could not be applied. These were predicted effects based on our model of PTSD with mTBI and the putative mechanisms of action of tVNS (3) and promising reports from animal work using implanted VNS (43, 44). These effects suggest that tVNS may modulate emotional state as reflected by downregulating fight-or-flight and upregulating a physiological state conducive to positive social engagement (45). These results show a direct impact on the hyperarousal symptoms of PTSD by tVNS.

The mechanism of action of tVNS on these systems that are core to the experience of PTSD is not fully elucidated. There are both afferent and efferent components that may be relevant including vagal inputs into the heart and neuroanatomical connections whose activity appear to be modulated by tVNS. Furthermore, VNS can upregulate NE (46). NE is a neuromodulator that plays an important role in the mediation of many behaviors, including emotional learning and attention systems (both critical to the behavior of individuals with PTSD) (47, 48). As with all neuromodulators, NE's influences on neural function from the cellular level through interacting brain systems level are complex. For example, NE has opposing effects on amygdala network activity through α-NE versus β-NE receptors (49). In the

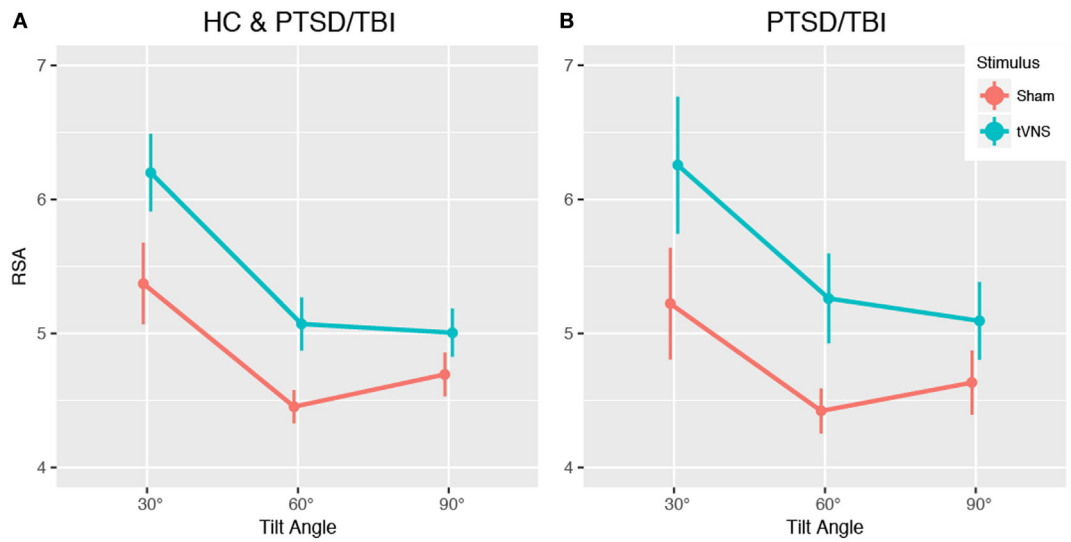

FIGURE 2 | Impact of transcutaneous vagal nerve stimulation (tVNS) on heart rate variability (HRV) during tilt-table experiment. Analysis of pilot data shows a trend toward increased respiratory sinus arrhythmia (RSA) high frequency HRV, indicating increased parasympathetic activity, across all tilt angles in the tilt-table experiment. **(A)** Main effect of tVNS, **(B)** impact of tVNS within PTSD and mTBI group. Data presented are mean ± SEM.

present study, a small subset of participants were prescribed prazosin and/or low-dose trazodone, which have α-adrenergic but not β-adrenergic pharmacology. As these participants took their last dose several half-life durations prior to participation, direct interaction with the NE impacts of tVNS was not expected. One participant was prescribed mirtazapine and another paroxetine, which affect α-adrenergic receptors but not β-adrenergic, so an interaction with the NE impacts of tVNS might have occurred; however, we would expect the most potent attenuation of tVNS to occur with β-adrenergic pharmacology. Instead of avoiding NE pharmacology, as we have done, future studies should consider pharmacologically dissecting the impacts of tVNS with α- and β-adrenergic blocking compounds.

The portions of the frontal lobe that compose networks that interact with the limbic system normally provide inhibition of the autonomic/automatic responses to stimuli previously associated with emotional responses. Consistent with this pattern of inhibition, these portions of the frontal lobes are important in the consolidation of extinction learning (50). In regard to fear learning, there is some evidence that NE-based prophylaxis with β-blockers such as propranolol can prevent the development of PTSD (51). However, this evidence is mixed and complicated by the predominant administration timeline beginning after the traumatic event. β-blocker usage as a treatment adjuvant is also complicated by its impairment of both reconsolidation and extinction learning as well as the attention modulation effects mentioned above.

Prefrontal networks have been strongly implicated in control of the ANS (52, 53). Consistent findings in animals have been found demonstrating a role of the prefrontal cortex in inhibiting sympathetic nervous system mobilization, possibly by modulating parasympathetic action (54, 55) including baroreceptor reflexes (56). Evidence from anatomical, lesion, and electrical stimulation studies suggest that medial prefrontal cortex is preferentially involved in modulating sympathoinhibitory responses, suppressing mobilization of the ANS for fight or flight (57). The full extent of how prefrontal cortex and nuclei involved in autonomic control interact is not known. There are cortical projections to the nucleus of the solitary tract (a major interaction vector for tVNS). These interconnections are involved in blood pressure, vasomotor, and heart rate regulation. In humans, increased heart rate and mean arterial pressure have been associated with decreased regional cerebral blood flow in prefrontal cortex (58).

Transcutaneous vagal nerve stimulation may be considered a parasympathomimetic treatment. It has both direct and indirect potential effects on HRV. Other treatments that manipulate the ANS have shown some promise in alleviating symptoms of PTSD including β-blockers, stellate ganglia blockade, and α channel blockers. Due to the direct impact of tVNS on the underlying brain and autonomic control systems affected by PTSD, tVNS may be a more effective and comprehensive approach to addressing symptoms of PTSD. Core to empirically supported treatments of PTSD are behavioral concepts of extinction and decoupling of a learned threat stimulus from perception of threat and autonomic mobilization. The combination of potential learning system effects (NE), autonomic behavior, and limbic activity may suggest a role of tVNS as a treatment adjuvant.

TABLE 2 | EDA reactivity to emotionally modulated startle shows a trend toward reduced reactivity with transcutaneous vagal nerve stimulation (tVNS) in pilot study.

Measure		Estimate	SE	*F*	*d*
Phasic max	Sham—tVNS	0.678	0.419	2.61	0.74
Max deflection	Sham—tVNS	0.114	0.093	1.49	0.56
Amplitude sum	Sham—tVNS	0.163	0.173	0.88078	0.43

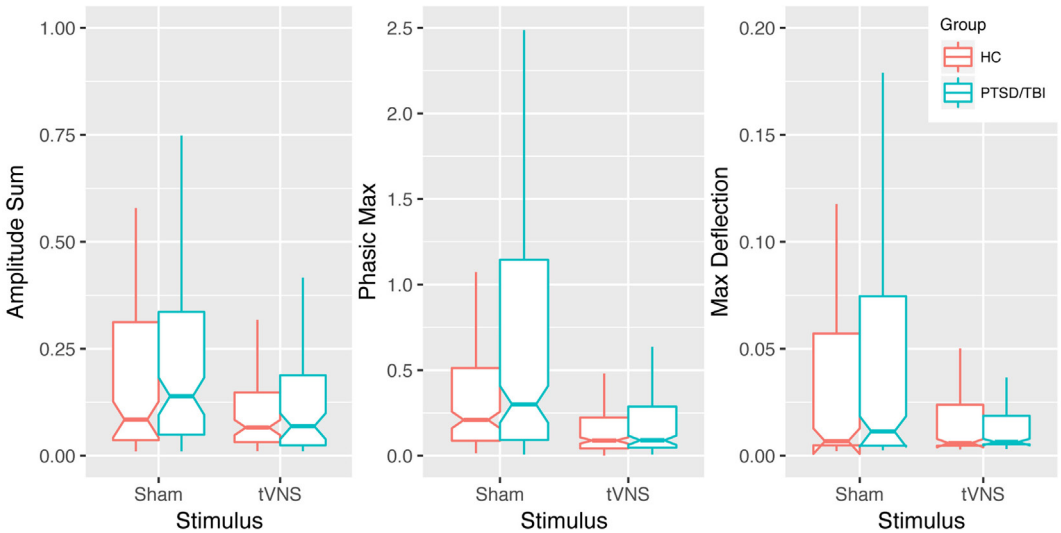

FIGURE 3 | Impact of transcutaneous vagal nerve stimulation (tVNS) on EDA measures. Box plots of phasic max, max deflection, and amplitude sum EDA measures showing a trend of reduced sympathetic activation in response to emotionally modulated startle with tVNS. Notches extend from the median ± 95% confidence interval ($1.58/\sqrt{n}$ × interquartile range), whiskers extend from lower and upper quartile to 1.5 × interquartile range.

This study has limitations, particularly the sample size is small. Thus, we are limited in the scope of the statistical models we can apply, so, we cannot analyze a full suite of predictors of individual differences in response. Further, the study was a between-subject, single session design. As such, though there was random assignment to condition, the data are between-subjects and, therefore, could reflect sampling. Finally, our subject blinding technique was psychological and not an active sham, i.e., there was no electrical stimulation in the sham condition. A large N crossover or longitudinal design would likely be more statistically powerful and would also allow for intraindividual comparison; however, the current design does avoid spillover effects and habituation as potential confounds.

CONCLUSION

To our knowledge, no studies have been published showing influence of tVNS treatment on alterations of baseline and emotionally modulated autonomic responses in individuals with PTSD. The results of the current preliminary study are promising and should be replicated and extended. What we observed in the current study is a baseline shift in physiological state, i.e., increased markers of parasympathetic nervous system activity. This change in parasympathetic nervous system activity may be interpreted as evidence of a tamping-down of defensive autonomic response and increased amenability to social engagement (45). Further supporting this interpretation, we observed decreased sympathetic nervous system response to emotionally modulated startle. One might conceptualize tVNS as a prosthetic for prefrontal action in inhibiting limbic activity and shifting emotional state to a more socially adaptive form. Autonomic behavior is central to symptoms of PTSD and effective modulation of these systems is associated with better emotional and health outcomes. Thus, further study of tVNS as a potential treatment or adjuvant for patients with emotional dysregulation in the continuum of PTSD is warranted. Follow-up mechanistic work is necessary for delivery impact and optimization and longitudinal effects the symptom clusters of PTSD as well as tolerability and other factors necessary for realization of this tool as a viable treatment approach. In addition to short-term impacts on emotional/autonomic features of PTSD as assessed in the present investigation, tVNS may also have long-term utility with repeated application in reducing symptoms of PTSD.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Florida Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Florida Institutional Review Board.

AUTHOR CONTRIBUTIONS

JW—conception of project, protocol creation, participant screening, data analysis, writing. DL—conception of project, protocol creation, execution of protocol with participants, data analysis, writing. EP—conception of project, protocol creation, data analysis (quantifying EDA), writing. GL—data analysis (ECG), writing.

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Full Length Article

Transcutaneous vagal nerve stimulation blocks stress-induced activation of Interleukin-6 and interferon-γ in posttraumatic stress disorder: A double-blind, randomized, sham-controlled trial

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ABSTRACT

Posttraumatic stress disorder (PTSD) is a highly disabling condition associated with alterations in multiple neurobiological systems, including increases in inflammatory function. Vagus nerve stimulation (VNS) decreases inflammation, however few studies have examined the effects of non-invasive VNS on physiology in human subjects, and no studies in patients with PTSD. The purpose of this study was to assess the effects of transcutaneous cervical VNS (tcVNS) on inflammatory responses to stress. Thirty subjects with a history of exposure to traumatic stress with (N = 10) and without (N = 20) PTSD underwent exposure to stressful tasks immediately followed by active or sham tcVNS and measurement of multiple biomarkers of inflammation (interleukin-(IL)-6, IL-2, IL-1β, Tumor Necrosis Factor alpha (TNFα) and Interferon gamma (IFNγ) over multiple time points. Stressful tasks included exposure to personalized scripts of traumatic events on day 1, and public speech and mental arithmetic (Mental Stress) tasks on days 2 and 3. Traumatic scripts were associated with a pattern of subjective anger measured with Visual Analogue Scales and increased IL-6 and IFNγ in PTSD patients that was blocked by tcVNS ($p < .05$). Traumatic stress had minimal effects on these biomarkers in non-PTSD subjects and there was no difference between tcVNS or sham. No significant differences were seen between groups in IL-2, IL-1β, or TNFα. These results demonstrate that tcVNS blocks behavioral and inflammatory responses to stress reminders in PTSD.

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, 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). In

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fact, a report from the Institute of Medicine stated that there is not sufficient evidence to conclude that the first line medication treatment, Selective Serotonin Reuptake Inhibitors (SSRIs), are effective for PTSD (Institute of Medicine of the National Academies, 2014). Based on these facts, new approaches to the treatment of PTSD are needed. Treatments that target the 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), may have promise for modulating the underlying basis for the disorder (Bremner, 2016; Shah et al., 2013).

Neuromodulation treatments that use electricity are a promising new approach to mental disorders that may act through effects on the underlying neurobiology of these disorders (Adair et al., 2020; Bikson et al., 2016, 2017b; 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., 1994, 1999; George et al., 1994; Handforth et al., 1998; Salinsky et al., 1999; The Vagus Nerve Stimulation Study Group, 1995) and treatment-refractory major depression (Berry et al., 2013; Brunoni et al., 2013, 2016, 2017; Dell-Osso et al., 2013; George et al., 2000, 2003, 2005; Marangell et al., 2002; Rush et al., 2000, 2005a, 2005b; Sackeim et al., 2001a, 2001b, 2007). FDA-approved VNS for these conditions involves surgical implantation in the brainstem with direct electrical stimulation of the vagus nerve (Aaronson et al., 2017; George et al., 2003; Terry, 2014). VNS has effects that may be beneficial for neurophysiological alterations associated with PTSD, 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). The requirement for surgical implantation, however, has limited the widespread implementation of VNS to psychiatry due to cost, inconvenience (Bremner and Rapaport, 2017; Marangell et al., 2002; Sackeim et al., 2001b), and lack of reimbursement by Medicare or other insurance companies (Feldman et al., 2013).

Dysregulated immune function is associated with stress and PTSD (Neigh and Ali, 2016; Passos et al., 2015). Mental stress in the laboratory in human subjects, including patients with coronary artery disease (CAD), is associated with increases in several inflammatory markers, including interleukin-6 (IL-6) (Hammadah et al., 2018; Marsland et al., 2017; Rooks et al., 2016), IL-1β (Lerman et al., 2016; Marsland et al., 2017), IL-10 (Marsland et al., 2017), and tumor necrosis factor (TNF)α (Marsland et al., 2017). Multiple studies show an increase in inflammatory factors at baseline in patients with depression (Akosile et al., 2018; Alcocer-Gómez et al., 2014; Capuron et al., 2008; Felger et al., 2016; Guo et al., 2015; Kiecolt-Glaser et al., 2007; Miller et al., 2009; Miller and Raison, 2016; Su et al., 2009; Vaccarino et al., 2008) and early trauma (Danese et al., 2007, 2008, 2011; Danese and McEwen, 2012; Rooks et al., 2012). Consistent with these studies, PTSD patients show increased inflammation (Gill et al., 2009), including increased baseline concentrations of leukocytes (Boscarino and Chang, 1999; Eswarappa et al., 2019), IL-6 (Gill et al., 2010; Gill et al., 2008; Guo and Tao Liu, 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 Kanel et al., 2010b), IL1β (Lindqvist et al., 2014; Passos et al., 2015; von Känel et al., 2007), TNF-α (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), IFNγ (Guo and Tao Liu, 2012; Hoge et al., 2009; Lindqvist et al., 2014; Passos et al., 2015; Woods et al., 2005; Zhou et al., 2014), intercellular adhesion molecule-1 (ICAM-1) (Plantinga et al., 2013; von Kanel et al., 2010a), vascular cell adhesion molecule-1 (VCAM-1) (von Kanel et al., 2010a), hsCRP (Eraly et al., 2014; Eswarappa et al., 2019; Heath et al., 2013; Lindqvist et al., 2017; Miller et al., 2001; Plantinga et al., 2013), and in one study, IL-2, IL-4, IL-8, and IL-10 (Guo and Tao Liu, 2012). Other studies showed no increase in IL-6 (Agorastos et al., 2019; Bruenig et al., 2018; McCanlies et al., 2011; Plantinga et al., 2013; von Känel et al., 2007), CRP (Baumert et al., 2013; Bruenig et al., 2018; Lindqvist et al., 2014; McCanlies et al., 2011; Sutherland et al., 2003; von Kanel et al., 2010b), IL-4 (von Känel et al., 2007), IL-10 (Lindqvist et al., 2017; Lindqvist et al., 2014; von Känel et al., 2007), IL-1β (Lindqvist et al., 2014), or IFN-γ (Bruenig et al., 2018). One study found increased diurnal cerebrospinal fluid (CSF) IL-6 but not plasma IL-6 in PTSD (Baker et al., 2001). Other studies showed altered genotype in genes modulating immune function in PTSD (Guardado et al., 2016). We recently found enhanced IL-6 response to mental stress involving public speaking in CAD patients with PTSD compared to CAD patients without PTSD (Lima et al., 2019). In summary, studies implicate altered immune function in PTSD, with a recent meta-analysis showing the largest effects for IL-6 and IFNγ (Passos et al., 2015).

VNS has effects on inflammation that may be beneficial for PTSD (Borovikova et al., 2000; Brock et al., 2017; Corcoran et al., 2004; Corsi-Zuelli et al., 2017; Das and Basu, 2008; Das, 2007, 2011; Li and Olshansky, 2011). IL-6 and TNF-α are modulable by the vagus nerve (Jan et al., 2010; Marsland et al., 2007). In animal studies VNS blocks lipopolysaccharide (LPS)-induced increases in IL-6, IL-18, IL-1β (Borovikova et al., 2000) and TNF-α (Bansal et al., 2012) but not IL-10 (Borovikova et al., 2000). Studies in patients with epilepsy and implanted VNS devices showed that long-term treatment resulted in decreased LPS-induced IL-6 (De Herdt et al., 2009) and neurotoxic kynurenic metabolites (Majoie et al., 2011) with no effect on IL-6, IL-10, IL-1β, or TNF-α (De Herdt et al., 2009).

A new generation of non-invasive devices have been developed for stimulation of the vagus nerve in the periphery (Bremner and Rapaport, 2017; Polak et al., 2009; Yoo et al., 2013). These non-invasive VNS (nVNS) techniques that stimulate the vagus in the ear (transcutaneous auricular VNS, or taVNS) or neck (transcutaneous cervical VNS (tcVNS)) have the potential for wide-spread implementation in patients with mental disorders (Bremner and Rapaport, 2017), however their effects on neurobiology, including immune function, have not been extensively studied. One study in healthy human subjects showed that tcVNS resulted in decreased TNF-α, IL-1β, IL-8, MIP and MCP-1 (Lerman et al., 2016), while another in PTSD patients showed reductions in TNF-α, but not IL-1β, IL-2 or IL-4 (Brock et al., 2017). tcVNS applied twice daily in an open-label, non-sham controlled study for 26 days in patients with Sjögren's Syndrome resulted in reductions in baseline levels of Il-6, TNF-α, IL-1β, and MIP (Tarn et al., 2019). No studies have looked at the effects of taVNS or tcVNS on stress-induced changes in immune function. We 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 (Gurel et al., 2020a, 2020b, 2020c), and other studies reported that taVNS blocked sympathetic function in patients with co-morbid mild Traumatic Brain Injury (mTBI) and PTSD (Lamb et al., 2017). We hypothesized these effects would be associated with a decrease in inflammation. In the current study, we examined the effects of tcVNS on peripheral cytokine response to personalized traumatic scripts and neutral mental stressors in the form of public speaking and mental arithmetic in traumatized subjects with and without PTSD. We hypothesized that tcVNS would block the effects of stress on IL-6 and IFNγ in PTSD.

2. Materials and methods

2.1. Human subjects

The research reported here (ClinicalTrials.Gov # NCT02992899) 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. Subjects

provided written, informed consent for participation. Subjects included physically healthy adults age 18–70 with a history of psychological trauma with and without the current diagnosis of posttraumatic stress disorder (PTSD) (Fig. 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 (CAPS) was administered to evaluate for presence and severity of both current and lifetime PTSD (Blake et al., 1995). Among 129 individuals who were screened for eligibility, 60 were enrolled and randomized to active or sham stimulation and 30 did not complete the protocol due to being lost to followup or technical reasons (Fig. 1). Thirty participants with a history of psychological trauma based on DSM criteria including 12 females completed the protocol at Emory University School of Medicine between May 2017 and October 2018. The Structured Clinical Interview for DSM-IV (SCID) was used to evaluate for psychiatric diagnosis (First and Gibbon, 2004). Ten subjects met criteria for current PTSD and 20 had a history of trauma without current PTSD. In the PTSD group, one (10%) met criteria for current co-morbid major depression and five (50%) for a lifetime history of major depression, two (20%) for current generalized anxiety disorder, one (10%) for current panic disorder with agoraphobia, one (10%) for current agoraphobia without panic disorder, one (10%) for current obsessive-compulsive disorder, one (10%) for current social phobia, one (10%) for a lifetime history of sedative/hypnotic abuse, one (10%) for a lifetime history of opioid abuse, and one (10%) for a lifetime history of cocaine abuse. In the non-PTSD group, 2/20 (10%) met criteria for current and lifetime major depression, one (5%) for a lifetime history of alcohol abuse, one (5%) for a lifetime history of marijuana abuse, one (5%) for a lifetime history of stimulant abuse, one (5%) for a lifetime history of opioid abuse, and one (5%) for a lifetime history of hallucinogen/PCP abuse. No subjects met criteria for current alcohol or substance abuse.

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 (Fig. 2) (Bremner et al., 2003, 2009; 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., 2003, 2009; Hammadah et al., 2017b). The first day included six traumatic recall scripts (approximately 1-min 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

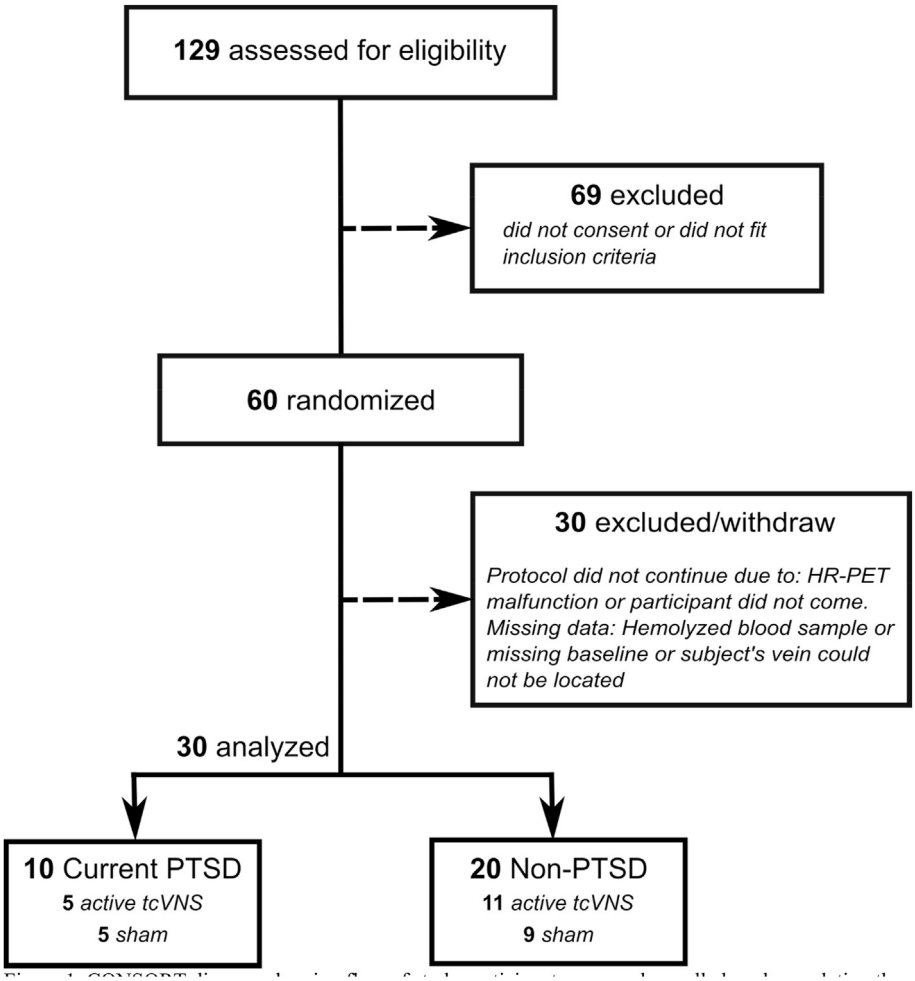

Fig. 1. CONSORT diagram showing flow of study participants screened, enrolled, and completing the protocol.

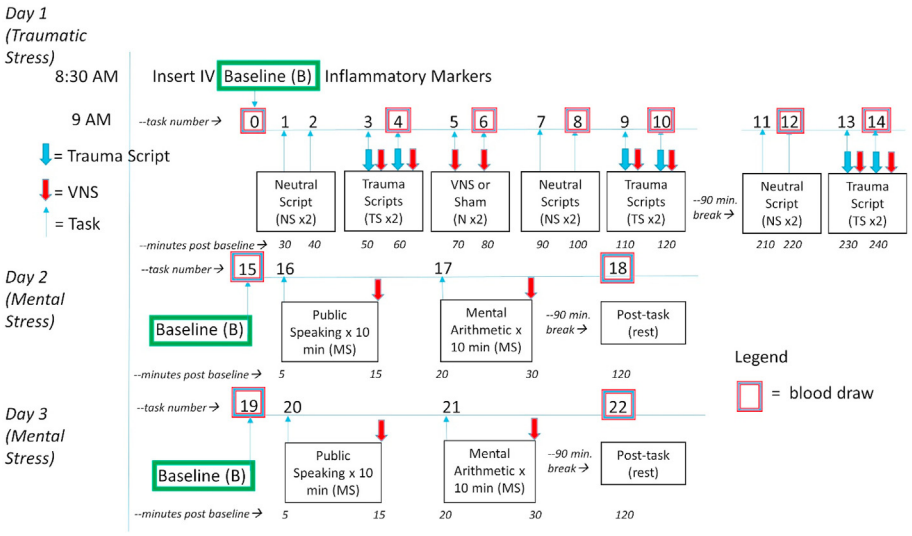

Fig. 2. Diagram of the study protocol. Traumatized participants with and without PTSD underwent three days of stress, one day (Day 1) with neutral scripts (NS) and personalized traumatic scripts (TS), and two days (Days 2 and 3) with mental stress (MS) involving public speaking and mental arithmetic 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 1 min and occurred in pairs with 10 min in between. Stress tasks were paired with stimulation with tcVNS or sham which began immediately after termination of the task and continued for 2 min 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 2 min in the absence of a task (N) repeated twice with 10 min in between followed by a blood draw. Neutral and traumatic script pairs were repeated followed by a 60 min 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 min 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 min into Day 1 (Traumatic Stress). On Day 2 after a baseline blood draw at rest (task #15) participants underwent mental stress (MS) involving 5 min of public speaking (task #16) with tcVNS or sham at the end, followed by an 8 min rest period, and another 5 min of mental arithmetic (task #17) followed by tcVNS or sham. After a 90 min 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 min time course of interleukin-6 (IL-6) response to stress based on prior studies. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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 after every four scans on this day. 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). Stimulations were applied immediately after the public speech and mental arithmetic tasks. First, the subjects underwent a public speech task for which they were required to provide a 2-min long defense statement in a scenario where they were accused of theft. After hearing the scenario details, they were given 2 min to prepare their defense and 2 min to present their statement. Stimulation was applied immediately after the public speech task. Later, the subjects rested for 8 min in silence. At the end of the 8 min, the subjects were given another task for which they were required to answer series of arithmetic questions for 3 min. A researcher provided negative feedback for incorrect answers and delayed response times. A second stimulation was applied immediately after the arithmetic task. After two mental stressors and two stimulation administrations, the subjects were given a 90-min break. After the break, a second blood draw was taken.

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 handheld GammaCore devices (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. Active

tcVNS devices produced an alternating current (AC) voltage signal consisting of five 5 kHz sine bursts (1 ms of five sine waves; pulse width = 40 ms) repeating at a rate of 25 Hz. 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., 2018a, 2018b, 2019; Bikson et al., 2017; Hays et al., 2013, 2014; Hulsey et al., 2017). The sham devices produce an AC biphasic voltage signal consisting of 0.2 Hz square pulses (pulse width = 5 s) eliciting a mild sensation. The peak voltage amplitudes for active and sham device are 30 V and 14 V, for active and sham, respectively. Throughout the protocol, the researcher gradually increased the stimulation intensity with a roll switch to the maximum the participant can tolerate, without pain. Amplitude dosing is dependent on subjective pain perception: researchers slowly increase the amplitude with a roll switch until the subjects instruct to stop. The active group received 17.8 V (± 6.6 SD), and sham group received 13.5 V (± 1.5 SD) averaged across all uses over three days, in this sample. An active stimulation amplitude higher than 15 V 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 2 min of stimulation. The stimulation intensity 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 30 V for active n-VNS, and from 0 to 14 V for the sham device. During each application, the stimulation intensity was increased to the maximum the subject could tolerate, without pain. The stimulation continued at the selected intensity. In this sample for blood draw analysis, the active tcVNS group (n = 16 participants) received 3.00 a.u. (±1.09) mean (±SD) and the sham group (n = 14 participants) received 4.74 a.u. (±0.69) averaged across all fourteen uses over three days, regardless of the disease status. In the active group, patients with PTSD (n = 5) received 2.94 a.u. (±1.09) and participants without PTSD received 3.02 a.u. (±1.09). In the sham group, patients with PTSD (n = 5) received 4.75 a.u. (±0.63) and participants without PTSD (n = 9) received 4.74 a.u. (±0.72). No participants reported lack of sensation.

2.5. Biomarker assay

We performed multiplex assays to measure IL-1β, IL-2, IL-6, TNFα, and IFN-γ purchased from Meso Scale Discovery. All experimental operations were in accordance with standard protocols. R2s of the standard curves for each plate were greater than 0.999.

2.6. Statistical analysis

Analysis of variance (ANOVA) tests were used to compare the demographic characteristics across the tcVNS treatment or sham stimulation group among patients with PTSD and healthy participants. We used ANOVA and linear regression models to measure the association between the cytokine levels and PTSD status, with or without tcVNS treatment effect. The beta coefficients (ß) from the mixed models indicate the adjusted average percent or absolute differences in the changes of parameters from the corresponding rest values, comparing active vs. sham device types. ß were reported along with 95% confidence intervals (CI) and P-values. A two-sided $p < 0.05$ denoted 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, education level and marital status (Table 1). The average age of this population was 30 (SD = 9), and the average BMI was 27 (SD = 5.60). Among all the participants, 18 (50%) were White / Caucasian, 21 (58%) were female, and consistent with prior reports (Kessler et al., 1995), 9/12 (75%) of the PTSD patients were female. All of the PTSD participants randomized to VNS were female (Fisher's Exact p = 0.045), and the gender proportion in the other groups was similar.

Table 1

	PTSD-VNS (n = 5)	PTSD-Sham (n = 5)	Non-PTSD-VNS (n = 11)	Non-PTSD Sham (n = 9)	Overall (n = 30)
Age					
Mean (SD)	29 (8)	32 (8)	30 (9)	34 (12)	31 (9)
Race					
White	1 (20%)	2 (40%)	6 (55%)	5 (56%)	14 (47%)
Black	3 (60%)	1 (20%)	3 (27%)	1 (11%)	8 (27%)
Other	1 (20%)	2 (40%)	2 (18%)	3 (33%)	8 (27%)
Sex					
Female	5 (100%)	2 (40%)	5 (45%)	5 (56%)	17 (57%)
Male	0 (0%)	3 (60%)	6 (55%)	4 (44%)	13 (43%)
BMI					
Mean (SD)	25 (8)	31 (5)	27 (6)	26 (4)	27 (6)
Education Level					
High school - graduate	3 (60%)	2 (40.0%)	5 (45%)	2 (22%)	12 (40%)
College graduate	2 (40%)	3 (60.0%)	6 (55%)	7 (78%)	18 (60%)
Marital Status					
Never married	4 (80%)	2 (40%)	7 (64%)	5 (56%)	18 (60.0%)
Married	0 (0%)	1 (20%)	3 (27%)	2 (22%)	6 (20.0%)
Divorced / Separated	1 (20%)	1 (20%)	1 (9%)	2 (22%)	5 (16.7%)
Widowed	0 (0%)	1 (20%)	0 (0%)	0 (0%)	1 (3.3%)
PTSD Score (PCL)					
Mean (SD)	44 (11)	52 (14)	29 (10)	30 (11)	35 (14)
PTSDSS Score					
Mean (SD)	29 (8)	24 (17)	17 (14)	19 (5)	18 (14)
Anger Index					
Mean (SD)	29 (7)	50 (30)	26 (13)	34 (13)	32 (9)
PSS-10 Score					
Mean (SD)	24 (4)	23 (3)	22 (4)	21 (8)	22 (12)
ESSI Score					
Mean (SD)	24 (5)	17 (8)	19 (9)	21 (5)	22 (2)
CADSS Score					
Mean (SD)	3 (4)	0 (0)	2 (5)	1 (3)	0 (0)

Exposure to personalized traumatic scripts resulted in greater increases in subjective anger on the VAS in PTSD patients compared to traumatized non-PTSD participants, and there was a pattern of greater blunting of response in the tcVNS compared to the sham stimulation group for PTSD patients (Fig. 3). Non-PTSD participants had minimal anger responses for both tcVNS and sham stimulation groups (Fig. 3).

Exposure to personalized traumatic scripts in conjunction with sham stimulation resulted in an increase in IL-6 in PTSD but not non-PTSD participants that was greater following repeated exposure to personalized traumatic scripts (Day 1) than for mental stress (public speaking and mental arithmetic on Days 2 and 3), that peaked about 90 min after exposure to the first traumatic scripts and was blocked by tcVNS (ß = 0.474, 0.009–0.939 95% CI, p = 0.046) (Figs. 4 and 5). There was minimal effect on IL-6 for neutral mental stress (public speaking and mental arithmetic) on days 2 and 3 in either the PTSD or non-PTSD, sham or tcVNS groups. Personalized traumatic scripts resulted in an immediate and marked rise in IFN-γ on Day 1 in the PTSD but not the non-PTSD participants (Fig. 6). The traumatic script-induced increase in IFN-γ was blocked by tcVNS versus sham (ß = -0.246, -0.470 – -0.022 95% CI, p = 0.032) (Fig. 5). There were no statistically significant differences between tcVNS of sham stimulation groups in IL-2, IL-1β or TNF-α (Table 2).

4. Discussion

Non-invasive transcutaneous cervical vagus nerve stimulation (tcVNS) in this study blocked an increase in the inflammatory marker interleukin-6 (IL-6) and Interferon-γ (IFN-γ) seen with personalized traumatic scripts in PTSD patients administered sham stimulation. Non-

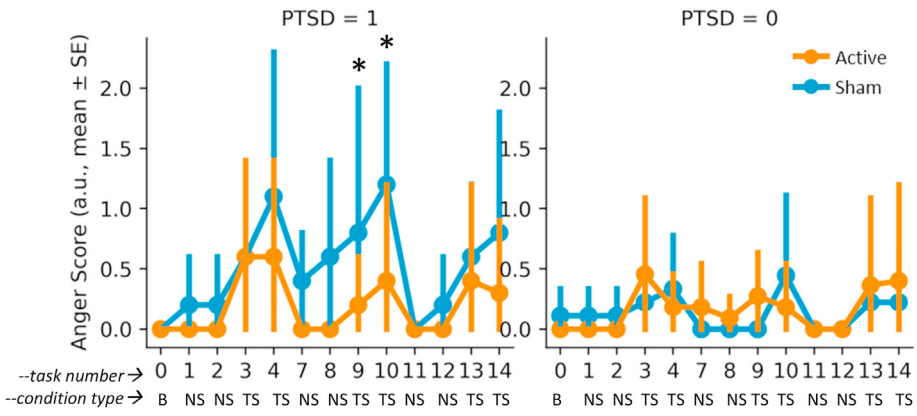

Fig. 3. Effects of tcVNS (red line) or sham (blue line) on subjective anger as measured with the Visual Analogue Scale (VAS) at baseline (B) and with neutral scripts (NS) and trauma scripts (TS). PTSD patients (left side) had greater anger responses to trauma scripts than non-PTSD traumatized participants, an effect that showed a pattern of being blunted by pairing with active tcVNS. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

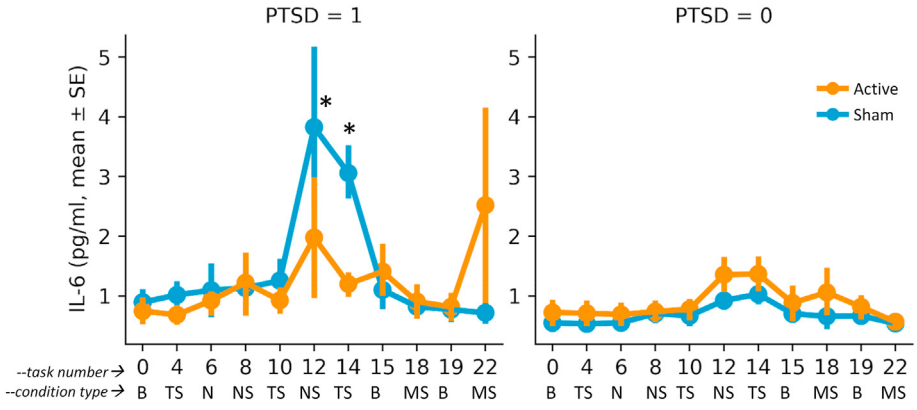

Fig. 4. Effects of tcVNS (red line) or sham (blue line) on interleukin-6 (IL-6) response to stress in patients with PTSD (left side) and traumatized participants without PTSD (right side). See Fig. 1 for a complete description of task numbers and condition types. On day 1 (personalized traumatic script day) blood was drawn at baseline (B), after the second presentation (task #4) of two trauma scripts 2 min in length paired with VNS or sham with 10 min between each script/stimulation pairing (TS), after the second (task #6) of two VNS/sham stimulations without task (N), after the second of two neutral scripts (NS) (task #8), after the second presentation (task #10) of two trauma scripts (TS) following the same protocol as before paired with VNS or sham. VNS or sham (TS), after the second of two neutral scripts (task #12), and after the second presentation (task #14) of two trauma scripts (TS) following the same protocol as before paired with tcVNS or sham. Toward the end of Day 1 with repeated TS there was an increase in IL-6 greater in sham versus tcVNS in PTSD patients (*) that occurred 90 min after the presentation of the first trauma scripts (Time points #12 and #14)($p < .05$). On Day 2 (D2) participants underwent a baseline blood draw at rest (task #15) and 90 min after mental stress (MS) in the form of public speaking and mental arithmetic paired with tcVNS or sham (task #18). On Day 3 (D3) participants again underwent a baseline blood draw at rest (task #19) and 90 min after mental stress (MS) using the same protocol as D2 (task #22). There were no significant differences between sham or active on days 2 or three with mental stress (MS, public speaking and mental arithmetic) compared to each days' baseline in PTSD. Non-PTSD participants showed no difference between active or sham for either trauma scripts (Day 1) or mental stress (Days 2 and 3). Statistical analysis showed a significant day by diagnosis by device effect ($p < .05$), with secondary analysis showing a significant increase in IL-6 in sham versus tcVNS in the PTSD group with traumatic scripts (Day 1, $p < .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

PTSD participants with a history of exposure to psychological trauma overall had minimal IL-6 or IFN-γ increases in response to personalized traumatic scripts. Personalized traumatic scripts had much greater effects than mental stress including mental arithmetic and public speaking on IL-6 and INF-γ in PTSD patients and therefore the blocking effects of tcVNS were more prominent. Active tcVNS also blocked subjective anger related to exposure to personalized traumatic scripts in PTSD patients.

The vagus nerve has both afferent fibers that go to the brain and efferent fibers that control peripheral organ, autonomic and immune function. Studies showing that peripheral IL-6 and TNF-α concentrations vary with changes in heart rate variability (HRV, a marker of para-sympathetic/sympathetic balance) are consistent with the current findings that the vagus modulates peripheral inflammation (Jan et al., 2010; Marsland et al., 2007) (Jan et al., 2010; Marsland et al., 2007). The

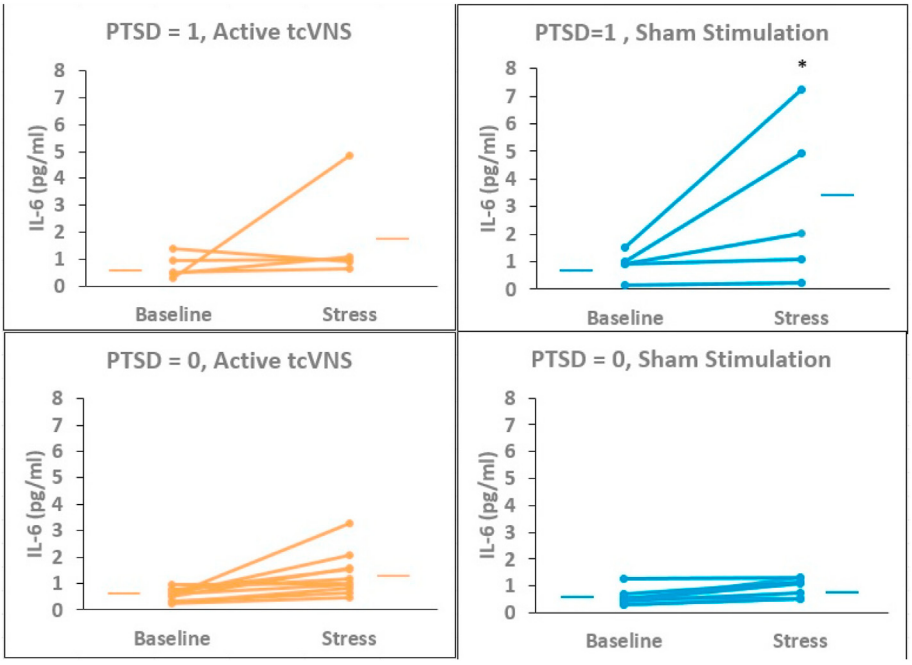

Fig. 5. Effects of tcVNS (red lines) or sham (blue lines) on IL-6 in individual traumatized participants with (PTSD = 1, top figures) and without (PTSD = 0, bottom figures) PTSD. Lines connect baseline to post-stress (traumatic scripts) measurements. There was a significant increase in IL-6 in PTSD patients undergoing sham stimulation. Traumatic scripts had little effect on IL-6 in non-PTSD participants. *p < .05. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

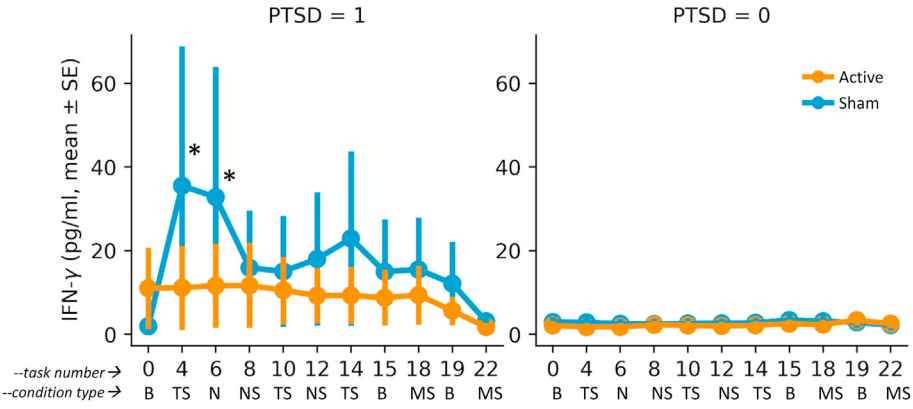

Fig. 6. Effects of tcVNS (red line) or sham (blue line) on Interferon-γ (IFN-γ) response to stress in patients with PTSD (left side) and traumatized participants without PTSD (right side). Overall there was a marked increase in IFN-γ in the PTSD but not the non-PTSD participants which was most pronounced after the first traumatic script (task #4) and was largely blocked by tcVNS but not sham, resulting in a significant increase in IFN-γ over the three day stress protocol in the sham group versus active tcVNS (*, p < .05). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

current study shows that PTSD patients have an enhanced inflammatory response to stress, with the greatest effects for personalized traumatic scripts. Our finding of blocked IL-6 and IFN-γ responses to stress with tcVNS adds to the growing literature on nVNS affecting central brain and peripheral autonomic function in human (Frangos et al., 2015; Frangos and Komisaruk, 2017; Gurel et al., 2020b, 2020c; Lerman et al., 2016, 2018, 2019; Yakunina et al., 2017) and animal studies (Brock et al., 2017; Chen et al., 2016; Oshinsky et al., 2014).

The effects of tcVNS blocking inflammatory responses and subjective anger to personalized traumatic script suggests clinical relevance for PTSD. Exposure to traumatic events can produce strongly encoded intrusive memories as well as lasting changes in neurobiology, brain circuits involved in the stress response, and symptoms of PTSD (Bremner and Pearce, 2016; Merz et al., 2016). Projections of the vagus through the nucleus tractus solitarius (NTS) extend to the locus coeruleus and hypothalamus, key areas involved in sympathetic hyperarousal in PTSD, as well as brain areas like the amygdala that are involved in the fear response and the medial prefrontal cortex / anterior cingulate, which is involved in both fear extinction and modulation of peripheral neurohormonal responses to stress (Hardy, 1995). tcVNS likely travels through these central pathways to effect changes in peripheral inflammation. Cytokines, inflammasomes, and other inflammatory markers have behavioral effects similar to stress-related psychiatric symptoms (Felger et al., 2013b; Miller and Raison, 2016), so reduction of spikes in IL-6 and IFN-γ that likely occur multiple times a day with traumatic reminders and daily stressors in PTSD patients to will likely benefit symptoms driven by inflammation and lead to improvements in clinical course. Reduction in subjective anger in addition to improving mental health also likely has beneficial health effects. For instance, in our studies of coronary artery disease (CAD) patients, we found not only an increase in mental stress-induced IL-6 in those with co-morbid PTSD (Lima et al., 2019) but also that psychological distress (including an aggregate measure of subjective anger, distress and PTSD) was associated with long-term adverse cardiovascular outcomes.(Pimple et al., 2019) Furthermore, CAD patients with mental stress-induced myocardial ischemia (MSI) had an increase in PTSD (Lima et al., 2020), and subjective anger response to stress was associated with MSI (Pimple et al., 2015).

IL-6 and IFN-γ are pro-inflammatory elements of a complex immune system that is responsible for fighting infections and is also responsive to stress (Miller et al., 2009). Data has accumulated in recent years that elevations in inflammatory markers are associated with stress-related psychiatric disorders, including major depression and PTSD (Miller et al., 2009). Studies in both animals and humans showed that catecholamines released during stress (including mental stress tasks) act

Table 2
Mean (SD) Concentrations of Interleukin-2 (IL-2), IL-1β and Tumor Necrosis Factor (TNF)-α Over Time in PTSD and Non-PTSD Participants with Active tcVNS or Sham Stimulation.

IL-2											
Time	0	4	6	8	10	12	14	15	18	19	22
PTSD Active	0.17	0.29	0.19	0.21	0.20	0.31	0.18	0.29	0.34	0.34	0.39
	(0.19)	(0.19)	(0.18)	(0.16)	(0.18)	(0.22)	(0.21)	(0.19)	(0.19)	(0.19)	(0.16)
PTSD Sham	0.28	0.31	0.23	0.32	0.30	0.34	0.40	0.22	0.20	0.22	0.21
	(0.21)	(0.18)	(0.20)	(0.18)	(0.19)	(0.19)	(0.16)	(0.19)	(0.17)	(0.17)	(0.17)
NonPTSD	0.34	0.23	0.32	0.34	0.29	0.37	0.35	0.23	0.32	0.22	0.39
Active	(0.22)	(0.21)	(0.22)	(0.22)	(0.24)	(0.21)	(0.20)	(0.21)	(0.23)	(0.22)	(0.19)
NonPTSD Sham	0.30	0.25	0.24	0.19	0.14	0.24	0.25	0.27	0.27	0.24	0.19
	(0.21)	(0.22)	(0.22)	(0.20)	(0.15)	(0.21)	(0.21)	(0.20)	(0.20)	(0.20)	(0.18)
IL-1β											
Time	0	4	6	8	10	12	14	15	18	19	22
PTSD Active	0.06	0.09	0.08	0.08	0.07	0.08	0.09	0.11	0.21	0.12	0.10
	(0.04)	(0.02)	(0.04)	(0.04)	(0.04)	(0.04)	(0.03)	(0.03)	(0.27)	(0.03)	(0.00)
PTSD Sham	0.10	0.07	0.07	0.08	0.06	0.03	0.05	0.11	0.09	0.11	0.10
	(0.08)	(0.04)	(0.04)	(0.04)	(0.03)	(0.01)	(0.01)	(0.08)	(0.05)	(0.06)	(0.05)
NonPTSD	0.05	0.06	0.08	0.05	0.08	0.07	0.05	0.12	0.14	0.12	0.12
Active	(0.03)	(0.04)	(0.04)	(0.04)	(0.07)	(0.04)	(0.04)	(0.08)	(0.10)	(0.08)	(0.09)
NonPTSD Sham	0.08	0.06	0.07	0.09	0.07	0.11	0.09	0.12	0.11	0.09	0.08
	(0.04)	(0.03)	(0.04)	(0.04)	(0.04)	(0.08)	(0.04)	(0.06)	(0.06)	(0.04)	(0.05)
TNFα											
Time	0	4	6	8	10	12	14	15	18	19	22
PTSD Active	2.60	2.27	2.47	2.41	2.45	2.32	2.27	2.44	2.46	2.55	1.94
	(0.85)	(0.88)	(0.76)	(0.97)	(0.60)	(0.62)	(0.82)	(0.72)	(0.64)	(0.83)	(0.17)
PTSD Sham	2.64	2.84	2.50	2.64	2.79	2.55	2.09	2.71	2.73	2.59	2.34
	(1.35)	(1.83)	(1.38)	(1.51)	(1.58)	(1.14)	(0.53)	(1.42)	(1.65)	(1.48)	(1.46)
NonPTSD	2.90	2.95	2.89	2.94	2.94	2.74	2.54	3.12	3.17	3.25	2.54
Active	(1.47)	(1.53)	(1.66)	(1.56)	(1.38)	(1.15)	(0.73)	(1.52)	(1.57)	(1.26)	(0.29)
NonPTSD Sham	2.22	2.11	2.10	2.23	2.30	2.14	2.23	3.43	3.43	2.67	2.12
	(0.50)	(0.65)	(0.68)	(0.42)	(0.55)	(0.52)	(0.44)	(2.80)	(2.69)	(1.05)	(0.60)

through the adrenergic receptor to activate the transcription factor, nuclear factor-κB (NF-κB), which leads to increases in cytokines, including IL-6 (Bierhaus et al., 2003). Raison and Miller have hypothesized that depression and inflammation may have links in evolution (Miller and Raison, 2016; Raison and Miller, 2013). Depression represents an illness-related behavior that serves to conserve energy and may be adaptive in survival, however interpersonal stress may have been a prelude to violent conflicts in primitive societies where an anticipatory outpouring of pro-inflammatory factors may have be critical for survival in the event of life-threatening wounds (Miller and Raison, 2016; Raison and Miller, 2013). Considerable evidence links elevated immune function to major depression and PTSD, and relevant to the current study, one meta-analysis showed that the statistically strongest findings in PTSD were for IL-6 and IFN-γ (Passos et al., 2015). Several studies showed that stress is associated with enhances release of IL-6 (Marsland et al., 2017), including mental stress tasks in patient with PTSD (Lima et al., 2019) and in individuals with early life stress who are vulnerable to the development of depression (Pace et al., 2006). Elevations in IFNγ and IL-6 are associated with decreases in tryptophan, the precursor of serotonin, a key neurotransmitter underlying the neurobiology of depression and PTSD, with associated increased symptoms of depression (Felger et al., 2013a; Raison et al., 2010). Diversion of tryptophan metabolism leads to increased metabolism along the kynurenine pathway, which has been linked to suicide and depression (Myint, 2012). Kynurenine also antagonizes the cholinergic anti-inflammatory effects of VNS (Myint, 2012; Nizri and Brenner, 2013; Olofsson et al., 2015) and is blocked by VNS (Majoie et al., 2011). Kynurenine can be converted to quinolinic acid, which enhances glutamatergic transmission with associated decreases in brain derived neurotrophic factor (BDNF) in the hippocampus, a mechanism implicated in PTSD and depression and the response to antidepressant treatments (Duman, 2004; Duman et al., 2001; Nibuya et al., 1995; Santarelli et al., 2003). These studies indicate that blocking of stress-induced IL-6 elevations with tcVNS may impact the underlying neurobiology of PTSD and have clinical utility for its treatment.

Findings of increased IFN-γ with stress in PTSD that are blocked by tcVNS have relevance for alterations in cell mediated immunity that may underlie symptoms of PTSD. Cell mediated immunity utilizes T cells including $CD8^+$ cytotoxic cells that lyse cells harboring microbes and $CD4^+$ cells that produce cytokines and activate phagocytes that engulf and kill microbes. These latter cells differentiate into Th1 and Th2 subsets, as well as Th17 subsets. Glucocorticoids including cortisol are anti-inflammatory and lower levels of cortisol as seen in patients with PTSD (Bremner et al., 2007; Yehuda et al., 1996) could result in enhancement of Th1 cell function in PTSD patients (Griffin et al., 2014; Zhou et al., 2014). Cytokines produced by Th1 cells include proinflammatory mediators (IFN-γ) and IL-2. IFN-γ is a potent macrophage activator which also has antiviral activity. Th2 cytokines are IL-4, IL-5, IL-10 and IL-13, which are mainly anti-inflammatory. Cytokine production is partly controlled by cholinergic neurotransmission and therefore the vagus nerve (Nizri and Brenner, 2013), and vagal nerve stimulation has been shown to shift the TH1/TH2 balance and dampen pro inflammatory responses (Olofsson et al., 2015). Several lines of evidence link altered cellular immunity to PTSD, including studies in women with PTSD showing enhanced cell mediated immunity (S.N. Wilson et al., 1999) and delayed-type hypersensitivity (DTH) reactions that are consistent with an enhancement of Th1 response and thus increased IFN-γ (Altemus et al., 2003). Other studies have linked DTH responses to elevated IFN-γ (Barth et al., 2003) and have shown increased IFN-γ in PTSD (Lindqvist et al., 2014; Passos et al., 2015; Woods et al., 2005). Vagus nerve stimulation activates T cells that produce acetylcholine, and by binding to the alpa-7 subunit of the cholinergic receptor inhibit NF-κB (Rosas-Ballina et al., 2011). VNS also inhibits High Mobility Group Box 1 (HMGB1), a proinflammatory master mediator, which is increased in PTSD (Huston et al., 2007; Wang et al., 2015). The findings of the current study of an increase in IFN-γ blocked by tcVNS in light of the findings reviewed above add more evidence for a clinically relevant impact on the underlying neurobiology of PTSD.

The current study has several important limitations. We examined multiple biomarkers which introduces the possibility of false positives, even though the primary hypothesis was based on IL-6. The sample size was small, and gender was not evenly distributed between groups. Our original hypothesis was that tcVNS would block IL-6 response to both

traumatic script stress and neutral mental stress (public speaking and mental arithmetic). The current study did not find as much of an IL-6 response to neutral mental stress as in our prior study of public speaking stress in patients with Coronary Artery Disease (CAD) and PTSD. That was a different sample, however, including older patients with CAD and more medical comorbidities than the current sample of younger uncomplicated PTSD patients (Lima et al., 2019). The prior study was also the first exposure to stress performed while sitting in a chair as opposed to lying in a scanner, which we have found presents a more direct interpersonal experience in the solicitation of stressful responses within a social context. In prior studies we found a reduction in cardiovascular reactivity on a following day when stress was repeated in a scanner (Bremner et al., 2018). In the current study, neutral mental stress came on subsequent days to personalized traumatic scripts, so a reduction in responsiveness is to be expected. Furthermore, our prior research has shown a more robust biological response in terms of heart rate and blood pressure and cortisol response to traumatic script stress (Elzinga et al., 2003) than neutral mental stress (Bremner et al., 2003) in patients with PTSD. The current findings of great effect on traumatic script stress are in line with these prior results. Also, since this was an exploratory study and nothing is known about the effects of tcVNS on different types of stressors it can be considered as hypothesis generating data for future research. Due to these factors it is possible that findings are related to false positives. Therefore the current findings should be considered exploratory and the results should be replicated in other samples with larger numbers of subjects. Another possible limitation concerns the comparison intervention or "sham stimulation" which involved an active electrical stimulation. Although we have not found that the parameters of the stimulation result in responses consistent with vagus nerve stimulation (Hays et al., 2013; Noble et al., 2017, 2018, 2019; Pena et al., 2014; Souza et al., 2019), it is possible that stimulation occurred in some individuals, or that stimulation of other sensory nerves would have an effect. Use of inert devices as controls could lead to participants perception that they were not getting active interventions, increasing the risk of positive results that are only due to a placebo effect, a constant risk in device research. High frequency voltage signals (such as the active stimulus) 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) 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 may deliver substantial energy 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 and current levels directly to the skin, it activates skin nociceptors, causing a similar feeling to a pinch. This sensation is necessary for blinding of the participants, and is thought as a critical detail by the authors for the evaluation of the potential treatment in psychiatric populations. Use of sham stimulation is a universal practice, regardless of voltage or current mode. For example, current-mode (i.e., auricular) stimulation studies typically use the earlobe as sham stimulation as the earlobe contains anatomically less nerve innervation (Burger et al., 2019; Kraus et al., 2007; Stavrakis et al., 2020; Verkuil and Burger, 2019; Yakunina et al., 2017, 2018). Compared to 'no stimulation' as a sham alternative, investigators think that any sort of sensation is necessary for blinding of the participants, and is thought as a critical detail for the valuation of the potential treatment in psychiatric populations. As this is a psychological study, use of an 'active sham' compared to no stimulation might mitigate psychological effects regarding treatment perception. It is important to note that each subject only uses one type of stimulation (either active or sham), devices assigned by staff who do not take part in data collection or analysis. Hence, subjects do not know how the other devices (that they did not use) feel like. If anything, however, factors reviewed above would have diminished our ability to detect differences between active and control devices, rather than increase them.

Findings of the current study that tcVNS blocks inflammatory responses to stress add to our recent studies showing that tcVNS blocks sympathetic arousal associated with exposure to personalized traumatic scripts and/or enhances parasympathetic function (Gurel et al., 2020b, 2020c). These physiological systems are known to be associated with anxiety and other symptoms relevant to PTSD. Other studies in implanted VNS suggest that tcVNS may have useful clinical applications based on its effects on memory and possible enhancement of neuroplasticity and/or facilitation of extinction of conditioned responses to reminders (Bremner, 2016; Bremner and Charney, 2010; Clark et al., 1999; Engineer et al., 2011; Noble et al., 2017; Pena et al., 2014; Peña et al., 2013). Future studies should investigate fundamental questions regarding parameter-specific effects of the stimulation (frequency, amplitude, waveform shape) on neuroinflammatory, cardiovascular, and peripheral function for improved autonomic control and the design of adaptive and personalized therapies (Ardell et al., 2015, 2017; Badran et al., 2018b; Gurel et al., 2020b)

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2020.100138.

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Quantifying acute physiological biomarkers of transcutaneous cervical vagal nerve stimulation in the context of psychological stress

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ABSTRACT

Background: Stress is associated with activation of the sympathetic nervous system, and can lead to lasting alterations in autonomic function and in extreme cases symptoms of posttraumatic stress disorder (PTSD). Vagal nerve stimulation (VNS) is a potentially useful tool as a modulator of autonomic nervous system function, however currently available implantable devices are limited by cost and inconvenience.

Objective: The purpose of this study was to assess the effects of transcutaneous cervical VNS (tcVNS) on autonomic responses to stress.

Methods: Using a double-blind approach, we investigated the effects of active or sham tcVNS on peripheral cardiovascular and autonomic responses to stress using wearable sensing devices in 24 healthy human participants with a history of exposure to psychological trauma. Participants were exposed to acute stressors over a three-day period, including personalized scripts of traumatic events, public speech, and mental arithmetic tasks.

Results: tcVNS relative to sham applied immediately after traumatic stress resulted in a decrease in sympathetic function and modulated parasympathetic/sympathetic autonomic tone as measured by increased pre-ejection period (PEP) of the heart (a marker of cardiac sympathetic function) of 4.2 ms (95% CI 1.6–6.8 ms, $p < 0.01$), decreased peripheral sympathetic function as measured by increased photoplethysmogram (PPG) amplitude (decreased vasoconstriction) by 47.9% (1.4–94.5%, $p < 0.05$), a 9% decrease in respiratory rate (−14.3 to −3.7%, $p < 0.01$). Similar effects were seen when tcVNS was applied after other stressors and in the absence of a stressor.

Conclusion: Wearable sensing modalities are feasible to use in experiments in human participants, and tcVNS modulates cardiovascular and peripheral autonomic responses to stress.

Introduction

Traumatic stress is associated with activation of the sympathetic nervous system (SNS), and can be associated with lasting alterations in autonomic function and in extreme cases symptoms of posttraumatic stress disorder (PTSD) [1]. Re-exposure to traumatic

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memories can be associated with a re-activation of the SNS, which can lead to symptoms of PTSD [2–6]. Vagal nerve stimulation (VNS) effectively modulates autonomic nervous system function and thus represents a potential treatment option for PTSD [7,8], however, implementation is limited by the implantation procedure. Widespread implementation of VNS has been limited by invasiveness of the therapy and high costs typically not covered by medical insurance [9–11]. Transcutaneous vagal nerve stimulation (tVNS) devices applied to cervical or auricular portions of the vagus nerve potentially offer substantially enhanced feasibility and tolerability [12–16], but their effects on physiology are not well understood.

The vagus nerve is a complex neural structure that contains descending efferent fibers that regulate peripheral organs and autonomic nervous system activity, and ascending afferent fibers to the brain via the nucleus tractus solitarus (NTS) [7]. The NTS projects to other brain areas such as the amygdala, hippocampus, locus coeruleus, and prefrontal cortex that play important roles in emotion regulation and have been implicated in stress-related mental disorders, including PTSD [17,18]. Efferent fibers modulate cardiovascular function and peripheral autonomic tone, which can also be modulated by afferent fibers via brain areas with effects on these parameters including the prefrontal cortex and insula [19]. Electrical stimulation of the vagus nerve, using implantable devices (direct VNS), has been demonstrated to be efficacious for the treatment of epilepsy and refractory major depression, and is approved by the Food and Drug Administration (FDA) for the treatment of these disorders [10,20–26]. The effects of direct VNS on autonomic imbalance likely explains much of its efficacy for these disorders, as well as its applicability to cardiovascular disorders [27,28]. The effects of direct VNS on enhancement of memory and neuroplasticity also suggest a role for treatment of cognitive disorders, stroke, and other conditions [29–34].

tVNS devices that target the auricular (taVNS) or cervical (tcVNS) portion of the vagus nerve have recently been developed that, due to their low cost and on-demand usability, have the potential to be widely implemented for rehabilitation, treatment of mental disorders, and performance improvement [35,36]. taVNS and tcVNS technologies are considered separately as they target different portions of the vagus nerve: the auricular branch is accessed from the ear and the cervical branch from the right or left side of the neck. Brain imaging studies reveal that taVNS devices can modulate the vagal afferents [15,37–39], along with studies that report improved vagal tone through heart rate, heart rate variability, and microneurography [40,41], increased salivary alpha amylase, and decreased salivary cortisol [42]. Beneficial outcomes have been noted by multiple groups on episodic migraine [39], epilepsy seizure frequency [14], major depression [43,44], and chronic tinnitus [45].

While fewer studies exist focused on tcVNS, several important results have been demonstrated. Imaging studies noted vagal afferents are accessible with tcVNS [46] and, recently, a multi-scale image-derived model of tcVNS was developed predicting the fiber activation due to tcVNS [47]. The downstream effects were observed in serum cytokines, chemokines, and cardiac vagal tone [48,49]. Clinically relevant outcomes for tcVNS were noted for trigeminal allodyna and migraine [50,51]. Analyses on the effects of taVNS and/or tcVNS on cardiovascular and autonomic function have produced mixed outcomes [40,48,51–53]. These studies used basic parameters such as heart rate (HR), heart rate variability (HRV) and blood pressure (BP), that are easy to attain, but are influenced by sympathetic and parasympathetic nervous systems along with subsequent peripheral vascular resistance, and therefore do not provide information on specific target pathways and physiological systems [54,55]. New advances in wearable sensing devices, incorporating seismocardiography, electrocardiography, ballistocardiography, movement, and peripheral vascular constriction, have improved specific assessment of sympathetic, parasympathetic, cardiovascular, and peripheral vascular function in conjunction with tasks such as mental stress, and could be applied to neuromodulation [56–58]. VNS results in changes in cardiovascular and peripheral function, reflected by a dynamic interplay between the activation of descending efferents and ascending afferents [59]. Due to this complex interplay, the effects of neuromodulation on *both* sympathetic and parasympathetic autonomic systems must be considered. Assessment of the effects of VNS on autonomic function also has clinical relevance as maladaptive autonomic regulation is the hallmark of many psychiatric disorders including PTSD.

Autonomic function plays a critical role in the stress response, but little is known about the effects of VNS on autonomic responses to stress. Exposure to traumatic events can produce strongly encoded intrusive memories associated with alterations in autonomic function that can persist in certain vulnerable individuals, and be associated with long-term changes in brain circuits involved in stress response, and possibly lead to PTSD [60,61]. Studying autonomic correlates of traumatic stress memories has clinical implications for patients with PTSD [62]. Both traumatic stress [63,64], and other stressors such as public speech or mental arithmetic [65–67] can be produced in the laboratory. These paradigms have been shown to reliably produce behavioral and physiological responses consistent with a stress response [65–68], although clinically there are fundamental differences between traumatic stress recall and “neutral” mental stress paradigms. Due to the role of traumatic reminders (conditioned fear) in PTSD, traumatic stress paired with direct or transcutaneous VNS has been studied in animal models and humans. In animal models, direct VNS with cuff electrodes has been shown to lead to improvements in fear response and pathological neural activity [8,69,70]. Human subject studies reported improvements in vagal tone in patients with PTSD through taVNS [71]. Some groups, through subjective scales, have reported improvements in fear and worry responses for taVNS for healthy populations [52,72,73], but no improvements in high worriers [53]. No changes were observed in physiological indices based on HR and HRV for fear and worry studies for taVNS, and pairing the less commonly used tcVNS with stress has not been explored. In the current study, we examined the effects of tcVNS applied in tandem with acute traumatic and mental stress on autonomic function in real time as measured with cardiovascular, peripheral, autonomic, and respiratory changes following tcVNS or sham administration with or without acute stress. We previously presented preliminary data from the initial participants of the current sample of traumatized healthy human participants without PTSD showing that tcVNS modulates autonomic responses to stress as measured by systolic time intervals and blood volume pulse [74,75]. In this study, we extended our measurements to electrocardiography (ECG), seismocardiography (SCG), photoplethysmography (PPG), respiration (RSP), electrodermal activity (EDA), and blood pressure (BP) signals, comparing two groups of participants receiving either active or sham tcVNS stimuli in conjunction with exposure to the stress of personalized traumatic scripts, mental arithmetic, and public speech tasks.

Materials and methods

Human subjects study

The study was performed under a protocol approved by the institutional review boards of Emory University (#IRB00091171), Georgia Institute of Technology (#H17126), SPAWAR Systems Center Pacific, and the Department of Navy Human Research

Protection Program. The study took place in Emory University School of Medicine between May 2017 and October 2018 (ClinicalTrials.gov # NCT02992899). Participants included healthy adults between ages 18–65 with a history of psychological trauma but without current posttraumatic stress disorder (PTSD) or other major psychiatric disorder. Participants were recruited and provided written, informed consent for participation. Fig. S1 presents the Consolidated Standards of Reporting Trials (CONSORT) diagram for the study, and Table S1 provides demographic data on the participants. Exclusion criteria were: pregnancy, traumatic brain injury (TBI), meningitis, active implanted device, current history of PTSD or other major psychiatric disorder including schizophrenia, schizoaffective disorder, bipolar disorder, severe major depression, bulimia or anorexia based on Diagnostic and Statistical Manual-5 (DSM-5) criteria [76] and the Structured Interview for DSM (SCID) [77], evidence or history of serious medical or neurological illness, post-menopausal status, positive toxicology screen, and carotid atherosclerosis. The Clinician Administered PTSD Scale (CAPS) was administered to evaluate for presence and severity of both possible current and lifetime PTSD [78] and participants who met criterion for current PTSD based on the CAPS were excluded. Among 46 individuals who were screened for eligibility, six declined to participate and 13 did not meet inclusion criteria. The remaining 27 were randomized to active or sham stimulus. Data were not available in three participants due to technical problems or withdrawals. This study presents data obtained from 24 participants including 12 females. Mean age of the participants was 31 (±9 SD). Sample size was pre-determined with power analysis. The active group and sham group participants were similar in age and sex. The active group participants (n = 12) had a mean age of 29 (±7 SD) and included five females; sham group participants (n = 12) had a mean aged of 32 (±11 SD), with seven females. SCID was used to evaluate for possible psychiatric diagnosis other than the diagnoses in exclusion criteria. In this sample, four (17%) met criteria for past depression, one (4%) for past PTSD, two (8%) for generalized anxiety disorder, one (4%) for past panic disorder, two (8%) for past alcohol abuse or dependence, and one (4%) for a past history of history of drug abuse or dependence.

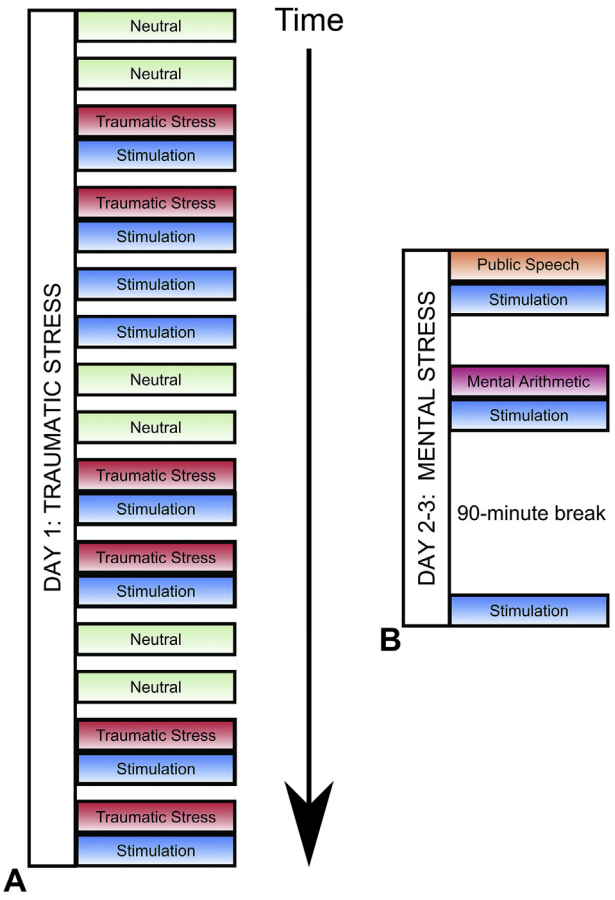

Fig. 1. Protocol description. **(A)** The first day included traumatic stress through headphones. After each traumatic stress prompt, stimulation (active or sham) was applied immediately. **(B)** Second and third days included two types of mental stress, public speech and mental arithmetic. After each stressor, stimulation was applied immediately. After a 90-min break from the mental stress protocol, participants received tcVNS or sham without acute stress.

Study design

Each participant was asked to write their traumatic events; later, personalized voice recordings based on these scripts were prepared using methods previously described [79]. The protocol consisted of three subsequent days for each participant, Fig. 1 presents the details for each day. The first day included six traumatic recall scripts and six neutral scripts presented audibly through headphones to participants inside a high-resolution positron emission tomography (HR-PET) scanner at 20 °C temperature (Fig. 1A), starting approximately at 8AM. All scripts were 60 s in duration. The neutral scripts were designed to induce positive feelings to the participant, such as the description of pleasant scenery, designed for the imaging part of the study. Traumatic scripts included personalized traumatic memories. Stimulation (active or sham) was applied immediately after the termination of the personalized traumatic script for 2 min by a research associate. On the same day two stimulation administrations (active or sham) were applied without any stressor. The second and third days were identical to each other, at 25 °C temperature (Fig. 1B), starting approximately at 8:30AM: First, the participants underwent a public speech task for which they were required to provide a 2-min long defense statement in a scenario where they were accused of theft. After hearing the scenario details, they were given 2 min to prepare their defense and 2 min to present their statement. Stimulation was applied immediately after the public speech task. Later, the participants rested for 8 min in silence. At the end of the 8 min, the participants were given another task for which they were required to answer series of arithmetic questions for 3 min. A researcher provided negative feedback for incorrect answers and delayed response times. A second stimulation was applied immediately after the arithmetic task. After two mental stressors and two stimulation administrations, the participants were given a 90-min break. After the break, another stimulation was administered without any stressor.

Blinding

The participants were randomized into either active tcVNS or sham stimulus groups with an online randomizer using simple randomization. The devices were pre-numbered by the manufacturer who were not involved in the research, and random allocation was conducted by an individual who did not take part in enrollment, data collection or analysis. Enrollment was done by clinical staff. The participants and clinical staff were blinded to the stimulus type, and each of the participants received only one type of stimulus. The researchers, who were also blinded to the stimulus type, conducted the questionnaire assessments, data collection, signal processing, and parameter extraction. Statistical analyses were carried out by a biostatistician who did not take part in data collection or processing. Stimulus grouping (active or sham) was un-blinded for the interpretation of statistical analysis.

Transcutaneous cervical vagal nerve stimulation

Both active tcVNS and sham stimuli were administered using hand-held devices (GammaCore, ElectroCore, Basking Ridge, New Jersey) with identical placement and operation. tcVNS or sham was applied using collar electrodes on the left side of the neck. The treatment area on the neck was located by finding the pulse on the carotid artery for each participant (Fig. 2B). Conductive electrode gel (GammaCore, ElectroCore, Basking Ridge, New Jersey) was used to maintain good contact between the skin and the electrodes. Active tcVNS devices produce an AC voltage signal consisting of five 5 kHz sine pulses, repeating at a rate of 25 Hz. Sham devices produce an AC biphasic voltage signal consisting of 0.2 Hz square pulses that delivers a mild buzzing sensation similar to the active device but does not result in stimulation of the vagus nerve. Both active and sham device operation stops automatically after 120 s. The stimulation intensity was adjustable using a roll switch that ranged from 0 to 5a.u. (arbitrary units) with a corresponding peak output ranging from 0 to 30 V (~0–60 mA) for active tcVNS, and from 0 to 14 V (~0–60 mA) for sham device. During each application, the stimulation intensity was increased to the maximum the participant could tolerate, without pain. At the start of stimulation, the intensity was increased gradually until each participant instructed to stop. The stimulation continued at the selected intensity. The amplitude levels participants received were 18 V (±4.8 SD) for active tcVNS, and 12.6 V (±2.8 SD) for sham stimulus.

Physiological monitoring

Physiological data were collected by the measurement of the following signals: 3-lead electrocardiography (ECG), respiration (RSP), seismocardiography (SCG), photoplethysmography (PPG), electrodermal activity (EDA), and blood pressure (BP). Fig. 2A shows the test setup employed for each participant. The ECG, RSP, PPG, and EDA signals were measured using wireless Bionomadix RSPEC-R and PPGED-R amplifiers (Biopac Systems, Goleta, CA). Adhesive Ag/AgCl electrodes were used for ECG recording. A respiration belt was used to measure thoracic expansion and contraction while breathing in order to measure RSP signal. For SCG measurement, a low-noise 356A32 accelerometer was used on the mid sternum (PCB Electronics, Depew, NY). Only the SCG signals in the dorsoventral direction were used in this study. Transmissive PPG measurement was taken from the index finger. EDA measurement was taken from the same hand where the PPG measurement was taken, using the inner palm. An isotonic electrode gel (GEL101, Biopac Systems, Goleta, CA) and pre-gelled isotonic electrodes (EL507, Biopac Systems, Goleta, CA) were used for EDA recording. All data were transmitted to the Biopac MP150 16-bit data acquisition system at a sampling rate of 2 kHz. Non-continuous systolic (SBP) and diastolic blood pressure (DBP) values were recorded periodically with an Omron blood pressure cuff during the rest, all stressors, and stimulation administrations.

Signal processing & parameter extraction

The signal processing and parameter extraction were carried out in MATLAB (R2017b, Natick, MA). The following parameters were extracted: heart rate (HR), pre-ejection period (PEP), amplitude of PPG, pulse arrival time (PAT_{FOOT}, PAT_{PEAK}), respiration rate (RR), width (RW), respiration prominence (RP), low frequency and high frequency heart rate variability (LF HRV, HF HRV), skin conductance level [80], skin conductance response [81], frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}).

Pre-Processing: Fig. 2C shows sections from collected physiological signals from a participant and the parameters computed from these signals. The ECG, SCG and PPG signals were filtered with finite impulse response [77] band-pass filters, with cut-off

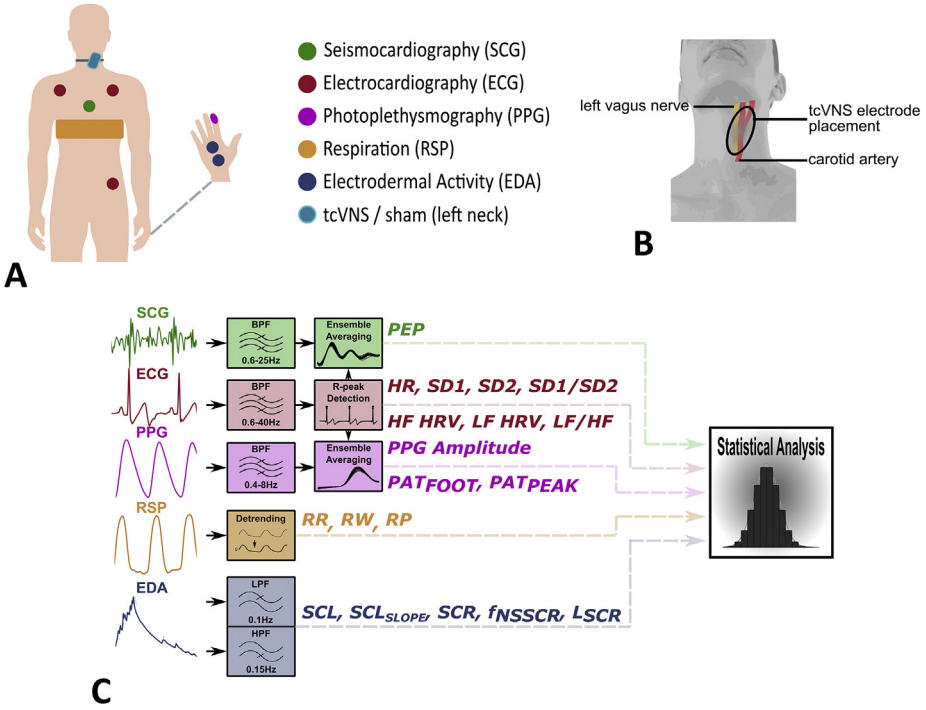

Fig. 2. Data collection and signal processing summary. **(A)** Non-invasive sensing modalities shown on participant, active or sham stimulation was applied from left neck. **(B)** Representation of relative locations of left carotid arteries and left vagus nerve. tcVNS electrodes were placed onto the area where the carotid pulsation was located. **(C)** Signal processing and feature extraction summary.

frequencies 0.6–40 Hz for ECG, 0.6–25 Hz for SCG, and 0.4–8 Hz for PPG, respectively, to preserve the waveform shape and cancel the noise outside their bandwidths [82,83]. The phasic component of EDA (for computing the parameters related to skin conductance response) was obtained using an FIR 0.15 Hz equiripple high-pass filter. The slowly varying RSP signal was used as is, as the module applies 10 Hz low-pass filter internally. The R-peaks of the ECG signals were detected using thresholding, and were used to calculate HR, HRV. SCG and PPG signals were ensemble averaged according to the R-peaks, using beat lengths of 150 ms for SCG and 600 ms for PPG. These lengths were sufficient to detect the fiducial points of each SCG and PPG beats. To reduce the effects of motion artifacts on the individually segmented beats, exponentially weighted moving ensemble averaging of successive beats was implemented for some parameters described below [82]. Exponentially decreasing weighting gives more emphasis to the more recent beats, while still providing noise reduction based on the averaging. Additional beat exclusion criteria were checked inside the algorithms, such as the identification of unrealistic timing intervals or unexpected morphology that may be caused by motion artifacts or momentary noise on the signal. For each parameter extraction, the beats were monitored to ensure that the time points were located correctly and the beats had acceptable morphology. The reader is referred to Fig. S2 for sample step-by-step PPG amplitude extraction from ECG and PPG signals. Similar ensemble averaging and feature extraction to extract amplitude and timing intervals were used for all continuous beat-by-beat signals.

Pre-Ejection Period: PEP, measured by the latency between the start of electrical depolarization of the ventricles to the opening of the aortic valve, is a non-invasive measure of cardiac contractility and cardiac sympathetic activity [84]. PEP provides limited insight on its own regarding baseline sympathetic tone or contractile state of the heart – two people with the same level of contractility may have different baseline PEP values due to differences in preload and afterload, which also impact the time it takes for the heart to proceed through isovolumetric contraction to systolic ejection of blood. However, *changes* in PEP have been associated with *changes* in contractility, specifically with a *decrease* in PEP indicating an *increase* in contractility. The increase in contractility leads to an increase in the maximal derivative of left ventricular pressure during isovolumetric contraction (i.e., dP/dt_{max}), and thus leads to a shortened PEP. In this work, we are examining the acute changes in PEP either associated with a stressor (e.g., traumatic stress) or tcVNS, or both, and thus are using PEP as an indicator of acute changes in cardiac contractility and thus sympathetic tone. The SCG signal provides high quality PEP estimation when combined with the ECG, computed by the time difference between the R-peak of the ECG to the second peak in SCG beat (aortic opening, AO point), known as R-Ao [57]. R-Ao values were computed following a three-beat exponential moving averaging procedure for noise reduction.

PPG Amplitude and Pulse Arrival Times: The PPG signal is known to be affected by sympathetic and vasomotor activity [83], therefore different parameters were extracted using this signal. Firstly, as a measure of peripheral sympathetic activity and vasomotor activity at the area of signal collection (index finger), the amplitude of each PPG beat was extracted. Secondly, pulse arrival time (PAT), representing the time delay from the electrical depolarization of the heart to the arrival of the pressure wave to the index finger was calculated from two reference points [85]. The first reference point was the foot of PPG signal, which was located by finding the maximum of the second derivative of the pulse wave before the maxima (PAT_{FOOT}). The second reference point was the peak (maxima) of the PPG signal (PAT_{PEAK}). A time constant of five beats was used for both PAT_{FOOT} and PAT_{PEAK} calculation.

Respiratory Measures: The respiratory parameters extracted were respiratory rate (RR), respiration width (RW), and respiration prominence (RP). Due to the loosening of the respiration belt over time while the participant was inside the PET scanner, the respiration signal occasionally had a DC offset. To remove this offset, a sixth order polynomial was fit to the signal in each interval (i.e. rest or stress), and the signal was detrended. From the detrended signal, the peaks representing inhalation and exhalation were located using thresholding. The rate of the peak appearance was extracted as RR. RR was considered as a continuous index of parasympathetic activity [86]. For RW, the width of each peak was computed as the distance between the points to the left and right of the peak, where the descending signal intercepts a horizontal reference line. The reference line was positioned beneath the peak at a vertical distance equal to half the peak prominence. The points themselves were found by linear interpolation. RP measured the prominence of a peak, i.e. how much the peak stands out due to its intrinsic height and its location relative to other peaks. It was calculated as the minimum vertical distance that the signal descends on either side of the peak before either climbing back to a level higher than the peak or reaching an endpoint.

Heart Rate Variability Measures: Two techniques were used to extract multiple HRV measures: Frequency-domain analysis and joint time-frequency analysis (Poincaré method). The first method, frequency-domain HRV, is the most commonly studied method for quantifying the sympathetic and parasympathetic branches of the autonomic nervous system, obtained from the non-constant R-R intervals from ECG R-peaks [87]. While the power in the high-frequency range (HF HRV, 0.15–0.4 Hz) is considered a measure of parasympathetic activity for humans, the low-frequency portion (LF HRV, 0.04–0.15 Hz) is mostly used for assessing the changes related to both sympathetic and parasympathetic influences [87]. The ratio of the two power bands (LF/HF) is often considered as a measure of sympathetic tone, while there are discrepancies in the literature [86,88]. For the second HRV analysis (Poincaré method), three standard indices were computed from the scatter plot of each R-R interval ($R\text{-}R_n$) versus the next R-R interval ($R\text{-}R_{n+1}$). In this procedure, an ellipse is fitted to the line-of-identity of the scatter plot ($R\text{-}R_n$ versus $R\text{-}R_{n+1}$). Three indices were extracted from the fitted ellipse: standard deviation of points perpendicular to the axis of line-of-identity (SD1), standard deviation of points along the axis of line-of-identity (SD2), and their ratio (SD1/SD2). SD1 measures short-term HRV which correlates with baroreflex sensitivity (BRS, change in the inter-beat interval duration per unit change in BP) and HF HRV. SD2 measures short- and long-term HRV and correlates with BRS and LF HRV. The ratio SD1/SD2 (the unpredictability of R-R intervals) is an indicator of the autonomic balance [89,90]. For both frequency-domain HRV and Poincaré analyses, ECG signals from the start and end of the days (longer than 5 min), ECG signals during stress (one to 3 min), stimulation (2 min), and post-stimulation (two to 8 min) were used. For each interval, the ECG signal was inspected visually to avoid ectopic, noisy beats and arrhythmias.

Despite the wide use of these HRV indices, there is still ambiguity in the research community emerging from the lack of clear documentation, validation, and standardization of different HRV signal processing methods. Here for HRV analysis, we used a MATLAB-based open source HRV toolbox that was previously validated with a variety of HRV measurement techniques and platforms to calculate LF HRV, HF HRV, LF/HF HRV, SD1, SD2, SD1/SD2 [91].

Electrodermal Activity Measures: The EDA signal is composed of two main components. The slow tonic component (skin conductance level, SCL) shows the general trend of the signal. The faster tonic component (skin conductance response, SCR) is

superimposed onto the tonic component. Electrodermal activity parameters extracted were SCL, SCL slope, SCR, frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}) [92]. For SCL, the DC level of EDA signal was extracted and the mean, minimum, maximum, standard deviation, slope of the first order polynomial fit (SCL slope), and area under curve properties were derived. SCR was analyzed in a similar manner to SCL. The peaks in SCR were located by thresholding, and the number of peaks per interval was computed to calculate f_{NSSCR}, excluding the first peak in the signal which corresponds to a specific event (i.e. stress start instance). For L_{SCR}, the latency from the start of the interval to the first peak appearance was calculated. The determination of the minimum peak amplitude was required to define the response occurrence. Although a minimum of 0.05 μS is common with hand scoring of SCR responses, this threshold is largely task- and subject-specific, and can be as low as 0.01 μS [92]. We determined the minimum peak amplitude to be two times the rest SCR mean amplitude for each participant, resulting in a mean of 0.06 ± 0.03μs for this study.

Statistical analysis

We compared participant characteristics between active and sham group using student t-tests (for normal continuous variables), Wilcoxon rank-sum tests (for non-normal continuous variables), and chi-squared tests (for categorical variables), as shown in Table S2. To understand the relative changes in the physiological parameters, data were separated into intervals reflecting the baseline of the corresponding day, stress, stimulation (active or sham), and post stimulation. Absolute and percent changes from the baseline state for each interval were computed and compared between-group differences across the intervals. For physiological parameter intervals (except HRV), data from 1 min of baseline rest, first 30 s of stress, last minute of stimulation, and 1 min from post-stimulation (3 min after the stimulation stops) were used. For speech and mental arithmetic tasks which corrupt the respiration waveform due to vocalization, the respiration beats just before the subjects start speaking were extracted as respiratory data during these stressors. These intervals correspond to the end of speech preparation (just before the subject starts speaking after 2-min preparation), and the interval just after the subjects heard the first mental arithmetic question (before answering). For non-continuous BP analyses, similarly SBP, DBP, PP values measured during baseline, stress, stimulation, and post-stimulation were used. Longer intervals for HRV measures were used to comply with the standards. The extracted parameters were evaluated with respect to the corresponding baseline values for each day, either as a ratio with baseline (percent changes) or subtraction from the baseline (absolute changes), for each interval. HRV indices were also evaluated as raw values for each interval. Data in bar plots were represented as mean ± 95% confidence interval, CI plotted from the raw unadjusted values. To evaluate if device type (active vs. sham) was associated with changes in parameters from the baseline value, we used mixed models with repeated measures that included random effect for each participant using unstructured correlation matrix (i.e., multiple traumatic scripts from the first day, two stimulations without acute stress on the first day, two stimulations without acute stress after a 90-min break on the second and third days, two stimulations followed by two public speech or two mental arithmetic tasks), and adjusted for age in the models. In a sensitivity analysis, we also tested the significance of the interaction between device type and time variable. Statistical analyses on both percent and absolute changes were carried out in all the models. The beta coefficients (β) from the mixed models indicate the adjusted average percent or absolute differences in the changes of parameters from the corresponding rest values, comparing active vs. sham device types. β were reported along with 95% CI and P-values in results and figure captions. A two-sided $p < 0.05$ denoted statistical significance. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and MATLAB (R2017b, Natick, MA).

Results

tcVNS has a similar effect on SNS activity both in the presence and absence of stress

To understand the physiological changes induced only by active or sham stimulation, the protocol included two stimulation administrations in the absence of traumatic scripts or mental stress (mental arithmetic and public speech) tasks, after a 90-min break from the mental stress protocol on the second and third days (Fig. 3 shows the data from the unadjusted raw changes from the baseline state during stimulation and post-stimulation intervals, results were expressed as mean values, 95% confidence intervals, p-values obtained after adjustments). Stimulation without stress tasks resulted in differences in physiological biomarkers associated with

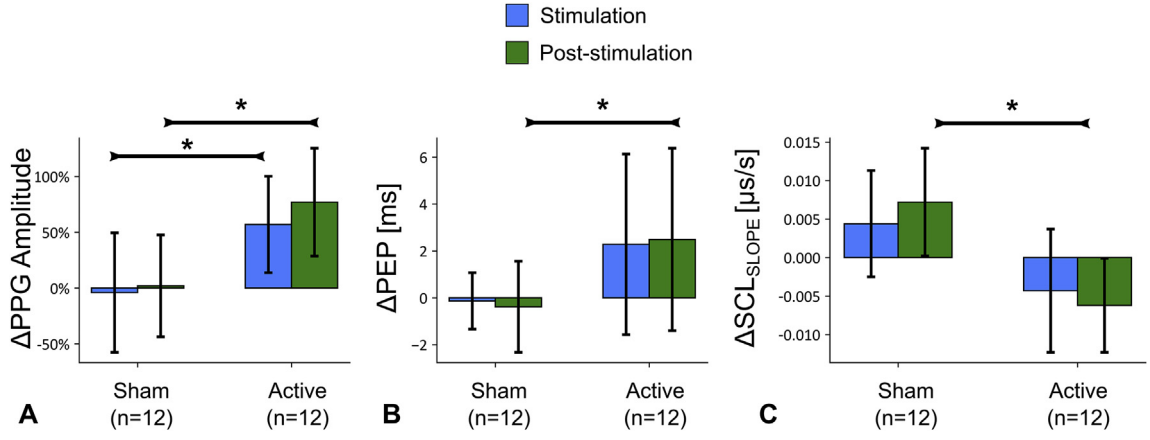

Fig. 3. Primary outcomes from physiological signal analyses for stimulation without acute stress from the second and third protocol days. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. **(A)** Active tcVNS group experienced an increase in PPG amplitude during stimulation ($p = 0.049$) and post-stimulation ($p = 0.021$) compared to the sham group. **(B)** Active tcVNS group experienced an increase in pre-ejection period during the post-stimulation interval ($p = 0.035$) compared to the sham group. **(C)** Active tcVNS group experienced a decrease in SCL slope during the post-stimulation interval ($p = 0.014$) compared to the sham group.

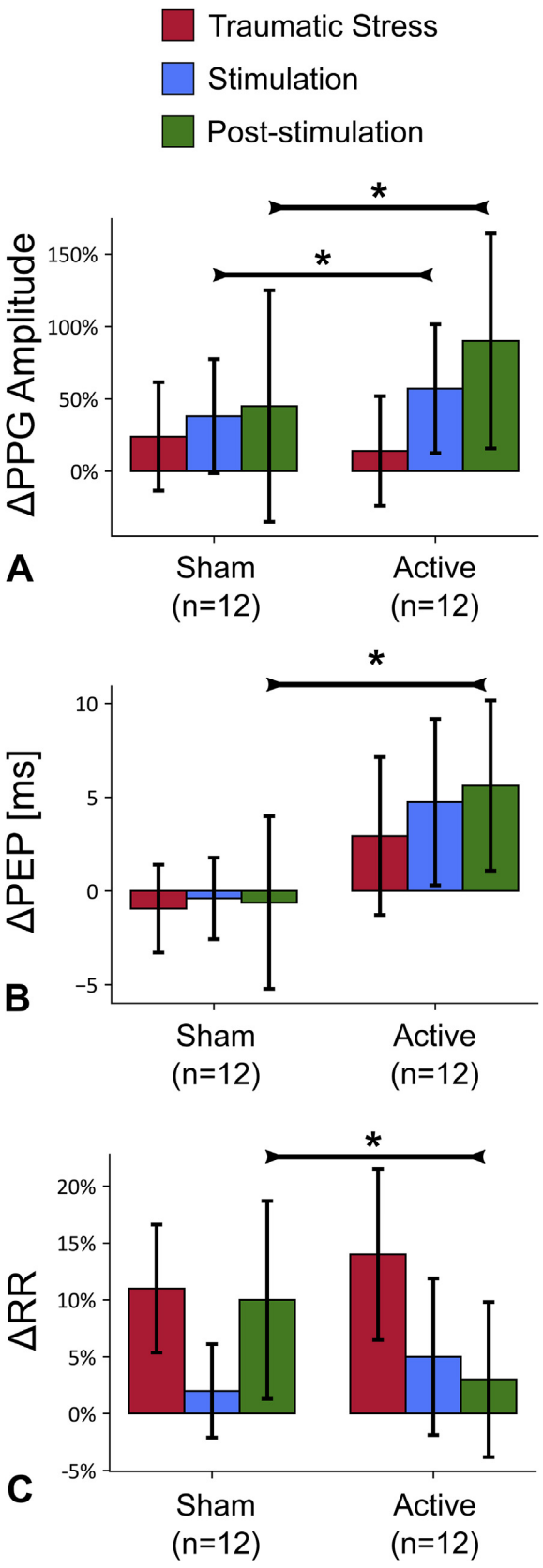

Fig. 4. Primary outcomes from physiological signal analyses for stimulation following traumatic stress. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. **(A)** The active tcVNS group experienced a greater increase compared to sham in PPG amplitude during stimulation ($p = 0.036$) and post-stimulation ($p = 0.044$). **(B)** The active tcVNS group experienced an increase in pre-ejection period during post-stimulation ($p = 0.003$) compared to sham. **(C)** Sham group experienced increase in respiratory rate (RR) during post-stimulation ($p = 0.002$).

sympathetic tone: PPG amplitude (Fig. 3A, measurement of peripheral vasoconstriction, inversely related to peripheral sympathetic activity) increased (indicating relative vasodilation and decreased sympathetic activity) during stimulation by 78.6% (95% CI, 0.5–156.7%, $p = 0.049$), and following stimulation by 95% (15.7–174.2%, $p = 0.021$) after adjustments in the active tcVNS group relative to the sham group. The pre-ejection period (PEP, Fig. 3B, inversely related to cardiac sympathetic activity) increased following stimulation by 3.3 ms (0.2–6.3 ms, $p = 0.035$) after adjustments in the active group compared to the sham group, indicating a decrease in cardiac contractility and sympathetic activity. Electrodermal activity slope (SCL slope, Fig. 3C, related to sympathetic activity) decreased during post-stimulation by −0.013 μS/s (−0.024 to −0.003 μS/s, $p = 0.014$) after adjustments in the active tcVNS group relative to the sham group.

tcVNS modulates autonomic tone following exposure to personalized traumatic scripts

Stimulation following exposure to personalized traumatic scripts revealed marked changes in autonomic reactivity between the active and sham groups. Fig. 4A–C illustrates changes in physiological parameters from the baseline state for the three intervals: traumatic stress, stimulation, and post-stimulation, data shown from unadjusted raw values. There were no significant differences in peripheral vasoconstriction measured by PPG amplitude during traumatic scripts between groups. There was an increase in PPG amplitude (indicating relative vasodilation and decreased peripheral sympathetic activity) during stimulation delivered immediately at the termination of traumatic scripts which persisted after the end of stimulation in the active versus the sham group. PPG amplitude was 43.7% higher (3.1%–84.3%, $p = 0.036$, Fig. 4A) during active versus sham stimulation and 47.9% higher (1.4%–94.5%, $p = 0.044$) in the post-stimulation interval after adjustments. As for PEP, there were no significant differences in PEP during traumatic scripts and during stimulation between groups. In the post-stimulation interval, an increase in PEP (indicating decreased cardiac sympathetic activity) was observed in the active versus sham group with an adjusted difference of 4.2 ms (1.6–6.8 ms, $p = 0.003$, Fig. 4B). Respiratory rate (RR) was similar between tcVNS and sham groups during traumatic scripts and stimulation, with an adjusted decrease in the active group relative to sham of −9% (−14.3% to −3.7%, $p = 0.002$, Fig. 4C) during post-stimulation indicating a release of parasympathetic activity.

Effects of tcVNS on PPG amplitude and respiration rate following mental stress

There were no statistically significant differences during the public speech task between the active and sham groups in PPG amplitude, RR, respiration prominence (RP), SCL slope (Fig. 5A–C, F). PPG amplitude increased during post-stimulation in the active group compared to sham by 61.3% (17.3%–105.3%, $p = 0.009$, Fig. 5A) after adjustments. RR decreased in the post-stimulation in active versus sham by an adjusted difference of −11.3% (−20.3% to −2.3%, $p = 0.017$, Fig. 5B). RP decreased during stimulation in active versus sham by −25.4% (−47.9% to −3%, $p = 0.028$, Fig. 5C) after adjustments. Lastly, SCL slope decreased during stimulation in active versus sham by −0.014 μS/s (−0.026 to −0.001 μS/s, $p = 0.027$, Fig. 5F) after adjustments.

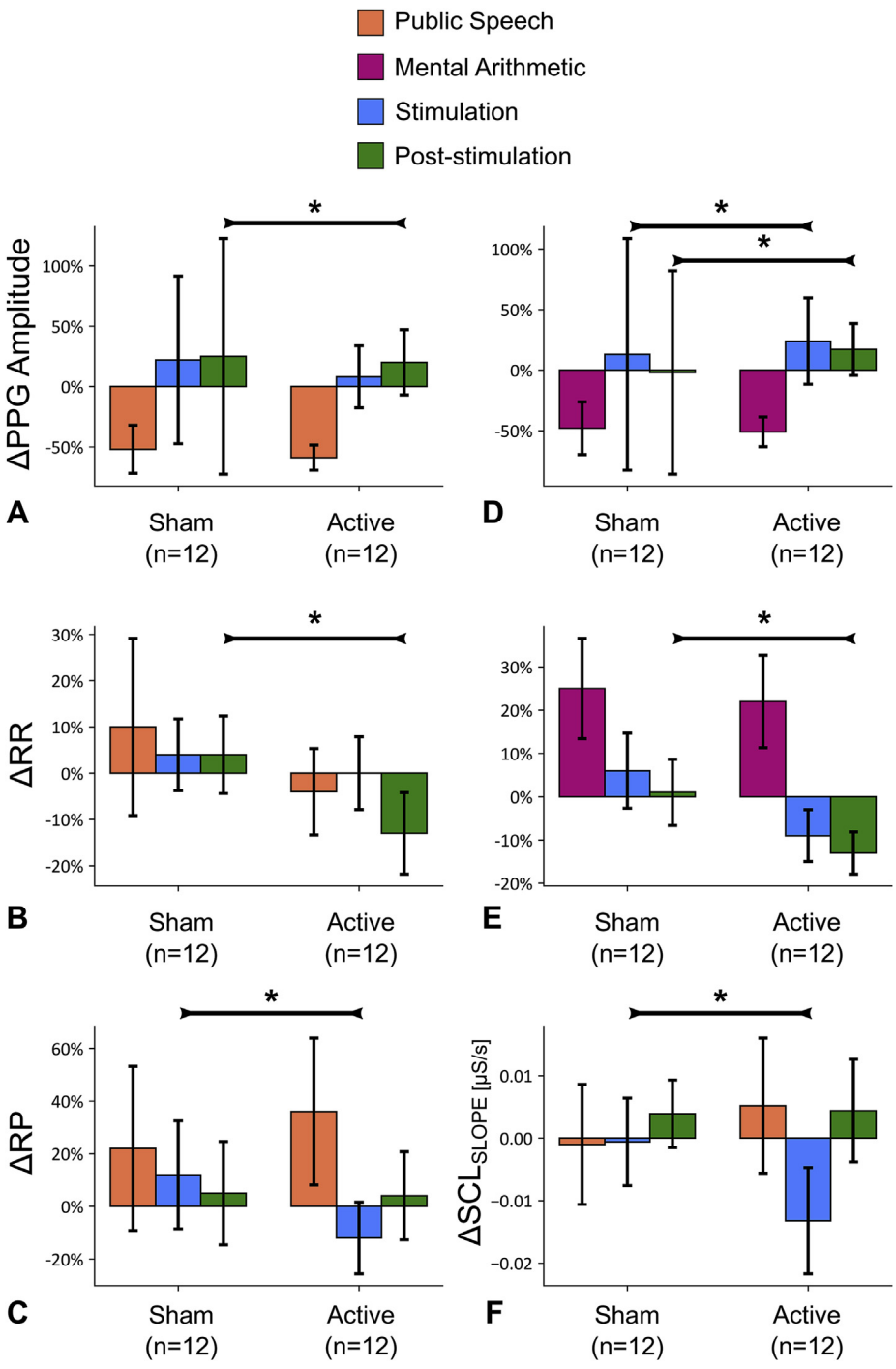

Fig. 5. Primary outcomes from physiological signal analyses for stimulation following two types of mental stress, public speech and mental arithmetic. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. **(A)** Increase in PPG amplitude for active group during post-stimulation ($p = 0.009$). **(B)** Decrease in respiratory rate (RR) for active group during post-stimulation ($p = 0.017$). **(C)** Decrease in respiration prominence (RP) for active group during stimulation ($p = 0.028$). **(D)** Similar to **(A)**, active group shows a consistent recovery in PPG amplitude during stimulation ($p = 0.005$) and post-stimulation ($p = 0.001$). **(E)** Decrease in RR during post-stimulation for active group ($p = 0.007$). **(F)** Decrease in SCL slope for speech task during stimulation for active group ($p = 0.027$).

Similar to public speech, there were no difference between active and sham groups during the mental arithmetic stress task in PPG amplitude or RR. Active stimulation relative to sham resulted in an adjusted increase in PPG amplitude of 95.8% (32.3%–159.2%, $p = 0.005$), with a post-stimulation adjusted increase of 70.4% (30.8%–110%, $p = 0.001$) (Fig. 5D). Following active tcVNS there was a decrease in RR of −14.6% (−24.8% to −4.3%, $p = 0.007$, Fig. 5E) after adjustments. Increased PPG following mental stress tasks and tcVNS indicates decreased peripheral sympathetic activity while decreased RR suggests a decrease in parasympathetic withdrawal. As for the two administrations without acute stress on the first day, PEP in active group compared to sham increased by 7.2 ms ($p = 0.027$) after adjustments following stimulation. There were no other marked differences in heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), respiration width (RW), low-frequency heart rate variability (LF HRV), or

high-frequency HRV, low-to-high HRV ratio (LF/HF), SD1, SD2, SD1/SD2, pulse arrival time (PAT), other parameters related to electrodermal activity, such as skin conductance level (SCL), frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}) that could distinguish active tcVNS and sham stimulation.

Discussion

This study demonstrated the feasibility and utility of quantification of cardiovascular and peripheral autonomic nervous system function using wearable sensing devices in conjunction with administration of tcVNS and a sham control and stressful tasks. tcVNS minimized sympathetic activation and/or withdrawal of parasympathetic tone following exposure to stress based on a range of physiological parameters. This was observed for different kinds of stressors, including exposure to recordings of personalized traumatic memory scripts, and "neutral" or "mental stress" tasks including mental arithmetic and public speech stress tasks. The findings suggest that wearable sensing devices could be used as real-time non-invasive physiological biomarkers of tcVNS to predict treatment efficacy and/or provide empirical evidence of proper tcVNS administration.

tcVNS shows effects in multiple physiological biomarkers

Active tcVNS compared to the sham group resulted in a decrease in peripheral and cardiac sympathetic activation for tcVNS alone as measured by increased PPG amplitude, increased PEP, and decreased SCL slope. There was also a reduction in peripheral sympathetic activation with tcVNS applied after both traumatic script and mental stress tasks as measured by increased PPG amplitude, and decreased cardiac sympathetic activation after traumatic scripts (but not mental stress) based on increased PEP. In a complementary manner, tcVNS resulted in reduced parasympathetic withdrawal after both traumatic scripts and mental stress tasks based on reduced RR. tcVNS also decreased SCL slope when followed by public speech task (but not arithmetic or traumatic stress). The use of various stressors revealed task-specific changes in autonomic nervous system activity: while an increase in PEP was observed for tcVNS when applied following a traumatic stressor, an increase in PEP was not observed upon stimulation for mental stressors. Similarly, reduction in SCL slope was observed for stimulation without acute stress and stimulation following public speech only. On the other hand, tcVNS resulted in increases in PPG amplitude and decreases in RR when applied after both traumatic script and mental stress tasks. Thus, there is not a single biomarker of tcVNS, rather its efficacy could be revealed from signals that are related to different pathways of autonomic reactivity to different types of stressors.

Changes in PPG-amplitude versus lack of changes in blood pressure

Increased PPG amplitude with tcVNS was one of the most consistent results across the various stressful tasks in this study. However, a myriad of factors is involved in affecting the amplitude of PPG signals, and thus associating changes in PPG amplitude changes with a particular underlying physiological origin is not straightforward. To the first order, the two main factors influencing PPG amplitude are pulse pressure and arterial compliance [93]. Thus, it is important to note that in this study we did not observe differences between the active tcVNS and sham groups in systolic, diastolic blood pressure, and pulse pressure for any of the intervals – this indicates that the changes in PPG amplitude that were significant, and quite substantial, were linked to local changes in arterial tone associated with vasoconstriction and vasodilation. Accordingly, the effects of tcVNS on PPG amplitude may be attributed to sympathetic regulation of vascular tone.

No effects of tcVNS on heart rate (HR) or heart rate variability (HRV)

The current study did not find differences between the active tcVNS and sham groups in ECG-based measurements of heart rate (HR) or heart rate variability (HRV) either for short intervals during and after stimulation, or for whole day measurements. HRV is commonly used as a proxy measure for peripheral autonomic function [87]. HRV measures have limitations, however, in terms of specificity and validity of assessment of specific aspects of sympathetic and parasympathetic autonomic activity [94]. Frequency-domain based HRV measures are convenient to measure, provided that the recording is long enough (at least 3–5 min) without ectopic and noisy beats. However, there has long been a debate about the relative contribution of sympathetic and parasympathetic activity to LF HRV, and there is general agreement that it is not a specific measure of sympathetic activity alone [86,95,96]. HR is also not specific, since changes in HR might reflect either an increase in SNS or decrease in PNS. HR carries information solely on the electrical activity, while PEP (controlled by the contractile force in the heart) incorporates information on electromechanical coupling of the heart [97–99]. Our findings suggest that tcVNS has specific effects on sympathetic and parasympathetic function distinct from other cardiovascular parameters as shown by specific effects on PEP, RR, PPG amplitude, and SCL slope, but not on HR, HRV, or EDA measures other than the slope. The HR measurements are not significantly different between active and sham groups for any of the intervals analyzed (Fig. S3). The changes in PEP thus provide more information than changes in HR alone. Our findings are also consistent with studies observing no HRV or EDA changes (SCR) following auricular VNS in combination with subjectively measured fear and anxiety [52,72,73]. A recent study on the effects of tcVNS with a noxious stressor (thermal stimuli) reported difference in EDA-related changes in active and sham group, specifically in SCL slope and latency of SCR (L_{SCR}) [100]. Our analysis has shown difference for SCL slope, but not for latency of SCR.

Use of seismocardiography (SCG) in the assessment of effects of tcVNS on peripheral autonomic function

The current study found PEP, a measure of sympathetic activity, to be useful in the assessment of the effects of tcVNS on autonomic function. PEP has been studied as a measure of cardiac sympathetic activity (or cardiac contractility), along with comparisons with HRV, EDA, and plasma catecholamines [84,97–99,101–103]. However, PEP is used less commonly in practice in clinical studies due to the need for multiple electrodes and the addition of another sensing modality (impedance cardiography or ICG) along with ECG. We observed in our study that tcVNS administration creates electrical stimulation artifacts on ICG signal as the stimulation bandwidth and ICG signal bandwidth coincide with each other (Fig. S4, SCG versus ICG during tcVNS), hiding the fiducial point to extract the PEP (known as B-point, representing the opening of aortic valve) [84]. SCG is a viable option to calculate PEP in clinical studies that use tcVNS as it is a mechanical signal reflecting the chest-wall vibrations of the heart, hence the electrical stimulation does not affect the waveform shape. Beat-by-beat analysis during the treatment is possible with SCG-derived PEP. SCG also does not require electrodes, unlike ICG. tcVNS in the current study induced robust changes in PEP with or without stress, and across of a broad range of different stressful tasks.

Translation to populations afflicted with maladaptive autonomic regulation

Patients with PTSD suffer from recurrent and intrusive thoughts about traumatic events. Our results regarding the use of tcVNS in tandem with traumatic stress motivate possible translation to PTSD populations, in the clinic or at-home, as an acute treatment for these recurrent memories [60]. However, these results, which focus on the physiological biomarkers of tcVNS for individuals with prior psychological trauma, do not yet address the question of how the population with maladaptive autonomic regulation (i.e. patients with PTSD) would respond to tcVNS treatment. Nevertheless, it is known that individuals with PTSD have abnormal oscillations in autonomic state and show hyperarousal after recalling traumatic memories supporting either elevated sympathetic activity or withdrawal, as observed by physiological signals [104], brain imaging [62], or serum biomarkers [105]. PTSD patients suffer from exaggerated responsivity to reminders of traumatic memories, and the changes induced by tcVNS observed in traumatized persons without PTSD may potentially be observed in this population as well. Our findings on tcVNS hold promise for PTSD as there is preclinical evidence for direct VNS to enhance he extinction of conditioned fear [8,69,70], and clinical evidence for taVNS to improve vagal tone in patients with PTSD [71], though with different stimulation targets, direct VNS and auricular VNS, respectively.

Limitations

The following limitations should be noted for this study. Prior animal studies initiated direct VNS or sham before the initiation of the fear-related stimulus [8,32]. Other studies in human subjects initiated taVNS or sham before or during the stimuli [52,71,73]. Therefore, stimulation prior to stress and concurrently with stress appear to improve the pathological response based on previous studies. This study employs a reactive acute treatment approach as stimulation administrations were applied right after the stressors ended. Subjects were instructed, however, to form an image from the traumatic scripts in their mind and hold it, and stimulation was applied immediately at the end of the script. Our prior experience with traumatized subjects including those with PTSD demonstrated that upsettedness typically continues after the termination of the script, stress- or fear-related task [63,106]. Therefore, we believe that the stimulation was applied at the peak of the behavioral effects of the task. Future studies should investigate the effects of preemptive versus reactive stimulation in the context of traumatic stress.

Due to the clinical nature of this study, the target engagement of the cervical vagus nerve could not be validated directly. This study relies on previous literature that reported the ability to reach the vagal afferents using tcVNS [46,47]. We replicated the stimulation application reported in Ref. [46] throughout the protocol, by locating the carotid artery as an anatomical reference. Although variation exists regarding the location and topographical anatomy of the cervical vagus nerve, a recent cadaveric study reports that cervical vagus nerve can be visualized in a 35×35 mm distance lateral of the laryngeal eminence and posterior to the skin of the neck, which typically falls under the area the electrodes are placed [107].

A natural restriction of this study is the possibility of therapeutic effects from traumatic exposure (traumatic stress rehearsal) [108]. Fig. 4 combines data from all six traumatic stress scripts per subject, showing increases only in RR during traumatic stress. To show how the subjects respond to traumatic stress initially, we also analyzed only the first traumatic script responses for the primary outcome variables, excluding all other repetitions, for each subject (see Fig. S5). It is seen that HR and RR increase, PPG amplitude decreases during traumatic stress. The lower stress reactivity in Fig. 4 might be due to the therapeutic effects of the repetitions as the data were merged from six traumatic scripts per subject (repetition numbers were included in our statistical analyses). Nevertheless, as our study focuses on tcVNS effects on stress, our main consideration was whether the active and sham groups received comparable amounts of stress. We did not observe significant differences in stress responses, which was an essential requirement to evaluate the effects of tcVNS on the recovery from stress. Therefore, although repeated exposure might change stress reactivity over the time, the reactivity remained similar between the active and sham groups, which facilitated comparison of the effects of active and sham stimulation.

The functional relevance of the PPG amplitude results could be attributed to changes in total peripheral resistance (TPR) or pulse pressure (PP), however there is no direct linear correlation to either. The PPG signal is an optical measurement, the amplitude of which is determined by the Modified Beer-Lambert Law [93]. PPG amplitude reflects the expansion and contraction of the vessel diameters in the region (index finger) being illuminated by the light source. This expansion and contraction of vessel diameter is proportional to both PP and arterial compliance. Compliance is the change in a vessel's volume for a given change in PP. Thus, while directional relationships between PPG amplitude changes and TPR can be quite informative, the attribution of a given change in amplitude to a particular change in TPR is complex. Nevertheless, the study did not find remarkable differences in non-continuous BP measures (SBP, DBP, PP) or pulse arrival time (PAT). This is an interesting result considering the relationship of PPG and BP waveforms [56,83]. PPG measurements (hence the extracted PPG amplitude and PAT) were continuous, and thus beat-by-beat assessment was feasible —a desirable measurement for the acute characteristics of this study. BP measurements were taken through a blood pressure cuff, and hence BP changes at beat-by-beat level could not be assessed. Future studies should examine whether continuous BP is affected by tcVNS.

While assessing the mental stress reactivity to tcVNS, it is important to clarify that the active and sham groups reacted similarly to mental stressors, which permits the comparison of stress response upon stimulation between the groups. The public speech task is a version of Trier Social Stress Test [109]. Traumatic stress protocol, public speech, and mental arithmetic tasks have been verified multiple times to induce significant psychobiological and cardiovascular responses on human subjects [62,110–112]. In this study, similar responsivity between the groups were seen in the measures analyzed during the stressors. The groups showed no significant difference during stress intervals in any of the cardiovascular, peripheral, electrodermal activity measures.

Conclusion

In summary, our investigation demonstrates that tcVNS has effects on peripheral autonomic function that can be feasibly and reliably measured with wearable sensing devices. Specifically, tcVNS both in isolation and following exposure to stress reduces sympathetic and enhances parasympathetic function, leading to a modulation in autonomic tone. These physiological biomarkers may be useful for long-term monitoring of tcVNS in the home setting to assess adherence and accuracy of neuromodulation treatments and to provide subject-specific dosage recommendations for tcVNS therapy. tcVNS also minimizes sympathetic activation in response to stress, which suggests that it may have clinical applications to stress-related psychiatric disorders characterized by

increased sympathetic activity that is correlated with symptoms of these disorders [113–118]. The fact that tcVNS reduces or blocks sympathetic arousal associated with exposure to personalized traumatic scripts suggests a clinical application to patients with PTSD in the context of modulation of indelible traumatic memories and possible enhancement of neuroplasticity and/or facilitating extinction of conditioned responses to reminders, which were previously studied in preclinical literature through direct VNS with implantable devices [8,32,69,70,119,120]. Although not assessed in the current study, emerging findings of the beneficial effects of direct VNS on cognition and memory suggest other possible benefits of tcVNS for patients with stress-related psychiatric disorders [121]. tcVNS could have a potentially broad impact in the domains of human performance and mood improvement, and wearable sensing devices can be used to quantify the stimulation. This could be applicable to other clinical and neuroscience research environments and in general wearable bioelectronic medicine, for patients with or without psychiatric disorders or other medical conditions.

Declaration of interests

Dr. Bremner reported having funding support from ElectroCore LLC. Other authors reported no potential conflicts of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.08.002.

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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 Kanel 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 interferonγ (IFNγ) 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)-α 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-α (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-α in human subjects, while another study showed long term treatment with tcVNS lowered both TNF-α and IL-6 in patients with Sjögren's Syndrome (Tarn et al., 2019). We showed that tcVNS blocks IL-6 and IFNγ 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-γ 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. CAll 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 α_2 NE inhibitory autoreceptor on the LC, such as clonidine

(Kinzie and Leung, 1989), while α_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 (Geracioti 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 findgs 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-κB (NF-κB), 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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Statement of interest

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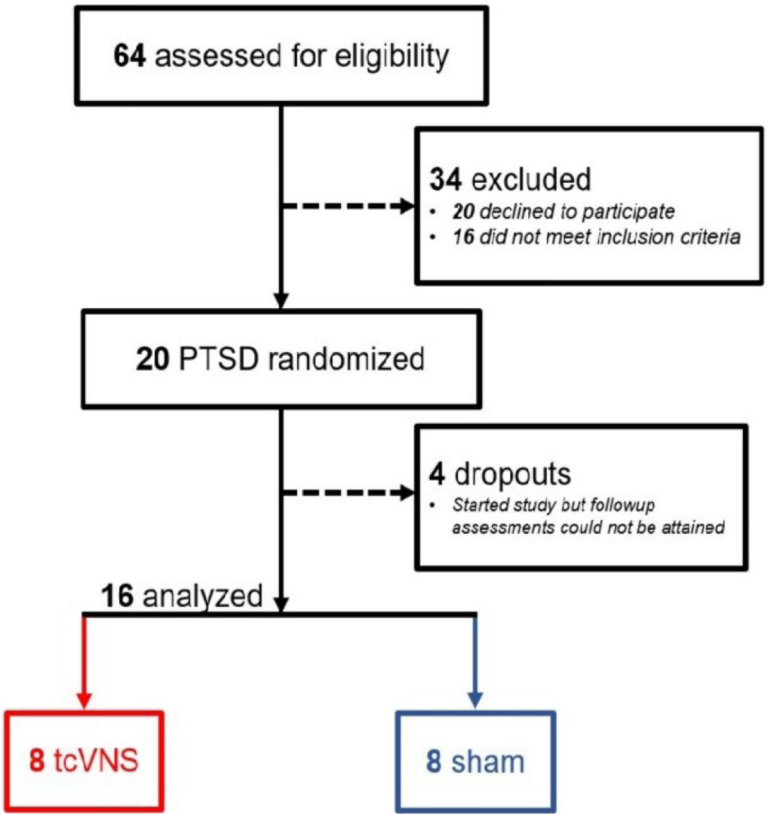

Figure 1.
CONSORT diagram showing flow of study participants screened, enrolled, and completing the protocol.

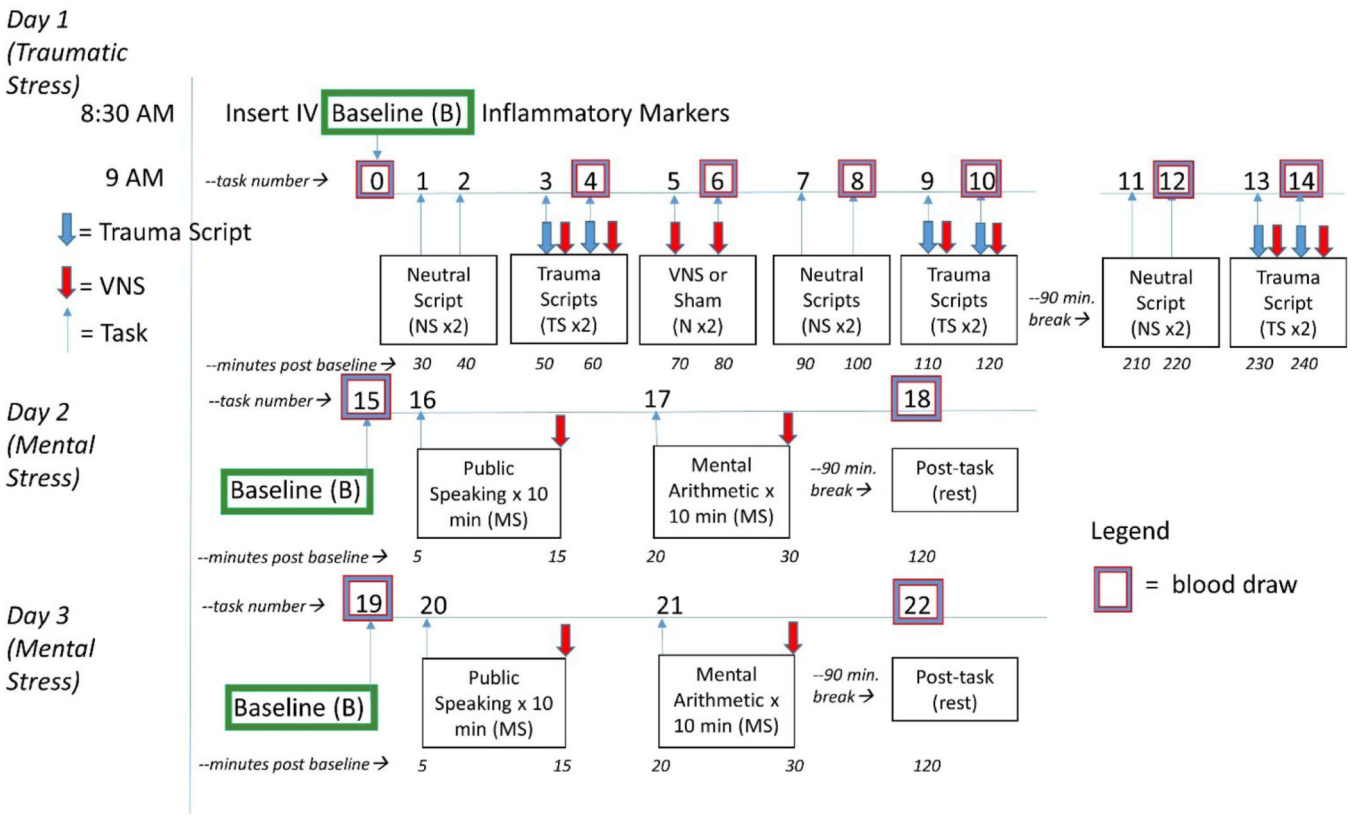

Figure 2.

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.

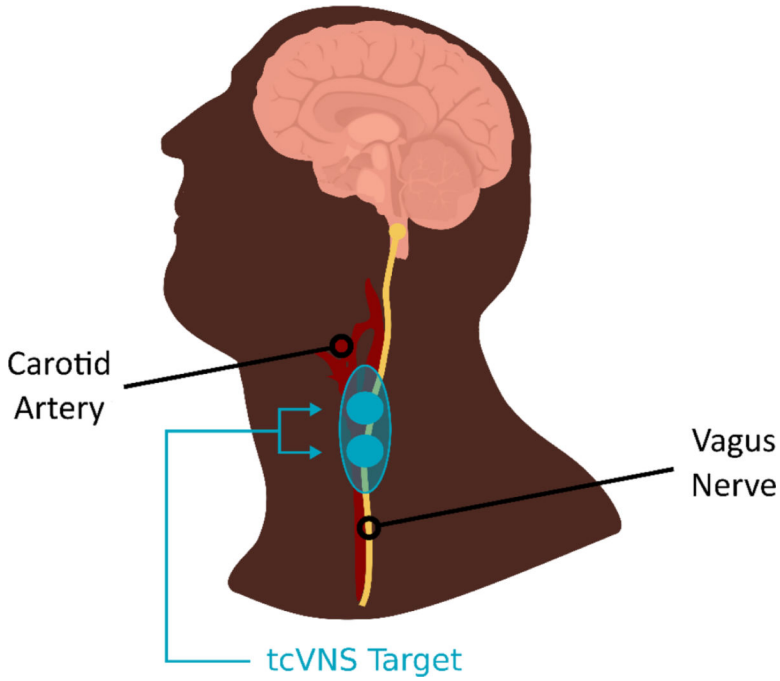

Figure 3.
Diagram showing placement of tcVNS device on the neck to target the vagus nerve as it travels through the carotid sheath.

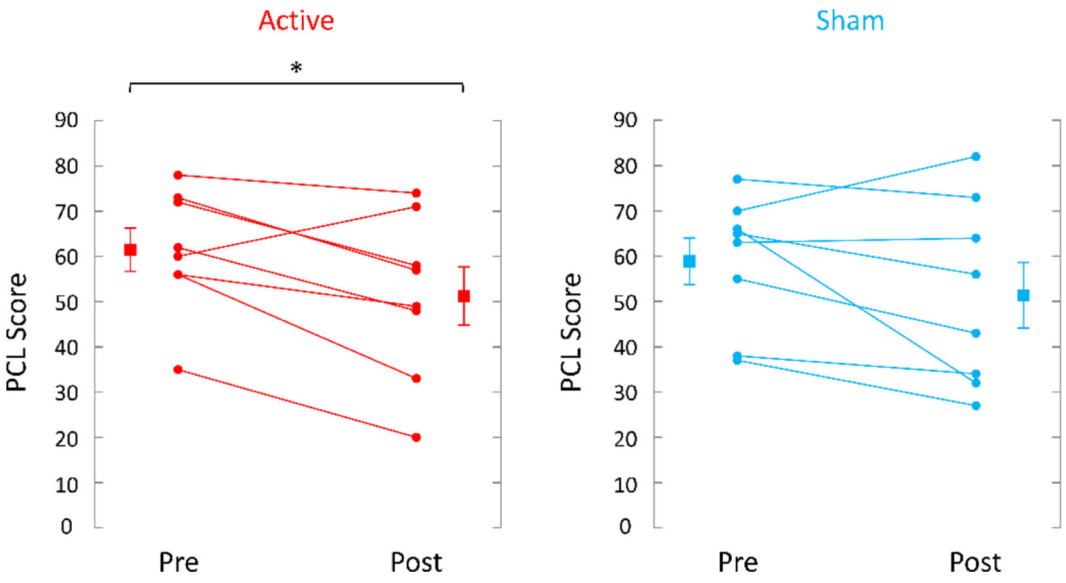

Figure 4.

Effects of up to three months of twice daily transcutaneous cervical Vagal 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).

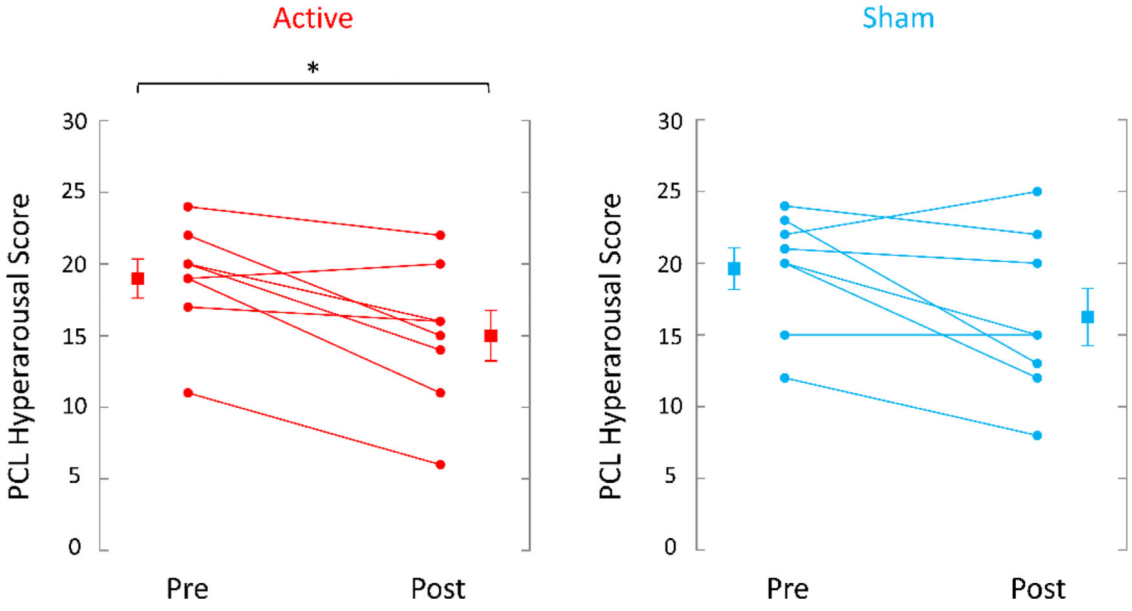

Figure 5.

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).

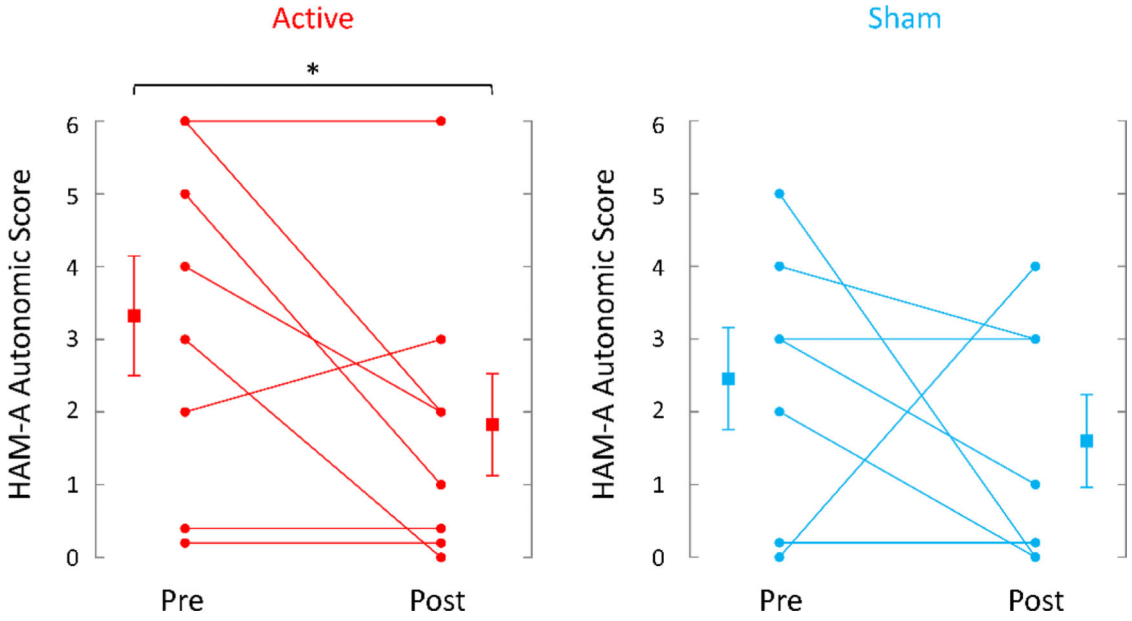

Figure 6.

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).

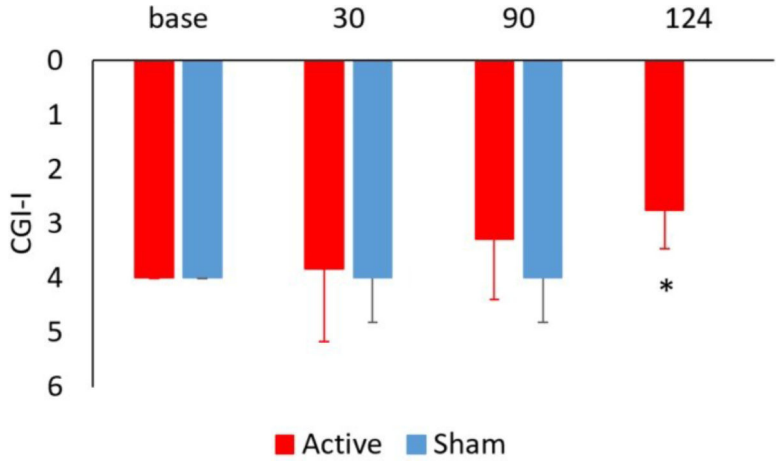

Figure 7.

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).

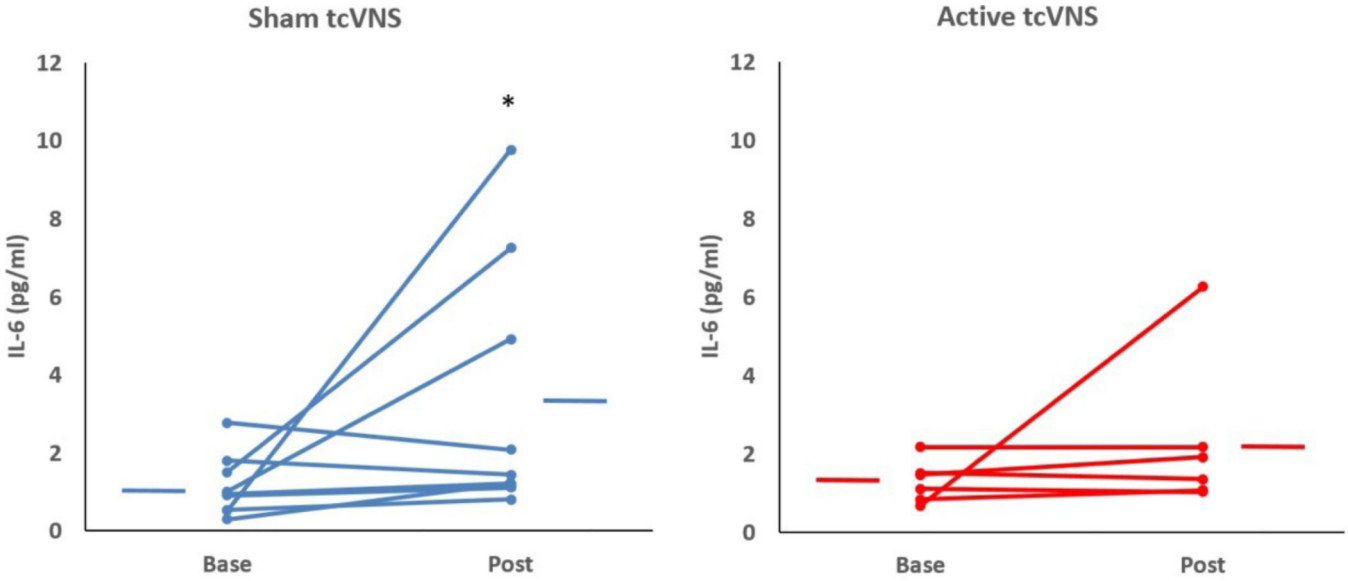

Figure 8.

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.

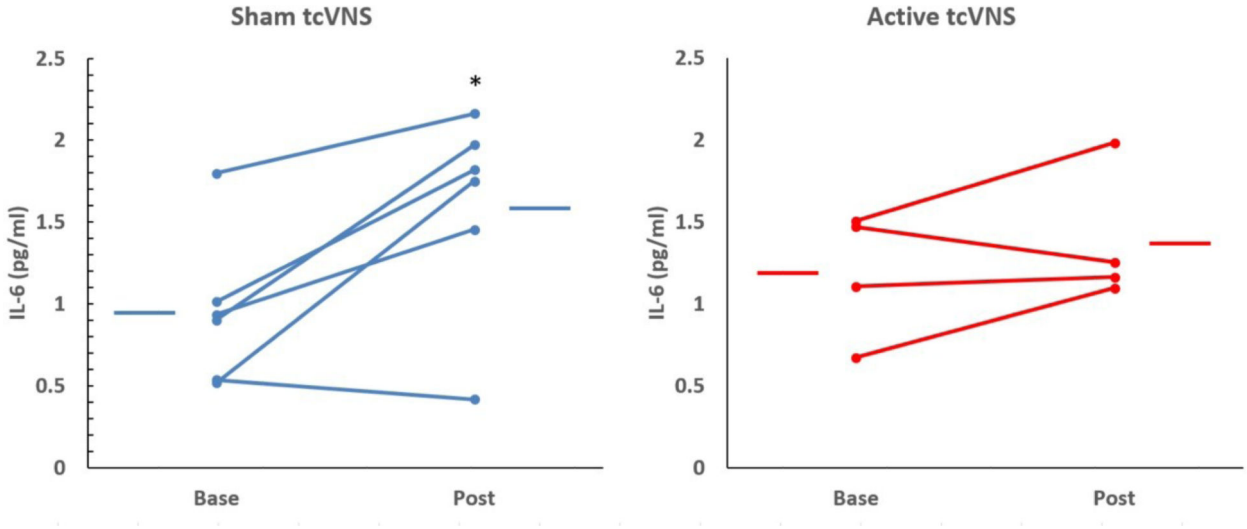

Figure 9.

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)
Age		
Mean ± SD	37 ± 13	40 ± 14
Race		
White	5 (55%)	5 (45%)
Black	4 (45%)	5 (45%)
Asian/Pacific Islander	0 (0%)	1 (9%)
Sex		
Female	6 (66%)	7 (64%)
Male	3 (33%)	4 (36%)
BMI		
Mean ± SD	30 ± 7	30 ± 6
Education Level		
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%)
Marital Status		
Never married	3 (33%)	5 (45%)
Married	3 (33%)	2 (18%)
Divorced / Separated	2 (22%)	3 (27%)
Widowed	1 (11%)	1 (9%)
PTSD Score (PCL)		
Mean ± SD	62 ± 14	61 ± 13
CAPS Score		
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
Ham-D Score		
Mean ± SD	19 ± 12	19 ± 5
Ham-A Score		
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

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Non-Invasive Cervical Vagal Nerve Stimulation Alters Brain Activity During Traumatic Stress in Individuals with Posttraumatic Stress Disorder

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Abstract

Objective: Posttraumatic stress disorder (PTSD) is a disabling condition affecting a large segment of the population, however current treatment options have limitations. New interventions that target the neurobiological alterations underlying symptoms of PTSD could be highly beneficial. Transcutaneous cervical (neck) vagal nerve stimulation (tcVNS) has the potential to represent such an intervention. The goal of this study is to determine the effects of tcVNS on neural responses to reminders of traumatic stress in PTSD.

Methods: Twenty-two participants were randomized to receive either sham (n = 11) or active (n = 11) tcVNS stimulation in conjunction with exposure to neutral and personalized traumatic stress scripts with High-Resolution Positron Emission Tomography scanning with radiolabeled water for brain blood flow measurements.

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DECLARATION OF INTERESTS: Dr. Bremner reported having funding support from ElectroCore LLC. No other authors report potential conflicts of interests.

Results: Compared to sham, tcVNS increased brain activations during trauma scripts ($p < 0.005$) within the bilateral frontal and temporal lobes, left hippocampus, posterior cingulate, and anterior cingulate (dorsal and pregenual), and right postcentral gyrus. Greater deactivations ($p < 0.005$) with tcVNS were observed within the bilateral frontal and parietal lobes and left thalamus. Compared to tcVNS, sham elicited greater activations ($p < 0.005$) in the bilateral frontal lobe, left precentral gyrus, precuneus, and thalamus, and right temporal and parietal lobes, hippocampus, insula, and posterior cingulate. Greater ($p < 0.005$) deactivations were observed with sham in the right temporal lobe, posterior cingulate, hippocampus, left anterior cingulate, and bilateral cerebellum.

Conclusion: tcVNS increased anterior cingulate and hippocampus activation during trauma scripts, potentially indicating a reversal of neurobiological changes with PTSD consistent with improved autonomic control.

Trial Registration: #NCT02992899

Keywords

Vagal Nerve Stimulation; PTSD; Trauma Scripts; Stress; Prefrontal Cortex; Anterior Cingulate

Introduction

Traumatic stressors such as early childhood trauma, combat exposure, or motor vehicle accidents, can lead to Posttraumatic Stress Disorder (PTSD), which affects ~8% of Americans (1). Symptoms of PTSD represent the behavioral manifestation of stress-induced changes in the brain including intrusive thoughts, avoidance behaviors, sleep disturbances, and hyperarousal. Previous studies also indicate PTSD may be associated with altered neural functional connectivity, elevated cardiovascular reactivity, and increased peripheral inflammatory biomarkers (2-6). Selective Serotonin Reuptake Inhibitor anti-depressants are the only approved medication treatment for PTSD, however patient response (60%) and full remission rate (20-30%) are poor (7, 8). A recent Institute of Medicine report did not find conclusive evidence supporting the efficacy of medication treatments for PTSD (9).

Neuromodulation represents a promising new treatment paradigm for stress-related psychiatric disorders (10, 11). In particular, vagal nerve stimulation (VNS) has shown the potential to mitigate exacerbated sympathetic nerve activity that may lead to intrusions and hyperarousal symptoms in PTSD (12, 13). Implantable VNS is approved for the treatment of depression and epilepsy and appears to partially mitigate peripheral cardiovascular and neural stress responses (14, 15), including during traumatic stress (16). Recently, studies have identified that transcutaneous stimulation of the auricular vagal afferents (taVNS) using surface electrodes can reliably produce vagus sensory evoked potentials (17, 18). Subsequent neuroimaging studies, employing taVNS and transcutaneous cervical vagal afferent stimulation (tcVNS), have replicated deactivations within the limbic brain areas commonly observed with invasive VNS (19, 20). Therefore, it appears that non-invasive VNS has promise as an effective alternative to invasive VNS without the side-effects of invasive surgery and high costs (20, 21).

Recently, we have observed that tcVNS attenuates neural activity in traumatized individuals without PTSD during exposure to personalized traumatic stress scripts within the insula, prefrontal cortex, orbitofrontal cortex, premotor cortex, hippocampus, and anterior cingulate (22). Additionally, tcVNS also elicited advantageous sympathetic downregulation during the traumatic scripts in peripherally measured physiological biomarkers (16). However, because PTSD disrupts many neurobiological systems and associated feedback loops (3, 5), it is unclear whether tcVNS is similarly effective in this population. For example, during personalized traumatic reminders, PTSD elicits hypoactivity in brain areas such as the anterior cingulate, hippocampus, and orbito- and medial pre-frontal cortex, that may remove inhibition and lead to a hyperactive amygdala (23). Because of these neurobiological changes and other mechanisms, PTSD patients experience exacerbated stress during traumatic scripts, as identified by increased endocrine response and aberrant neural activity compared to traumatized non-PTSD controls (24, 25). Therefore, a non-invasive device with the potential to decrease the stress reactivity in PTSD may alleviate the typified acute and chronic neurobiological changes present in this population.

The purpose of this study was to examine how tcVNS alters neural responses to personalized traumatic scripts in PTSD patients. We hypothesized tcVNS would significantly improve activation in brain areas altered by PTSD—the anterior cingulate, hippocampus, and medial/orbital frontal cortex—along with blocking stress-related activation within stress-related brain areas such as the insula.

Materials and Methods

Emory University (#IRB00091171), Georgia Institute of Technology (#H17126), SPAWAR Systems Center Pacific, and the Department of Navy Human Research Protection Program institutional review boards provided approval for this study which is posted on ClinicalTrials.gov (ClinicalTrials.gov # NCT02992899). It should be noted that, although the ClinicalTrials.gov study description included the neuroimaging methods presented in this study, brain activity findings were not listed as primary or secondary outcomes. Verbal / written informed consent was provided by all participants before enrollment.

Participants

Healthy individuals between the ages of 18 and 70 with a history of prior trauma were recruited. Supplementary Figure 1 (Supplemental Digital Content) presents the Consolidated Standards of Reporting Trials (CONSORT) for this study. Of the 127 individuals assessed for eligibility, 100 were excluded based on declining to participate (n = 37) or not meeting inclusion criteria (n = 63) including non-PTSD traumatized controls analyzed separately (22). The diagnosis of schizophrenia, schizoaffective disorder, bipolar disorder, bulimia, or anorexia, as defined by The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (26), excluded individuals from participating. Further exclusion criteria were current pregnancy, traumatic brain injury, meningitis, presence of an active implanted device, evidence or history of serious medical or neurological illness, or positive toxicology screen. Trained staff administered the Structured Interview for the Diagnostic and Statistical Manual of Mental Disorders (SCID) (27) for psychiatric diagnosis. The Clinician-Administered

PTSD Scale for DSM-5 (28) was used to detect both the severity and presence of current and lifetime PTSD. The remaining 27 participants with PTSD were randomized into either sham or tcVNS groups using an online tool. Five participants were excluded from data analysis due to a temporary malfunction of the HR-PET scanner (n = 4; all participants in the current manuscript were enrolled after repair) or withdrawing from the study due to adverse reaction to trauma script (n = 1). The remaining 22 participants were included in the analysis, with n = 11 receiving tcVNS and sham (Table 1). Across all participants: one (4.5%) had a history of agoraphobia, one (4.5%) had a history of cocaine abuse, two (9%) had a history of alcohol abuse, twelve (54.5%) had a history of major depression (seven tcVNS, five sham), one (4.5%) had a history of obsessive-compulsive disorder, one (4.5%) had a history of opioid abuse, three (13.6%) had a history of panic disorder, one (4.5%) had a history of sedative-hypnotic-anxiolytic abuse, two (9%) had a history of social phobia, and one (5%) had a history of stimulant abuse.

Supplementary Table 1 details the individual traumatic experiences of the study sample. The most common traumatic experiences were molestation, rape, and/or sexual abuse (41%). Among the other traumatic memories, the remaining were related to physical abuse by family or partner (32%), death of a family member or child (27%), emotional abuse (18%), military-related stress, violence, or death (23%), observed violence or a suicide attempt or threatened with violence (18%), car accident (4.5%), a victim of gun violence (4.5%), and death of a friend (4.5%).

Study Design

Data collection occurred between May 2017 and September 2019 at Emory University School of Medicine. The tcVNS manufacturer (*ElectroCore, Basking Ridge, NJ*) provided pre-numbered active and sham devices to a member of the research staff not involved in any other aspect of the protocol. Furthermore, research staff conducting enrollment and data collection were blinded to stimulus type. Research staff conducting analyses were unblinded to device type but were not involved in data collection.

At the initial screening, a trained member of the research staff conducted a psychiatric interview and facilitated a written traumatic history of the participant. From there, the written traumatic experiences were converted into a 60-second script which was recorded by a member of the research team (29). Participants also completed the PTSD Checklist (30), Beck Depression Inventory (31), Clinician-Administered Dissociative States Scale (32), Hamilton Anxiety Scale (33), Early Childhood Trauma-Self Report (34), Adulthood Trauma Inventory (35), and State Trait Anger Scale (36).

During the second visit, participants underwent a high-resolution positron emission tomography (HR-PET) scan session (Figure 1) consisting of auditory delivery of a series of both neutral and traumatic scripts. In total, there were fourteen HR-PET scans (neutral scans = 1-2, 7-8, 11-12; active scans = 3-4, 9-10, 13-14; tcVNS/sham only scans = 5-6), with a 90 min break in between scans 10 and 11 employed to assess the prolonged effects of tcVNS. The neutral scripts were descriptions of nature designed to induce neutral-to-positive affective responses. All scripts were delivered using headphones as the participant lay supine in the HR-PET scanner. Following traumatic scripts, stimulation (tcVNS or sham) was

delivered to the left side of the neck during an automated two-minute interval. For scans five and six, the stimulation was delivered while the participant rested with eyes open. Following all scans, participants were asked to complete the Subjective Units of Distress Scale, rating their level of distress from 0 (no distress) to 100 (extreme distress).

Non-Invasive Transcutaneous Cervical Vagus Nerve Stimulation

All stimulation (tcVNS or sham) was administered with hand-held devices, identical in appearance and operation (*GammaCore, ElectroCore, Basking Ridge, NJ*), using collar electrodes applied to the left side of the neck for 120 seconds using previously described placement protocols (18). The tcVNS devices produced an AC voltage signal of five 5kHz sine pulses repeating at 25 Hz. The sham devices produced an AC biphasic voltage of 0.2 Hz square pulses which create a mild sensation similar to tcVNS. Sham stimulation is commonly used in tcVNS protocols (37); the sham signal in this study, being low frequency, is mainly attenuated at the skin-electrode interface due to the high impedance and therefore is unlikely to activate the afferent nerve fibers. However, the sham device does deliver relatively high voltage and current at the skin, and thus the nociceptors are activated, feeling like a pinch, and giving a similar sensation as active tcVNS. Stimulation intensity was adjustable from 0-5 arbitrary units with outputs of 0-30 V (~0-60 mA) and 0-14 V (~0-60 mA) for tcVNS and sham, respectively. Stimulation intensity was recorded as the maximum tolerable without pain following an acclimation period where the level was gradually increased. The stimulation intensity was 3.9 ± 1.1 (range: 2 – 5) and 4.9 ± 0.3 au (range: 3.5 – 5) for active tcVNS and sham, respectively. All participants reported feeling the stimulation for both active and sham.

Neuroimaging and Analysis

Regional cerebral blood flow was measured using HR-PET (*CTI, Knoxville, TN*) (38). Before the scans, participants were instructed to minimize all movement. Five seconds before the script (or stimulation period in scans five and six), a 20 mCi of radio-labeled water ($H_2[O^{15}]$), produced in an onsite cyclotron, was intravenously administered. Following the radio-labeled water injection, the HR-PET scan was initiated along with the script recording to measure brain blood perfusion.

HR-PET image analysis was completed similar to previous research (39) within the statistical parametrical mapping (SPM12; www.fil.ion.ucl.ac.uk/spm) suite. Scans were pre-processed by spatially normalizing to a mean intensity image across the fourteen individual scans, transformed into a common anatomical space (SPM PET Template), smoothed using a three-dimensional Gaussian filter at 5-mm full width half maximum, and then normalized to whole-brain activity. First level (individual) models were computed using the neutral script and traumatic script conditions with the factor of scan pairs. Because the first trauma script occurs without prior stimulation, this scan was omitted from the analyses. The first level model was grand mean scaled, estimated, and contrasts computed for activation (trauma scripts – neutral scripts) and deactivation (neutral scripts – trauma scripts) for each trauma script block (n = 3). Second-level analysis (between-participant) was completed for both activation and deactivation in separate models using the first-level contrast images in a flexible factorial model with stimulation type (independent) and trauma

script block (dependent) as factors. The second-level analysis also included the covariates of age (numeric) and sex (binary).

Statistical Analysis

Data normality was assessed using the Shapiro-Wilk test in R (*v3.4.0;* www.r-project.org), with comparisons between tcVNS and sham groups completed using a two-sample t-test or Mann-Whitney-Wilcoxon test for continuous and Fisher's exact test for discrete variables, respectively. For assessments during the scripts, linear mixed-effects models were fit to the data (lme4; cran.r-project.org/web/packages/lme4) using between-participant fixed effects of stimulation type (sham, tcVNS) and within-participant fixed effects of script type (neutral, traumatic) and duration (before, after break), random effect of participant, and baseline value as a covariate. The before- and after-break time points were chosen as more finite time decompositions (i.e., trauma script blocks, scan number) produced singularity or lack of convergence model fit errors. Model selection was based on the lowest Akaike Information Criterion with residual and qq plots checked to ensure appropriate fit.

Regional brain blood flow changes (activation, deactivation) between device types were encoded similar to previous recommendations (40) resulting in t-statistic brain maps. For both activation and deactivation analyses, a threshold of $p < 0.005$ (uncorrected) and a minimum voxel size of eleven was employed to minimize Type I and Type II errors in neuroimaging research (41). Significant cluster peaks were identified using the distance from the anterior commissure with x, y, and z coordinates transformed from Montreal Neurological Institute (MNI) space to those of the Talairach stereotaxic atlas (42). Cluster peaks were identified using Brodmann Areas (BA) from the Talairach daemon (www.talairach.org). The *a priori* α level for non-brain imaging data was chosen at 0.05. All data are presented as mean ± SD.

Results

Demographic

Demographic variables and psychosomatic scales were not significantly different between participants receiving tcVNS or sham (Table 1). tcVNS and sham also demonstrated similar PTSD checklist and CAPS-5 scores which were indicative of current PTSD (Table 1).

Psychometric Measures During Traumatic Scripts

Across all participants, the experimental environment elicited an increased state of distress ($t(21) = 5.7$, $p < 0.0001$; range: 0 - 80); baseline distress was also a significant predictor of distress across stimulation and script type ($p < 0.0001$). Furthermore, subjective distress decreased following the break (by 13.5 ± 16.8, $p = 0.001$). Given the greater initial baseline, trauma scripts (mean difference: 1.7 ± 8.4, $p = 0.36$) and tcVNS (mean difference: −3.4 ± 26.8, $p = 0.56$) did not alter subjective distress compared to neutral scripts and sham, respectively (script by stimulation interaction $p = 0.22$). A similar pattern of results was observed for visual analog ratings (anxiousness, fear, nervousness, high, and anger) and CADSS (moving slow, unreal, separation).

Neuroimaging

Figure 2 and Supplementary Tables 2-3 present the overall patterns of activation and deactivation while listening to the traumatic scripts in individuals with PTSD. When the study sample was examined as a whole (collapsed across stimulation type), trauma scripts resulted in significant ($p < 0.005$) activity within the bilateral ventromedial prefrontal cortex including the subgenual anterior cingulate (BA 10-11, 25), right temporoparietal areas (BA 7, 39), superior frontal lobe and dorsal anterior cingulate (BA 6, 32), bilateral insula, bilateral post- and pre-central gyri, and bilateral parietal lobe (BA 39, 40). Across the whole sample of individuals with PTSD, trauma scripts elicited significant areas of hypoactivation ($p < 0.005$) within the bilateral parietal precuneus, bilateral superior frontal gyrus (BA 11), right temporal lobe (BA 39), bilateral occipital lobe (BA 18), bilateral parahippocampal gyrus, bilateral posterior cingulate (BA 29,30).

Figure 3 and Supplementary Table 4 present brain areas with greater activations and deactivations during the traumatic scripts when sham stimulation was applied compared to active tcVNS. Compared to active tcVNS, sham elicited greater ($p < 0.005$) activations within the left medial, middle, and inferior gyri (BA 6, 11, 47), left precentral gyrus (BA 4, 44), left precuneus (BA 7), right postcentral gyrus and inferior and superior parietal lobules (BA 3, 5, 7, 40), left thalamus, left lentiform nucleus, right inferior frontal lobe (BA 46), right insula (BA 13), right superior temporal gyrus (BA 22), and right posterior cingulate (BA 31). Compared to active tcVNS, sham elicited greater ($p < 0.005$) deactivations within the right inferior (BA 20) and superior (BA 39) temporal lobe, left middle occipital gyrus (BA 19), right parahippocampal gyrus (BA 36) and hippocampus, right posterior cingulate (BA 29, 30), left middle temporal gyrus (BA 21), and left anterior cingulate (BA 24).

Figure 4 and Supplementary Table 5 present brain areas with greater activations and deactivations during the traumatic scripts when active tcVNS was applied compared to sham. Compared to sham tcVNS, active elicited greater ($p < 0.005$) activations within the left hippocampus and parahippocampal gyrus (BA 36), right parahippocampal gyrus (BA 35), left inferior, medial, middle, and superior temporal gyri (BA 10, 20, 21, 38), right middle and superior temporal gyri (BA 22, 38, 39), right postcentral gyrus (BA 2), left medial frontal gyrus (BA 10), left fusiform gyrus (BA 18), left occipital lobe (BA 18, 19), left posterior cingulate (BA 23, 29-31), left anterior dorsal anterior cingulate (BA 24, 33), and left pregenual anterior cingulate (BA 24), and left cerebellum. Compared to sham tcVNS, active elicited greater ($p < 0.005$) deactivations within the right inferior, medial, and middle frontal lobe (BA 6, 8-10, 45), left medial, middle, and superior frontal lobe (BA 6, 8, 10), bilateral pre- and post-central gyri (BA 1-4, 6, 7, 40), bilateral inferior and superior parietal lobules (BA 7, 40), bilateral cerebellum, left occipital lobe (BA 19), left thalamus, left caudate, and left lentiform nucleus.

Figure 5 and Supplementary Table 6 present brain areas with greater activity during only stimulation. Compared to active tcVNS, sham stimulation had greater ($p < 0.005$) activity during the application of the device within the right inferior and superior frontal gyrus (BA 9, 45), right pre- and post-central gyrus (BA 2, 6), right inferior lobule (BA 40), right temporal lobe (BA 38), left middle and superior frontal gyrus (BA 6, 8, 9), and right insula (BA 13). Compared to sham stimulation, active tcVNS had greater ($p < 0.005$) activity

within the bilateral fusiform gyrus (BA 20, 37), right posterior cingulate (BA 31), right middle temporal gyrus (BA 21, 22), right precuneus (BA 7, 39), left superior temporal gyrus (BA 38), left uncus, and bilateral occipital lobe (BA 18, 19).

Discussion

Application of tcVNS in PTSD patients exposed to traumatic scripts resulted in increased brain activity within the anterior cingulate, including the subgenual (sgACC), anterior dorsal (adACC), and posterior dorsal (pdACC) subdivisions. Prior studies have observed a relative decrease in sgACC activity, located primarily within left BA 24, in PTSD patients exposed to traumatic reminders, including traumatic scripts (23, 43, 44), and cognitive challenges such as oddball paradigms (45, 46). Downregulated activity of the sgACC in PTSD is thought to reflect deficient emotional regulation associated with traumatic remembrance (23) possibly via reduced inhibition of the amygdala by the anterior cingulate/medial prefrontal cortex as these areas have shown an inverse relationship during an emotional conflict task (47). Furthermore, greater sgACC activity was also associated with decreased arousal levels as measured by skin conductance (47). Therefore, increased sgACC activity would facilitate inhibition of the amygdala leading to improved emotional regulation. Since skin conductance is driven by the sympathetic nervous system, increased activity of which underlies hyperarousal symptoms in PTSD (48), the findings in the current study indicate that tcVNS may have the capacity to improve hyperarousal symptoms through a well-defined neural mechanism (23) associated with PTSD during a simulated intrusive memory which may have direct clinical application.

The adACC plays a central role in the integration of cognitive and emotional neural processes. Located within BA 24 and 32, the adACC is part of the dorsal cognitive division, which is a distributed attentional network with reciprocal connections with the lateral prefrontal cortex, parietal cortex, and motor areas (premotor cortex, supplementary motor area) (49, 50). Studies show a relative decrease, in function in this area during exposure to traumatic scripts in PTSD (23, 44). In the current study, there were similar results with traumatic script exposure paired with sham stimulation when compared to tcVNS. The adACC has three general functions: monitor, controller, and economic, which, according to a recently published integrative theory (51), allows it to act as a ‘storage buffer’ following the processing of an initial stimulus before guiding the individual to appropriate action. This processing appears advantageous in cognitive processes leading to fear extinction and/or emotional reappraisal (52). Therefore, greater activity within the adACC may represent a similar mechanism to the sgACC—and indeed areas within the anterior cingulate are functionally connected (52)—to improve emotional processing and regulation. PTSD also decreased activity during executive cognitive processing within the adACC, and this relationship was associated with PTSD severity (53). These results additionally present a direct clinical application for tcVNS and suggest the breadth of benefits may span across multiple aspects of emotional regulation and cognitive function.

The second main finding of this study is that tcVNS increased brain activity within the left hippocampus during personalized traumatic stress. The hippocampus is involved in processing explicit memory and fear extinction and is seen during context-specific traumatic

reminders (54). In PTSD, a failure of hippocampal activation is commonly observed during trauma reminders (44), potentially as a result of PTSD-mediated crenation (55) leading to declarative memory deficits (56). Additionally, PTSD disrupts a memory network consisting of the hippocampus, anterior cingulate, and orbitofrontal cortex, that is employed in the recall of emotionally valenced words (57).

PTSD also appears to disrupt the resting functional connectivity of the hippocampus. In previous traumatized control participants, the anterior and posterior hippocampus differed in their connection to areas within the default mode and salience network (58). However, this separation was not observed with PTSD, in addition to exhibiting a less inhibitory effect of the hippocampus on the precuneus (58). The left precuneus was more active in the sham condition in the current study, reinforcing this inefficient inhibition and potentially indicating a lessened ability to inhibit the default mode network at-large which is known in PTSD (59).

The hippocampus is also sensitive to stress. For example, injecting yohimbine, an α-2 antagonist which stimulates norepinephrine release, decreased hippocampus metabolism in PTSD patients (60), suggesting a high sensitivity to norepinephrine. One potential mechanism of action for tcVNS is that stimulation will decrease downstream norepinephrine release from the locus coeruleus during traumatic stress (61), which would decrease noradrenergic innervation of the hippocampus and therefore increase metabolic activity. While circulating norepinephrine levels are not available in the current study, previous studies in both the periphery (16) and brain (22, 62) indicate tcVNS can decrease reactivity to both somatosensory and traumatic stressors. Furthermore, whether this potential benefit of tcVNS is limited to acute effects or has the potential to modulate long-term neurobiological changes—such as increasing hippocampal volume through neural regeneration—requires investigation.

The third major finding of this study was that tcVNS decreased brain reactivity to traumatic stress in a similar manner to our previous non-PTSD findings (22). In non-PTSD individuals experiencing previous trauma, tcVNS decreased activity within similar areas as observed in the current study such as the left frontal lobe (middle, inferior, and superior gyri), left precentral gyrus, left thalamus, and insula (although discordant hemispheres) (22). Many of these areas coincide with the conjunction analysis for cohort-specific brain activations (Figure 1) and are commonly activated in response to traumatic stress in both healthy and PTSD patients (43, 63). These findings provide evidence that tcVNS may decrease general emotional stress reactivity in PTSD and provide support for use as a treatment modality. Interestingly, nodes of the default mode network, such as the posterior cingulate and precuneus, were more active with sham stimulation. Therefore, an alternative interpretation of greater activity with sham during trauma scripts is a greater efficacy of tcVNS to silence resting-state networks during cognitive processing.

In addition to decreasing general emotional stress reactivity to trauma scripts, tcVNS may block activation to areas important in regulating the peripheral stress response such as the insula (64). In the current cohort, tcVNS blocked activation within the mid-to-posterior insula, which is the subdivision that processes somato-visceral information from the body

and translates to an emotional state (65). Furthermore, there is emerging evidence that the right insula processes sympathetic effects (66), with greater activity observed with more physiological stress (67). These data support earlier findings (22) in a non-PTSD sample and reinforces the potential efficacy for tcVNS to decrease both central and peripheral arousal during traumatic stress.

While the current study presents novel findings of neural activity responses during traumatic stress in PTSD with tcVNS, the study is not without limitations. While multiple studies have observed evidence of tcVNS stimulating the vagal nerve (18, 68), similar data are not available for the current manuscript. However, it should be noted that the presence of sham or active tcVNS could be predicted with an accuracy of 96% using peripheral biomarkers (69), providing evidence of a distinct change in physiology with stimulation of the vagus nerve. Secondly, it should be noted that changes in brain activity were observed without concomitant changes in perceptual measures of distress. This was likely an artifact of participants reporting elevated baseline stress, potentially in response to the upcoming traumatic reminder scripts, which limits the degree into which changes values be calculated. This limits interpretability of the study results, as a direct causal relationship between perceptual distress and changes in neural activity cannot be established. Further studies are required to fully examine the relationship between perceptual responses during traumatic stress, tcVNS, and brain activity in PTSD. Thirdly, while the application of tcVNS to a clinical population is novel, this is a cross-sectional study and therefore longitudinal changes cannot be determined. This is particularly salient in PTSD with known neural changes, possibly related to altered neurotransmitter sensitivity/levels (48), and future studies are required to determine the long-term efficacy of tcVNS. This study would be improved by probing whether participants thought they received either sham or active tcVNS.

In conclusion, this study has provided novel insight into the potential benefits of tcVNS applied following personalized traumatic reminders in PTSD. Stress-related disorders such as PTSD are important public health problems, affecting ~8% of Americans (1). Furthermore, approved pharmacological treatments of PTSD suffer from poor response, elevated remission rates, and insufficient evidence supporting their efficacy (9). The current study uniquely addressed whether a novel, non-invasive neuromodulation device, tcVNS, altered neural activity during personalized traumatic scripts. Personalized trauma scripts allow for an individualized approach to inducing hyperarousal symptoms and provides insight into potential efficacy in real-world scenarios where tcVNS may have an application.

Specific to PTSD, the application of tcVNS following traumatic scripts increased activity in brain areas known to be hypoactive, the anterior cingulate and hippocampus. Reversal of this hypoactivity likely indicates tcVNS-mediated improvements in emotional processing, memory retrieval, and a decreased fear response, providing neural evidence for the clinical efficacy of tcVNS. Like non-PTSD but previously traumatized individuals, tcVNS also was able to decrease trauma script-related brain activity and therefore may be indicative of lessened arousal. These findings also indicate differences between PTSD and non-PTSD, supporting the need for a targeted investigation into PTSD populations. Specifically, whether the potential beneficial effects of tcVNS on the anterior cingulate and hippocampal circuitry persist during prolonged usage merits further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

PTSD	Posttraumatic Stress Disorder
HR-PET	High-Resolution Position Emission Tomography
VNS	vagal nerve stimulation
taVNS	transcutaneous auricular vagal nerve stimulation
tcVNS	transcutaneous cervical vagal nerve stimulation
DSM	Diagnostic and Statistical Manual of Mental Disorders
SCID	Structural Clinical Interview for DSM
SPM	Statistical Parametric Mapping

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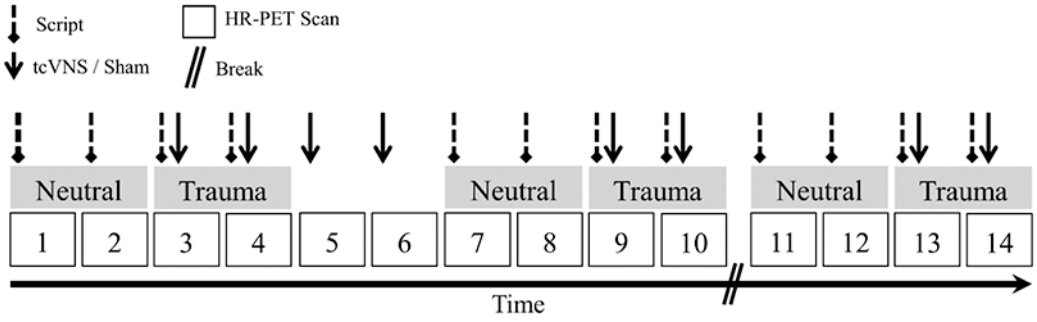

Figure 1:

Protocol timeline for the current study depicting timing of script delivery and non-invasive cervical vagal nerve (tcVNS)/sham stimulation. All scripts were delivered via headphones with stimulation initiated immediately following. Total session duration was five hours including a prolonged (90 min) rest period.

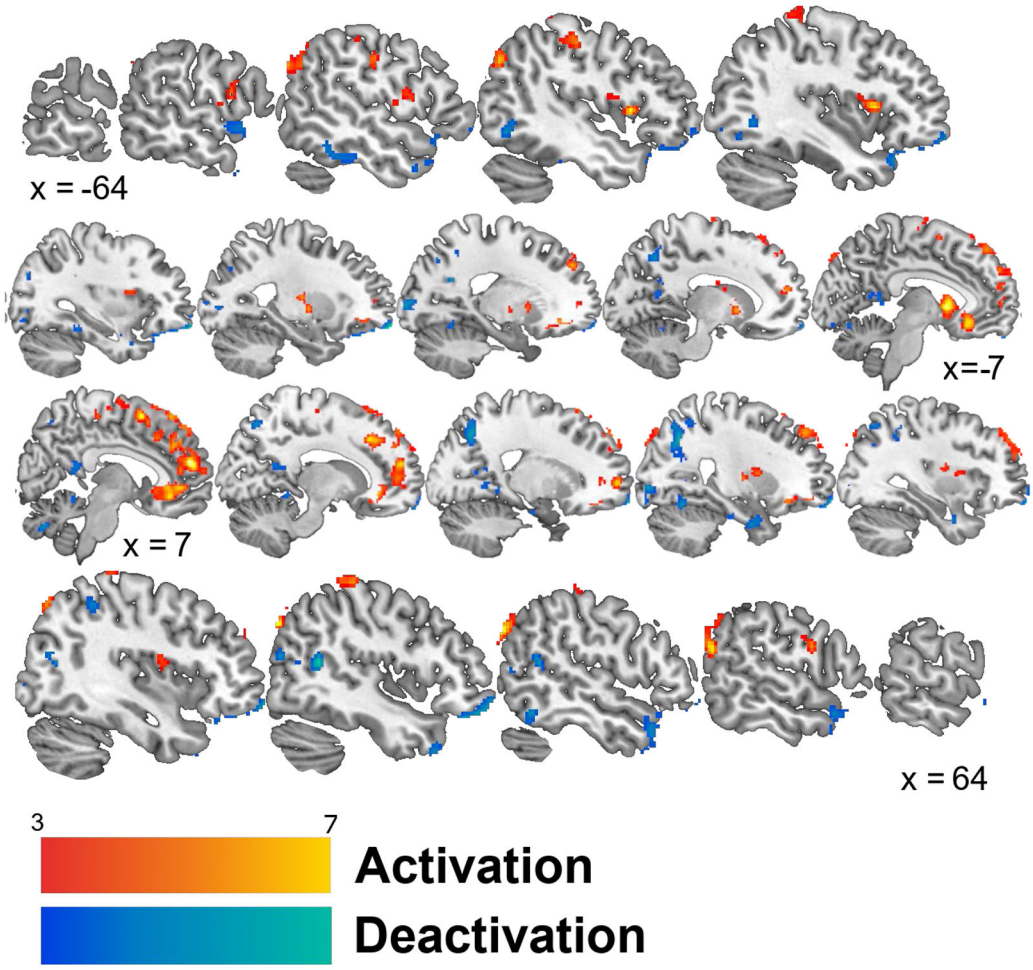

Figure 2:

Sagittal slices presenting significant ($p < 0.005$) activation (red) and deactivation (blue) while listening to personalized trauma scripts in participants receiving cervical non-invasive vagal nerve and sham stimulation with posttraumatic stress disorder. Talairach x coordinates below slices indicate the location with negative and positive coordinates located in the left and right hemispheres, respectively. Color bars indicate z-values of the cluster.

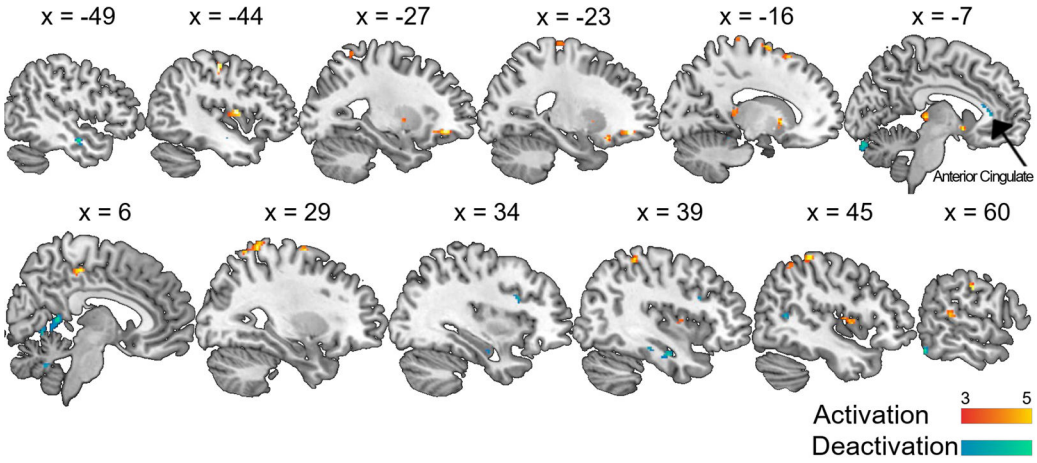

Figure 3:

Sagittal slices presenting greater ($p < 0.005$) activation (red) and deactivation (blue) while listening to personalized trauma scripts in participants with posttraumatic stress disorder receiving sham cervical non-invasive vagal nerve stimulation compared to active. Talairach x coordinates above slices indicate the location with negative and positive coordinates located in the left and right hemispheres, respectively. Color bars indicate z-values of the cluster.

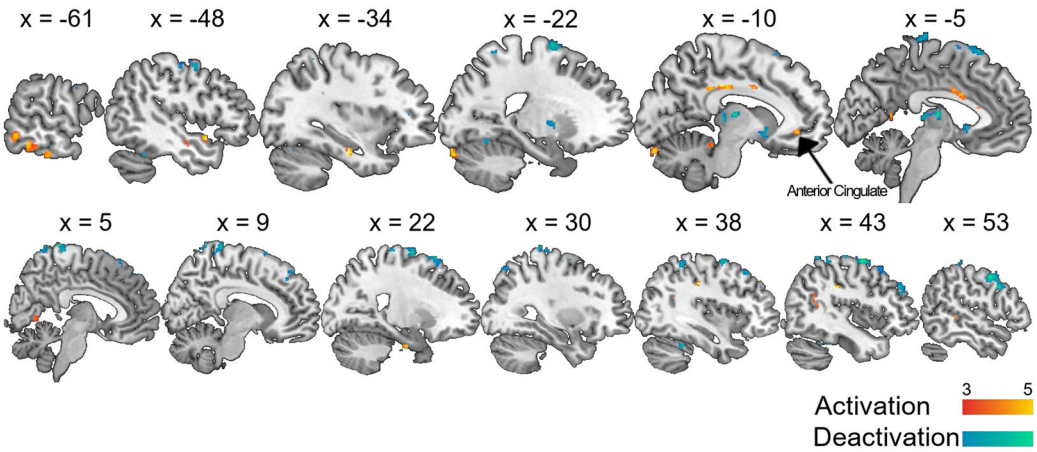

Figure 4:

Sagittal slices presenting greater ($p < 0.0025$) activation (red) and deactivation (blue) while listening to personalized trauma scripts in participants with posttraumatic stress disorder receiving active cervical non-invasive vagal nerve stimulation compared to sham. Talairach x coordinates above slices indicate the location with negative and positive coordinates located in the left and right hemispheres, respectively. Color bars indicate z-values of the cluster.

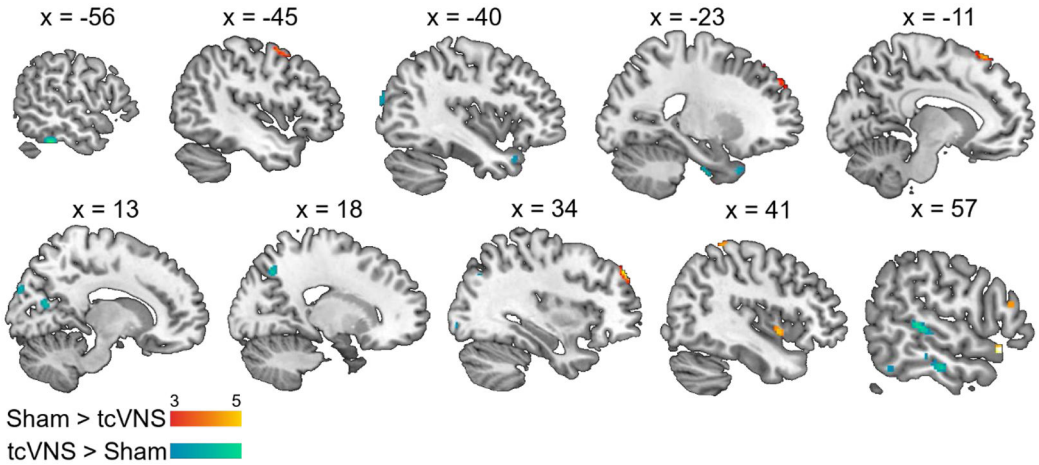

Figure 5:

Sagittal slices presenting greater ($p < 0.005$) activity during either sham (red) and active transcutaneous vagal nerve stimulation (tcVNS; blue) during periods of only stimulation in participants with posttraumatic stress disorder. Talairach x coordinates above slices indicate the location with negative and positive coordinates located in the left and right hemispheres, respectively. Color bars indicate z-values of the cluster.

Table 1:

Participant Demographics for the active non-invasive vagal nerve stimulation (tcVNS) and sham groups.

PTSD = Posttraumatic stress disorder, CADSS = Clinician-Administered Dissociative States Scale, ETI-SR = Early Trauma Inventory-Self Report, ATI = Adulthood Trauma Inventory, SUDS = subjective units of distress, CAPS = Clinician-Administered PTSD Scale

Measure	tcVNS (n = 11)	Sham (n = 11)	p value
Age (y)	35.3 ± 12.7	37.6 ± 13.7	0.69
Sex	9 F, 2 M	6 F, 5 M	0.36
BMI (kg·m^{-2}),	29.6 ± 7.3	28.6 ± 4.0	0.71
Race/Ethnicity			0.66
White/Caucasian	5 (45.4%)	7 (63.6%)	
Black/African American	6 (54.5%)	3 (27.3%)	
Asian	-	1 (9.1%)	
Education			0.20
High School - Graduate	1 (9.1%)	1 (9.1%)	
College – Not Complete	5 (45.5%)	1 (9.1%)	
Associate Degree	2 (18.2%)	2 (18.2%)	
Bachelor's degree	3 (27.3%)	7 (63.6%)	
Marital Status			0.92
Never Married	5 (50.0%)	5 (41.7%)	
Married / Civil Partnership	2 (20.0%)	2 (16.7%)	
Divorced / Separated	2 (20.0%)	4 (33.3%)	
Widowed	1 (10.0%)	1 (8.3%)	
PTSD Measures			
PTSD Checklist Score	56.1 ± 15.3	57.1 ± 13.7	1.0
CAPS-5 Total Severity	16.3 ± 2.8	14.5 ± 4.3	0.55
Psychometric Measures			
Beck Depression Inventory	28.7 ± 16.2	27.5 ± 11.5	0.84
CADSS	8.1 ± 9.0	8.3 ± 9.7	0.84
Hamilton Anxiety	22.3 ± 8.8	17.1 ± 7.6	0.16
ETI-SR	24.3 ± 10.9	17.9 ± 13.4	0.24
ATI	7.0 ± 1.9	6.8 ± 3.2	0.87
Anger			
Total	127.4 ± 19.4	120.0 ± 11.0	0.31
State	22.0 ± 11.1	16.8 ± 2.4	0.21
Trait	22.3 ± 7.2	21.0 ± 4.0	0.62
SUDS (Baseline)	34.5 ± 29.4	33.9 ± 28.5	0.87

REVIEW ARTICLE OPEN

Check for updates

The role of the immune system in posttraumatic stress disorder

Seyma Katrinli[1]✉, Nayara C. S. Oliveira[1,2,3], Jennifer C. Felger[4,5], Vasiliki Michopoulos[4] and Alicia K. Smith[1,4]

Posttraumatic stress disorder (PTSD) develops in a subset of individuals upon exposure to traumatic stress. In addition to well-defined psychological and behavioral symptoms, some individuals with PTSD also exhibit elevated concentrations of inflammatory markers, including C-reactive protein, interleukin-6, and tumor necrosis factor-α. Moreover, PTSD is often co-morbid with immune-related conditions, such as cardiometabolic and autoimmune disorders. Numerous factors, including lifetime trauma burden, biological sex, genetic background, metabolic conditions, and gut microbiota, may contribute to inflammation in PTSD. Importantly, inflammation can influence neural circuits and neurotransmitter signaling in regions of the brain relevant to fear, anxiety, and emotion regulation. Given the link between PTSD and the immune system, current studies are underway to evaluate the efficacy of anti-inflammatory treatments in those with PTSD. Understanding the complex interactions between PTSD and the immune system is essential for future discovery of diagnostic and therapeutic tools.

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder characterized by re-experiencing of trauma, avoidance of trauma reminders, and hyperarousal symptoms that cause negative alterations in cognition, mood, and physiologic health [1]. PTSD is unique among other psychiatric disorders, as it requires trauma exposure to develop. Although over 70% of the population is exposed to at least one traumatic event during their lifespan, it is not clear why only some individuals develop PTSD [2]. Given the high comorbidities of inflammatory and metabolic disorders with PTSD [3–9], some studies have focused on the potential for an immune-related or inflammatory etiology for PTSD [10–12], whilst others suggest that PTSD promotes inflammation [13, 14] or that a bidirectional relation between PTSD and inflammation exists [15, 16].

In this review, we first summarize potential mechanisms connecting PTSD and the immune system. We describe studies of peripheral immune markers and mechanisms through which immune alterations affect neurotransmitter systems and brain regions that contribute to PTSD symptomatology. We also highlight the contribution of chronic inflammation in conditions often co-morbid with PTSD. Finally, we explore plausible therapeutic strategies targeting the immune system, based on its interaction with PTSD.

PTSD AND INFLAMMATION

The neuroendocrine, psychophysiological, and neurobiological changes in PTSD etiology and outcome have been extensively studied [17, 18]. Growing evidence in the past two decades points to mechanisms related to the innate (i.e., non-specific first line of defense regulated by innate immune cells, including monocytes, macrophages, dendritic cells, and microglia) and adaptive (i.e., antigen-specific immunity regulated by T and B lymphocytes) immune systems in the pathophysiology of PTSD [17–19]. The initial evidence for the relationship between PTSD and the immune system comes from individual studies and subsequent meta-analyses reporting alterations in peripheral inflammatory markers, such as C-reactive protein (CRP), interferon-gamma (IFN-γ), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) in individuals with PTSD (Fig. 1) [10, 20–23]. Moreover, hypothesis-free genome-wide [24, 25], epigenome-wide [26–30], and transcriptomic studies [31–35] of PTSD have identified multiple genes related to the immune system.

Alterations in peripheral immune markers in PTSD

The inflammatory environment in PTSD is characterized by increased levels of pro-inflammatory markers (e.g., CRP, IL-6, IL-1β, IL-2, TNF-α, IFN-γ) and decreased levels of anti-inflammatory markers (e.g., IL-10) (Fig. 1) [10, 20–22]. Elevated inflammatory markers in PTSD may create a positive feedback loop to promote inflammation, such that IL-6, IL-1β, and TNF-α induce CRP to activate the complement system, which triggers a cascade of events to promote inflammation [18]. Nonetheless, it is still not clear whether the inflammatory milieu is the outcome of PTSD, or if pre-existing or trauma-induced inflammation increases the risk of PTSD. Notably, a bidirectional relationship between PTSD and inflammation is supported by recent reports [15, 16, 19], including a large-scale genetic study reporting a bidirectional genetic association between PTSD and CRP [16].

Longitudinal studies investigating whether PTSD development leads to inflammation reported that PTSD caused increases in inflammatory markers, including IL-1β, IL-8, CRP, and tumor necrosis factor receptor II (TNFRII) [36, 37]. On the contrary, Glaus

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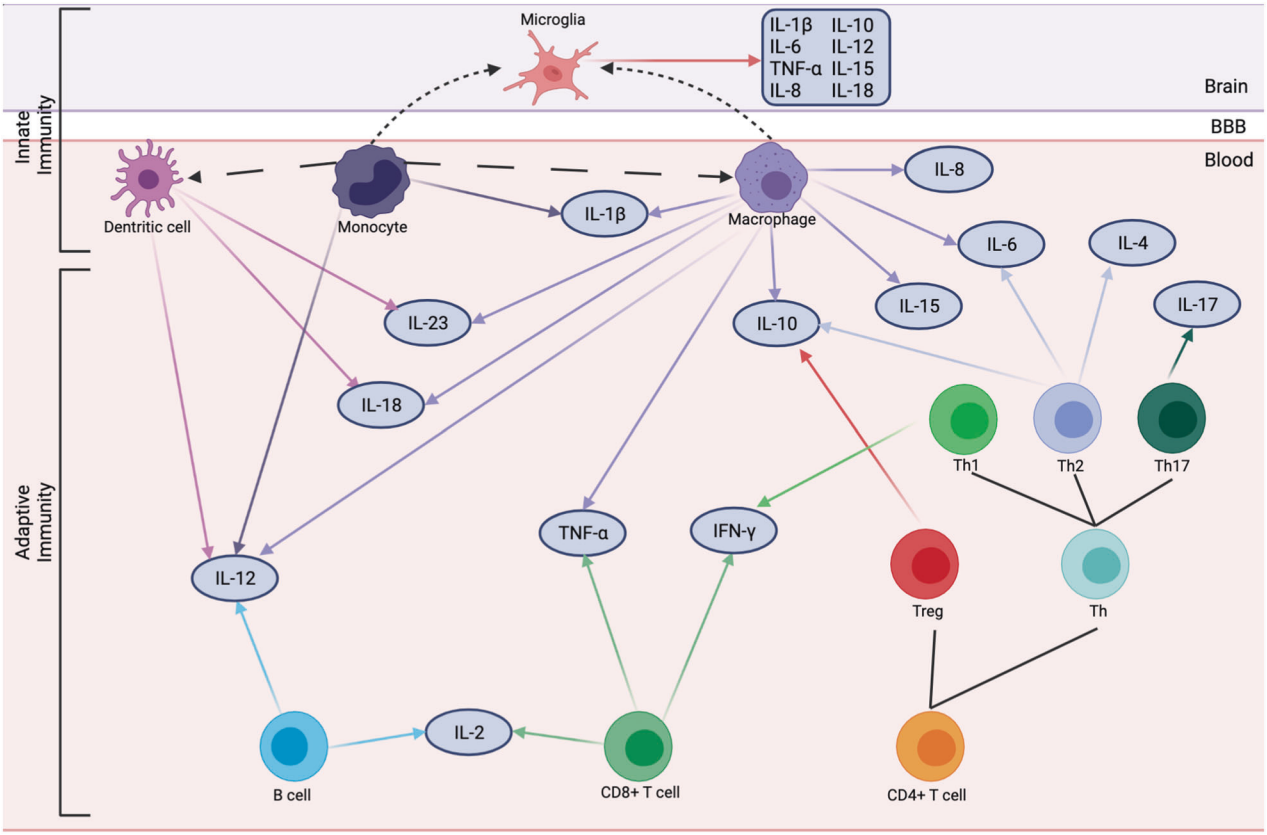

Fig. 1 Immune cells and cytokines implicated in PTSD. Long dashed lines represent differentiation. Short dashed lines represent trafficking into the brain. BBB blood–brain barrier, IL interleukin, Th T helper cell, Treg regulatory T cell.

et al. [38] observed lower IL-6 levels following PTSD diagnosis, indicating a decrease in inflammation. Longitudinal studies evaluating whether pre-existing inflammation is a risk factor for PTSD reported that increased levels of CRP and TNFRII predicted PTSD diagnosis [10, 15]. Transcriptomic studies conducted on US Marines revealed that upregulation of immune-related genes and overexpression of genes in networks associated with the innate immune response and interferon signaling at pre-deployment predicted post-deployment PTSD [39, 40]. Here, one can query the possible causes of pre-existing inflammation. Some possible drivers of this pre-existing inflammation (e.g., metabolic conditions, biological sex, and genetics) will be discussed below. Trauma and stress exposure across the lifespan might also contribute to inflammation prior to the incident trauma that results in PTSD [19]. Notably, a transdiagnostic meta-analysis of trauma exposure reported increased peripheral CRP, IL-1β, IL-6, and TNF-α concentrations in participants who experienced traumatic events (e.g., childhood maltreatment, natural disaster, violence) across their lifespan [41]. Specifically, early life adversity, including maltreatment, parental separation, and low socioeconomic status in childhood, is associated with increased CRP, IL-6, and TNF-α levels in adulthood [27, 42, 43]. Concordantly, studies that assessed inflammatory markers in the acute aftermath of trauma showed that increased levels of IL-6, IL-8, and CRP were associated with PTSD at follow-up [11, 12, 44]. In contrast, Michopoulos et al. [45] reported that decreased levels of TNF-α and IFN-γ upon trauma predicted chronic PTSD trajectory. However, since these studies measured inflammatory markers right after the trauma exposure, it is not clear whether the inflammatory response precedes the trauma or is the result of an acute posttraumatic response. The inability to assess the origin of the inflammatory response (i.e., pre- or post-trauma) may account for the inconsistent findings across the studies.

The relationship between inflammation and traumatic experiences is also supported by animal repeated social defeat stress (RSDS) models. For instance, IL-17A secreted from meningeal T cells in the brain was reported to control anxiety-like behavior in mice through neuronal IL-17a receptor subunit (IL-17Ra) signaling [46]. Following RSDS, anxiety-like behaviors are associated with increased levels of peripheral cytokines, including IL-2, IL-10, IL-17A, IL-22, and TNFα [47]. In contrast, Hodes et al. [48] showed increased peripheral cytokines in mice susceptible to social stress after RSDS.

Link between the HPA axis, autonomic nervous system, and inflammation in PTSD

The neuroendocrine stress response is comprised of the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis, which relay signals to the peripheral organs and the immune system (Fig. 2). Upon acute exposure to stress, corticotrophin-releasing hormone (CRH) is secreted from the hypothalamus, thereby activating the HPA axis [49]. The binding of CRH to its receptor on pituitary corticotropes triggers the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the systemic circulation, stimulating glucocorticoid (cortisol in humans) synthesis from the adrenal cortex [49]. In parallel, stress exposure also triggers the sympathetic nervous system (SNS) to release catecholamines (e.g., epinephrine and norepinephrine), which are responsible for physiological changes, such as increases in heart rate and blood pressure [50]. In response to norepinephrine, monocytes are mobilized from the bone marrow into the periphery, where they encounter danger-associated molecular patterns (DAMPs), activating nuclear factor kappa B (NF-kB) mediated production of pro-inflammatory cytokines [51, 52]. Indeed, individuals with PTSD exhibited increased peripheral NF-κB activity and NF-κB-mediated transcriptional changes in monocytes,

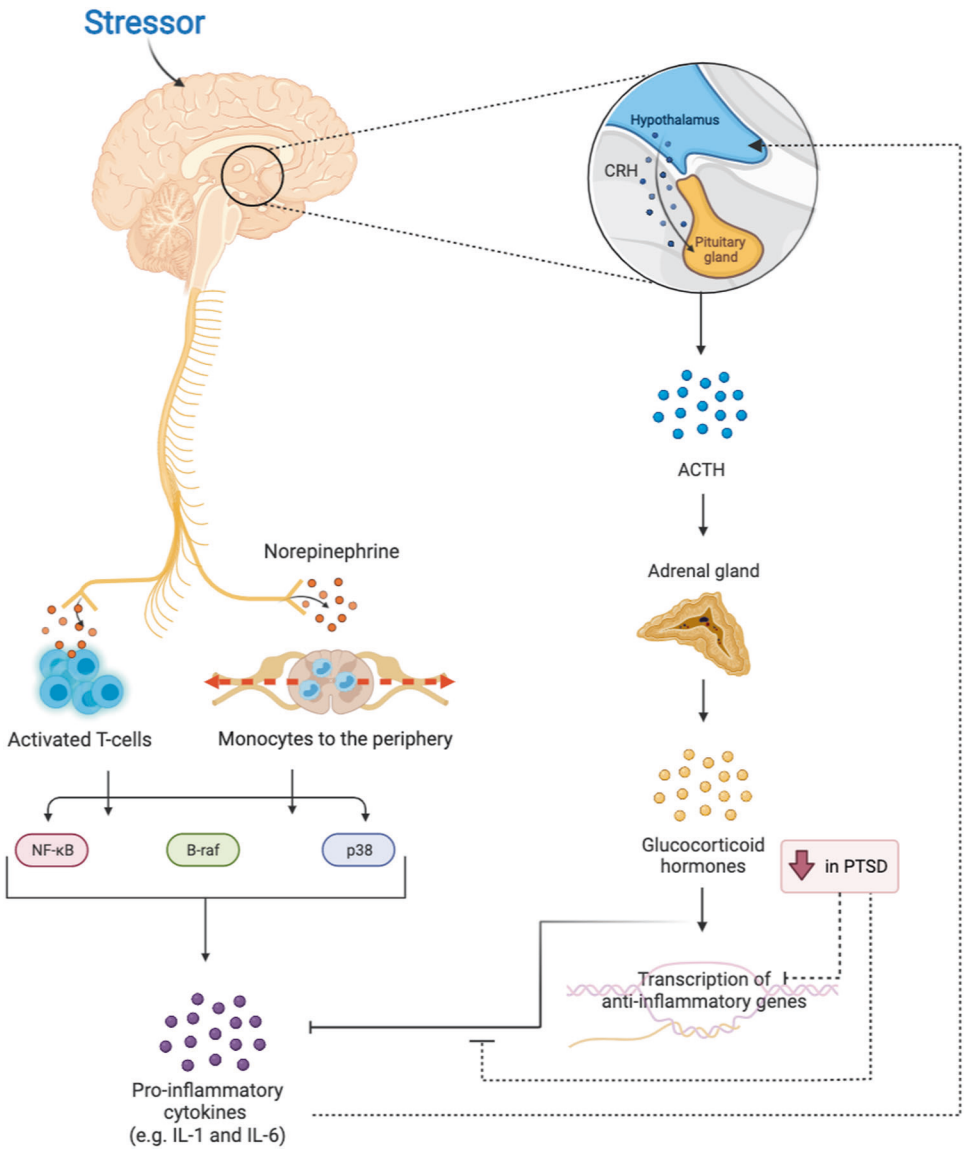

Fig. 2 Relationship between the HPA axis, sympathetic nervous system, and inflammation in PTSD. Stress exposure stimulates sympathetic nervous system (SNS). Norepinephrine release from activated SNS fibers stimulates proinflammatory cytokines production through the NF-kB, B-Raf, and p38 pathways. The HPA axis is also activated upon exposure to stress, stimulating inflammatory responses that limit HPA reactivity. The reduced ability of glucocorticoids to inhibit inflammatory processes contributes to the proinflammatory environment in PTSD. CRH corticotrophin-releasing hormone, ACTH adrenocorticotropic hormone, NF-κB nuclear factor-κB, IL-1 Interleukin-1, IL-6 Interleukin-6.

which contribute to the inflammatory environment [34, 35]. Similarly, norepinephrine release from activated SNS fibers further stimulates the NF-κB, B-raf-ERK1/2, and p38 pathways in activated T cells to produce pro-inflammatory cytokines [53–55]. Along with SNS activation, decreased parasympathetic activity in PTSD contributes to the inflammatory milieu [56]. Elevation in these pro-inflammatory cytokines, in turn, leads to HPA axis reactivity [17].

In PTSD, HPA axis hyperactivity following repeated trauma may disrupt glucocorticoid signaling, which leads to peripheral and central nervous system inflammation [17]. Normally, following the binding of glucocorticoids to the glucocorticoid receptor (GR), the stress response is dampened via a negative-feedback loop [18]. The glucocorticoid–GR complex also suppresses inflammatory responses either by stimulating the transcription of anti-inflammatory genes in the nucleus or by inhibiting the expression of proinflammatory proteins in the cytosol [18]. However, chronic exposure to stress may result in glucocorticoid resistance, wherein cortisol cannot inhibit NF-κB-mediated pro-inflammatory cytokine release, dampening glucocorticoid negative feedback of the HPA axis [17]. Overall, the inflammatory state in PTSD is propagated through a combination of glucocorticoid resistance along with increased sympathetic and decreased parasympathetic nervous system activity [17].

Chronic exposure to trauma and stress can also stimulate the production and release of DAMPs, such as mitochondrial reactive oxygen species (ROS) [57, 58]. DAMPs are key contributors to local or systemic inflammatory responses in the absence of pathogens or tissue damage [57, 58]. Plasma levels of the astroglial protein S100 calcium-binding protein B (S100b), one of the most studied DAMPs in the field of psychiatry, were reported to be higher in veterans with PTSD compared to healthy veterans [59]. Plasma levels of the nuclear protein high mobility group box 1 protein (HMGB1) were increased in severe blunt chest trauma patients

with PTSD compared to those without PTSD [60]. In response to stress exposure, these DAMPs bind pattern recognition receptors (PRRs), including the receptor for advanced glycation end-products (RAGE) and toll-like receptors (TLR) on innate immune cells, activating the NF-kB pathway to produce pro-inflammatory cytokines [57, 58].

Comorbidity between PTSD and immune-related diseases

PTSD can be highly co-morbid with serious physical illnesses, including asthma [61], autoimmune diseases [7, 8], and cardiovascular diseases (CVD) [4, 5]. Multiple studies identified PTSD as a risk factor for CVD and related cardiovascular events, including heart failure and ischemia [62–65], while others propose that CVD symptoms, treatment, and surgery may serve as a trauma that increases PTSD prevalence following acute coronary events [66, 67]. Emerging PTSD symptoms following these cardiovascular events may in turn increase the risk of severe cardiovascular outcomes, including recurrence and mortality [68, 69]. This bidirectional relationship between PTSD and CVD may be due, in part, to contributions from multiple common underlying mechanisms [5, 67, 68], including the ANS [70, 71], HPA axis, oxidative stress [72], and inflammation [5, 73–76].

PTSD is also strongly linked with asthma [3]. The relationship between PTSD and asthma also appears to be bidirectional, as numerous studies report increased asthma prevalence in individuals with PTSD [77–79], while others show a higher odds ratio for PTSD in individuals exhibiting symptoms of asthma [80]. The comorbidity of asthma and PTSD may be explained by shared inflammatory mechanisms. In asthma, the binding of allergens to PRRs triggers both innate and adaptive immune responses [3]. In severe cases of asthma, the T helper 2 (Th2) immune response is augmented by Th17 cells that produce IL-17A, enhancing the pro-inflammatory response [3]. Clinical studies report that those with PTSD do not exhibit differences in Th2 cell proportions [81], but have higher IL-17A levels [82, 83]. Consistent with this finding, those with more severe PTSD symptoms have elevated Th17 cell counts [81]. Overall, this evidence suggests that the link between PTSD and asthma may be driven by an increased Th17 immune response.

PTSD is co-morbid with autoimmune diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), multiple sclerosis (MS), and psoriasis [7–9]. Notably, the risk of an autoimmune disorder is higher in individuals with PTSD, compared to individuals with other psychiatric disorders [8]. Even though the direction of the association between PTSD and autoimmune diseases is not clear, the fact that this association is not affected by prior trauma and healthy behaviors [7] may suggest that PTSD precedes autoimmune diseases. This hypothesis was supported by a retrospective study of Swedish civilians reporting an increased risk of autoimmune disease development in those with PTSD [84]. Indeed, inflammation is one of the biological mechanisms suspected to link PTSD and autoimmune disorders. Elevated leukocyte, total T-cell counts, and cell-mediated immunity in PTSD may contribute to the development of autoimmune disorders [9, 85, 86].

The inflammatory environment in PTSD may also be exacerbated by co-morbid metabolic conditions [6]. Individuals with PTSD are most likely to suffer from type 2 diabetes mellitus, metabolic syndrome (MetS), and its individual components, including obesity, insulin resistance, and dyslipidemia [8, 87, 88]. This increased comorbidity can be explained by unhealthy lifestyles associated with PTSD (e.g., disrupted sleep patterns, unhealthy diet, tobacco and substance use, physical inactivity), which contribute to inflammation [89–92]. In both MetS and PTSD, the noradrenergic system is activated to trigger an innate immune response [6]. Like PTSD, MetS and obesity are also characterized by an increase in proinflammatory markers, such as CRP, IL-6, and TNF-α [20–22, 93, 94]. Inflammation can promote obesity and insulin resistance, and the resulting fat accumulation, in turn, may lead to elevated levels of proinflammatory cytokines [93, 94]. The connection between PTSD and MetS is supported by a recent hypothesis-free metabolomic study of PTSD that reported dysregulated production and utilization of carbohydrate, lipid, and amino acids, as well as alterations in energy-related pathways [95]. This metabolic evidence indicated inflammation, inefficient energy production, and possibly mitochondrial dysfunction in individuals with PTSD [90]. Mitochondrial dysfunction may lead to increased production of ROS in peripheral organs and immune cells, which contribute to peripheral inflammation. Kusminski and Scherer proposed that inflammation, oxidative stress, and metabolism can be linked together by mitochondrial dysfunction [96]. Overall, a "mitochondrial allostatic load" model may explain the link between these adverse metabolic conditions, inflammation, and PTSD. This model suggests that metabolic dysregulation in PTSD may disrupt mitochondrial activity, resulting in increased ROS production and inflammation [97].

Other drivers of inflammation in PTSD

Gut microbiota plays an important role in the communication between the brain and the gastrointestinal tract, called the "gut-brain axis". This axis regulates gastrointestinal homeostasis and links areas of the brain with intestinal functions through the vagus nerve, SNS, and both the endocrine and immune networks [98]. The composition of gut microbiota significantly influences the regulation of the gut-brain axis by stimulating immune cells that contribute to neuroinflammation [99]. Stress, diet, and other environmental factors can disrupt the gut microbiome, which signals the intestinal epithelium to produce pro-inflammatory cytokines [99] and may ultimately lead to permeability in the intestinal tract and excessive antigen trafficking and inflammation [99]. Growing evidence implicates dysregulated gut-brain axis signaling in the pathogenesis of stress and mood disorders and reports gut microbiome alterations in individuals with PTSD [100–103]. Gut microbiome alterations may also mediate the association between early life adversity and symptoms of anxiety in adulthood [104]. These data, in conjunction with evidence showing that PTSD is highly co-morbid with inflammatory gastrointestinal diseases (e.g., IBD), [8, 105] may implicate gut microbiota dysbiosis in the inflammatory environment of PTSD.

Since PTSD disproportionally affects women over men, [106] sex may also modulate the immune response in PTSD [107]. The higher prevalence of PTSD in women can be explained by higher trauma vulnerability, dysregulated fear processing, more sensitive HPA axis, and fluctuating HPA axis activity with the menstrual cycle, as well as aberrant immune responses [17, 106, 107]. Multiple studies reported PTSD-associated differences in immune markers based on sex (reviewed in ref. [108]). A gene co-expression study showed upregulation of an IL-12 signaling module in men but not women with PTSD [31]. Neylan et al. [109] reported increased activation of pathways related to the immune response in monocytes of women, but not men with PTSD. In addition, Kim et al. [110] reported sex-specific differences in peripheral blood leukocyte composition, as men but not women with lifetime PTSD have increased monocyte proportions. Evidence from post-mortem brain samples revealed decreased microglia proportions in women with PTSD [108, 111]. The key factor underlying the sex-specific immune response in PTSD may be estrogen, as studies showed that lower estrogen levels are associated with increased PTSD symptoms [112, 113]. A recent study showed the indirect effect of sex on non-remitting PTSD development through pro-inflammatory cytokines [114]. Authors also showed that this indirect effect of sex was moderated by estradiol, such that men with higher estradiol levels have elevated pro-inflammatory cytokine levels, which was associated with a lower risk of non-remitting PTSD [114]. This relationship can be explained by activation of the HPA axis and inhibition of the SNS

by elevated estrogen levels that suppress the production of pro-inflammatory cytokines from T cells and macrophages [107, 115].

Another driver of inflammation in PTSD might be genetics, considering SNP-based heritability estimates of 5–20% that vary based on sex [25]. Supporting evidence comes from studies reporting associations between PTSD and polymorphisms in genes involved in the immune system, including the human leukocyte antigen (HLA) locus [25, 116], *CRP* [117, 118], *TNF-α* [119], and ankyrin repeat domain-55 (*ANKRD55*) [24]. The variations in these immune genes may contribute to pleiotropy between PTSD and immune-related disorders, underlying the shared etiology of these complex and co-morbid disorders. The association between PTSD and the HLA locus is of particular interest, as HLA alleles resulting from combinations of different polymorphisms are heavily implicated in autoimmune disorders (reviewed in [120]). For instance, HLA-A*02:01, which was identified as a protective allele for MS, [121] was less frequent in individuals with PTSD [116], suggesting a plausible shared genetic etiology between PTSD and MS. As HLA alleles have distinct antigen-binding properties, PTSD-associated HLA alleles might have enhanced antigen presentation capacity that impacts T-cell activation and inflammation.

Impact of inflammation on brain and behavior

Since PTSD is a brain disorder, there has been a great interest in understanding the mechanisms of neuroinflammation and communication between the brain and the immune system, starting with how peripheral inflammatory responses affect brain function. To date, several mechanisms have been proposed to explain how peripheral inflammatory signals impact the brain (reviewed by [122, 123], Fig. 3): (1) cytokine-specific saturable transporters actively transport some peripheral cytokines (e.g., IL-1α, IL-1β, IL-6, TNF-α), (2) peripheral cytokines pass through the leaky regions in the blood–brain barrier (BBB), (3) activated cytokine receptors on afferent nerve fibers (e.g., vagal nerve) transmit cytokine signals to relevant regions of the brain, and (4) microglial cells that are activated in response to peripheral cytokine signaling produce monocyte chemoattractant protein (MCP-1), which attracts activated peripheral cell types, including monocytes, macrophages and T cells to the brain. Activated microglia and astrocytes also produce cytokines to promote neuroinflammation [18].

Microglia are the resident innate immune-cell type in the brain that are responsible for trophic support, chemotaxis, synaptogenesis, and neurogenesis [124]. Peripheral inflammation activates microglia and prevents microglia from exerting their homeostatic functions [124]. Instead, activated microglia produce pro- and anti-inflammatory cytokines that modulate the stress response in the brain, as noted in animal models of stress and PTSD [125–127]. The inflammatory markers released from microglia induce astrocytes to produce cytokines, which, in turn, activate microglia in a positive feedback loop [128]. Multiple studies in animal models demonstrate microglial activation upon stress through increased pro-inflammatory cytokine response [125, 126, 129] or elevated microglial markers [129, 130]; yet studies of depressed patients and controls showed no correlations between microglial activation and peripheral pro-inflammatory cytokine levels [131, 132]. In contrast, a recent study reported lower microglial activation in post-mortem brain samples of PTSD patients [133]. The authors also demonstrated a negative correlation between microglial activation and plasma CRP levels [133]. The inconsistencies in stress-induced microglial activation might be due to differences between species, stress type, or analysis strategies to assess microglial activation (e.g., immunohistochemistry vs. neuroimaging).

Effect of inflammation on neurocircuitry relevant to fear and anxiety. PTSD is associated with alterations in the brain regions relevant to fear, anxiety, and threat detection, such as the amygdala, hippocampus, medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), and insula [17, 123]. Hence, evaluating the effect of inflammation on these regions is relevant to understanding the behavioral changes associated with PTSD.

The amygdala is the brain region responsible for fear and anxiety responses and is hyperresponsive in individuals with PTSD

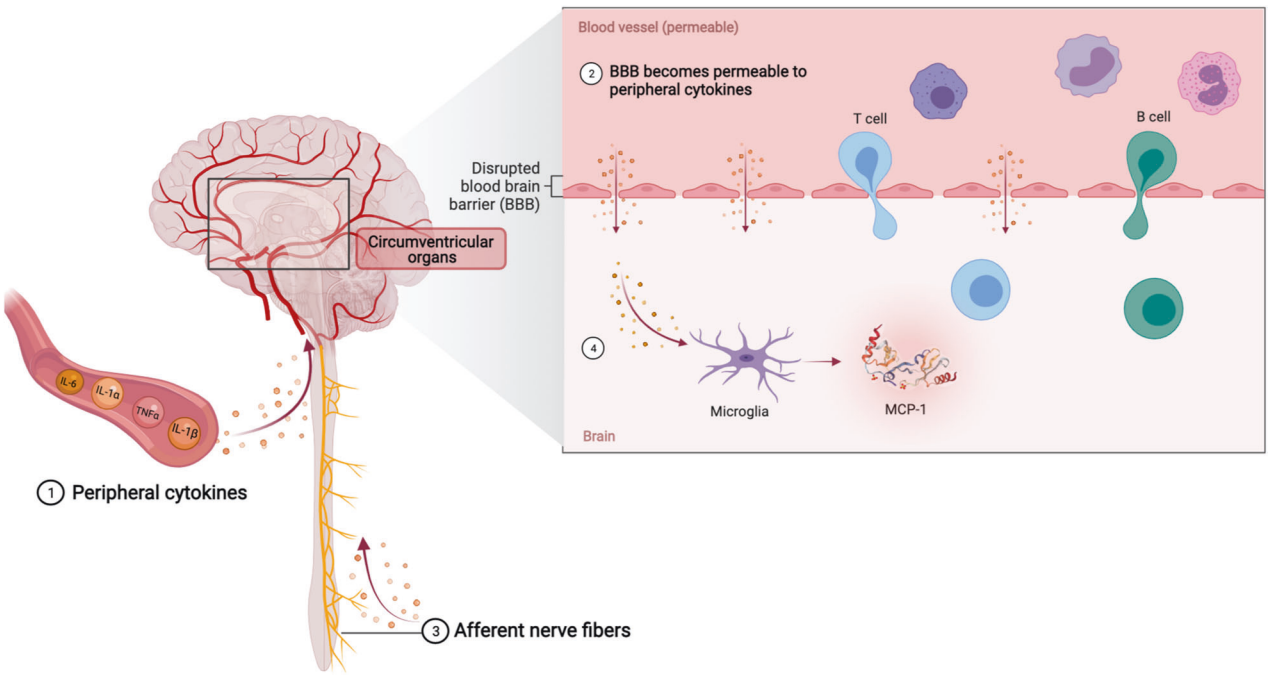

Fig. 3 Trafficking of peripheral inflammatory signals to brain. (1) Active transport of peripheral cytokines. (2) Passage of peripheral cytokines through leaky regions of blood–brain barrier (BBB). (3) Transmission of peripheral cytokine signals to the brain by activated cytokine receptors on afferent nerve fibers. (4) Trafficking of peripheral cell types (e.g., monocytes, macrophages, and T cells) in response to monocyte chemoattractant protein (MCP-1) release by activated microglia.

[123]. Multiple neuroimaging studies reported an association between heightened amygdala activation upon stress and increased proinflammatory cytokine levels. For instance, increased IL-6 and TNF-α concentrations following endotoxin administration in healthy individuals resulted in increased amygdala activity in response to socially threatening images [134]. Increased amygdala response to congruent and incongruent stimuli was also associated with increased IL-6 levels upon vaccination [135]. Notably, increased pro-inflammatory cytokine levels and amygdala activation are also associated with social disconnection, depressed mood, cognitive disturbance, and fatigue [135, 136].

The hippocampus is involved in fear and memory processing [137, 138]. Importantly, individuals with PTSD have smaller hippocampal volume [139]. In addition, reduced hippocampal volumes are associated with increased inflammation [140]. The effect of inflammation on the hippocampus was assessed in rodent models [141, 142], which suggests that microglial release of cytokines suppresses neurogenesis and stimulates apoptosis of neuronal progenitor cells [143]. Studies also showed the inhibitory effect of IL-1β on long-term potentiation in the hippocampus [144], as well as spatial and contextual memory processing [145]. Hence detrimental effects of inflammation on the hippocampus may be an underlying contributor to cognitive and emotional problems associated with PTSD.

Through their connections to the amygdala and hippocampus, the mPFC regions, including the rostral ACC, subgenual ACC (sgACC, Brodmann's Area 25), and the medial frontal cortex, play an important role in emotional regulation and fear extinction in PTSD [17]. Multiple studies investigated the effect of peripheral inflammatory markers on mPFC activity in response to stress or upon cytokine inducement [146, 147]. For instance, activation of the ventral mPFC, including the sgACC and the orbitofrontal cortex (OFC), in response to a grief-elicitation task associated with elevated IL-1β and sTNF-RII levels in grieving women [147]. Likewise, elevated IL-6 levels following typhoid vaccination led to increased sgACC activity, which is correlated with mood deterioration, and decreased connectivity of the sACC to the amygdala and mPFC [146]. In addition, increased plasma levels of CRP and IL-6 were correlated with reduced connectivity between the striatum and the ventral mPFC in depressed patients [148]. Finally, exposure to an acute laboratory-based social stressor led to an increase in IL-6 levels, which was associated with stronger functional connectivity between the right amygdala and the dorsomedial PFC [136]. Overall, this evidence links mPFC activation and inflammation in emotion processing following trauma or stress.

The dorsal ACC (dACC, Brodmann's Area 24) is involved in emotional and physical stress response through threatening social and physical pain stimuli detection and response [123]. Hyperactivation of the dACC is associated with PTSD [137, 149–152] and has been shown to mediate hyperarousal symptoms of PTSD [153]. Neuroimaging studies also demonstrated activation in response to inflammation. For example, IFN-α treatment of hepatitis C patients led to heightened dACC activation, which is associated with visual–spatial-attention errors [154]. Similarly, elevated IL-6 concentrations following typhoid injection are associated with increased dACC activation [135]. Finally, increased IL-6 levels upon endotoxin administration have been shown to be associated with augmented neural activity related to social pain in the dACC of women [155], consistent with sex-specific immune responses in PTSD. Taken together, these data suggest that dACC hyperactivation in response to inflammation may underlie some of the behavioral changes observed in PTSD.

The insula is involved in the emotional distress symptoms of PTSD and plays an important role in interoception (i.e., sense of body's physiological state) [123]. The insula is activated by peripheral inflammatory stimuli, which is expected considering the role of this region in perceiving the signals from the body [135]. Lower insula activation in response to changes in interceptive responses associated with PTSD in women exposed to intimate partner violence [156]. Likewise, women with PTSD related to intimate partner violence exhibited heightened activation of the insula and the amygdala, as well as weaker functional coupling among the insula, amygdala, and ACC during an emotional face-matching task [157]. Notably, the insula is also a target of the peripheral inflammatory response, such that IL-6 increases following stimulation of innate immunity led to heightened insula activity in response to congruent and incongruent stimuli [135]. Moreover, increased IL-6 and TNF-α levels following endotoxin administration are associated with increased glucose metabolism in the insula, as well as behavioral changes, including fatigue and lower social interest [158]. Similarly, increased IL-6 levels are associated with higher insula activity in response to social pain in women [159]. Hence, pro-inflammatory cytokines may lead to insula hyperactivity and alter the neural circuitry of the amygdala, mPFC, and ACC, thereby contributing to PTSD symptomatology relevant to fear and emotion processing.

Possible mechanism by which inflammation alters neurotransmitter function. Cytokines are critical to maintaining neural homeostasis, by participating in neural plasticity, including neurogenesis, synaptic pruning and remodeling, long-term potentiation, learning, and memory [145]. However, increased inflammatory signaling has detrimental effects on neurotransmitter systems related to the behavior and emotional characteristics of PTSD, such as serotonin, norepinephrine, dopamine, and glutamate [17, 122]. Inflammatory cytokines can alter neurotransmitter functions by influencing synthesis, reuptake, and release of neurotransmitters [122].

Serotonin is a monoamine neurotransmitter that is widely implicated in the etiology and pathophysiology of PTSD [160]. Serotonergic signaling may be influenced by the immune system in PTSD. Rats immunized with *Mycobacterium vaccae*, which exerts immunoregulatory properties through the production of anti-inflammatory cytokines, showed enhanced fear extinction and altered serotonergic gene expression in the brainstem [161, 162].

Cytokines can influence serotonin synthesis through the kynurenine pathway (Fig. 4). Pro-inflammatory cytokines increase the activity of indoleamine 2,3-dioxygenase (IDO), which converts tryptophan, the primary amino acid of serotonin, into kynurenine [122]. Hence, increased IDO activity in response to inflammation leads to serotonin depletion in the brain, as observed in animal models [163, 164]. In human studies, IFN-α therapy led to increased kynurenine and decreased tryptophan levels that were associated with symptoms of depression and anxiety, as well as cognitive problems, including memory disturbances and confusion [165, 166]. Another study of IFN-α therapy reported higher TNF-α and lower serotonin concentrations associated with somatic symptoms, including fatigue, loss of appetite, and irritability [167]. These findings suggest that pro-inflammatory cytokines may reduce serotonin concentration by acting on the kynurenine pathway, leading to cognitive and somatic symptoms relevant to PTSD.

Cytokines also downregulate serotonin synthesis by decreasing the activity of tetrahydrobiopterin (BH4), an enzyme co-factor of tryptophan hydroxylase and a rate-limiting enzyme in serotonin synthesis [122] (Fig. 4). Cytokines can increase the expression and function of serotonin transporters by stimulating the p38 mitogen-activated protein kinase MAPK pathway. Several in vitro studies have shown increased expression and activity of the serotonin transporter through activation of MAPK following TNF-α and IL-1β stimulation [168, 169]. Importantly, fluoxetine, a selective serotonin reuptake inhibitor (SSRI), suppressed the expression of IL-1β, IFN-γ, and TNF-α in the rat hippocampal dentate gyrus and downregulated MAPK signaling [170].

Dopamine plays an important role in PTSD symptom clusters, including re-experiencing symptoms and negative mood and

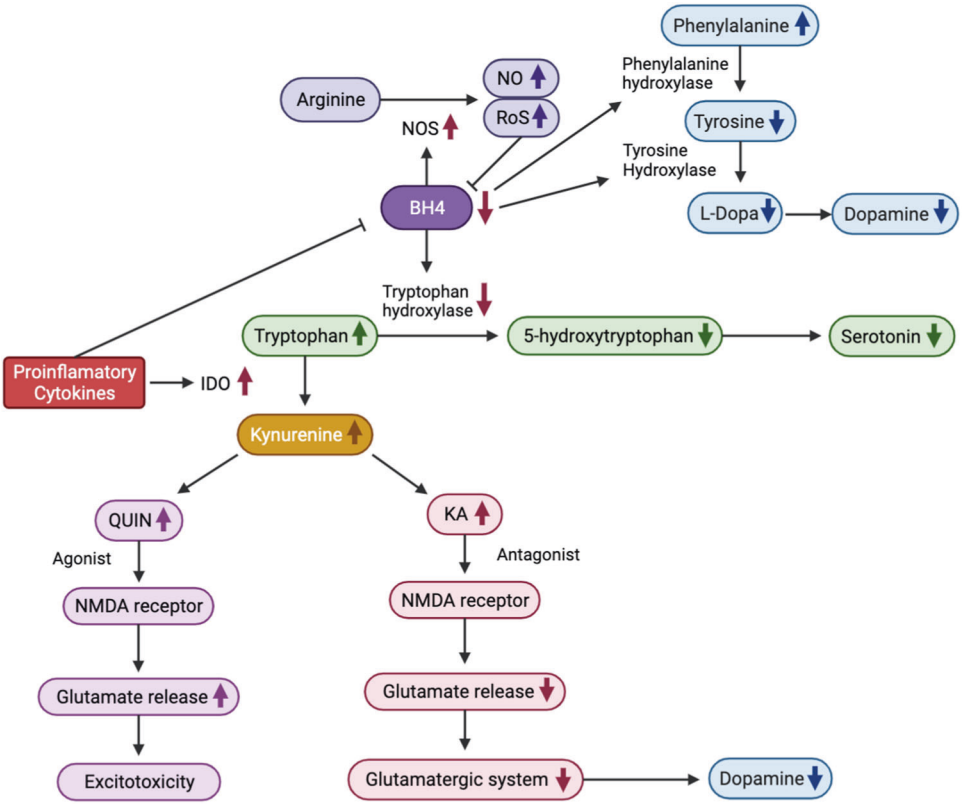

Fig. 4 Pro-inflammatory cytokine-induced changes in neurotransmitter systems. Mechanisms by which proinflammatory cytokines affect the synthesis of monoamine neurotransmitters (i.e., serotonin and dopamine) are illustrated. BH4 tetrahydrobiopterin, IDO indoleamine 2,3-dioxygenase, KA kynurenic acid, NMDA N-methyl-D-aspartate, NO nitric oxide, NOS nitric oxide synthases, QUIN quinolinic acid, ROS reactive oxygen species.

cognition [171]. Cytokines reduce dopamine levels by diminishing the activity of BH4, a co-factor of tyrosine hydroxylase and phenylalanine hydroxylase, the rate-limiting enzymes for dopamine synthesis [122] (Fig. 4). Inflammation can also induce nitric oxide synthase (NOS), the enzyme responsible for converting arginine into nitric oxide (NO), which uses BH4 as a co-factor [122]. Depletion of BH4 in turn leads to NOS uncoupling and production of ROS in the brain. Since BH4 is highly sensitive to oxidative stress, ROS promotes irreversible degradation of BH4, further limiting BH4 availability [123]. In fact, treatment of sympathetic neurons with IL-6 led to lower BH4 levels [172]. Similarly, IFN-α-treated patients exhibited decreased BH4 activity [173, 174], which was associated with lower dopamine levels in their cerebrospinal fluid (CSF) [173].

Glutamate is involved in motivation and motor functions. Inflammation leads to increased glutamate levels, contributing to symptoms of emotional numbness in PTSD [123]. The glutamatergic system partly regulates dopamine release, such that the effect of cytokines on the kynurenine pathway also impacts dopamine synthesis. As discussed above (Fig. 4), cytokines enhance IDO activity, leading to an increase in kynurenine production. Kynurenine can be broken down to kynurenic acid (KA) in astrocytes and quinolinic acid (QUIN) in microglia [123]. Notably, patients undergoing IFN-α treatment showed increased KA and QUIN concentrations in plasma and CSF [122, 123]. KA is an N-methyl-D-aspartate (NMDA) receptor antagonist that inhibits glutamate release and has downstream effects on dopamine [175, 176]. QUIN, an N-NMDA receptor agonist, can activate glutamate release from astrocytes, thereby contributing to excitotoxicity in the brain [123, 175]. Patients undergoing IFN-α treatment showed increased glutamate to creatinine levels in the dACC, which was associated with depressive symptoms [177]. Elevated CRP levels associated with symptoms of depression led to increased basal ganglia glutamate levels [178]. The increased glutamate release from astrocytes reduced brain-derived neurotrophic factor (BDNF), which is essential for neurogenesis and associated with disrupted contextual fear memory in PTSD [179, 180].

The effect of inflammation on other neurotransmitter systems related to PTSD, such as gamma-aminobutyric acid (GABA) and acetylcholine was less studied. PTSD patients were shown to have lower GABA levels in insula [181]. Notably, GABA decreased the production of inflammatory cytokines by suppressing the NF-kB and p38 MAPK pathways in rodents [182]. In addition, the expression and activity of acetylcholinesterase were induced by proinflammatory cytokines, which inhibited the release of acetylcholine from hippocampal neurons [183, 184]. Inhibition of acetylcholine release may, in turn, contribute to inflammation, as acetylcholine can downregulate peripheral cytokine production via the "cholinergic anti-inflammatory reflex." [122]. These data suggest that cytokines may reduce the release of GABA and acetylcholine, which both have anti-inflammatory effects, thereby leading to an inflammatory environment.

Taken together, studies collectively suggest that trauma may lead to HPA axis and SNS activation that increases proinflammatory cytokine production and subsequent neurotransmitter signaling that increases the risk of fear and anxiety symptomatology. Ultimately, this cascade may contribute to the risk of PTSD onset.

POTENTIAL ANTI-INFLAMMATORY THERAPEUTIC APPROACHES

Currently, only two selective SSRIs, paroxetine and sertraline, are approved by the FDA for the treatment of PTSD [18]. However, the response rates of these SSRIs are lower than 65% [185–188], indicating that a large proportion of PTSD patients do not respond

to SSRI treatment. Hence, given the inflammatory characteristic of PTSD, strategies that reduce inflammation and/or its effects on the brain may provide new avenues for the development of curative or preventative treatments to be used as an adjuvant to SSRIs or in combination with behavioral approaches.

Monoclonal antibodies to cytokines and their receptors, including TNF, IL-1, IL-6R, IL-12/23, and IL-17, are approved by the FDA for the treatment of autoimmune diseases and cancers. Given the increased IL-1β, IL-6, and TNF-α levels in PTSD, blocking these cytokines may be a straightforward treatment strategy. Although there are no reports of monoclonal antibody use for the treatment of PTSD, multiple studies reported that etanercept (TNF inhibitor), adalimumab (TNF inhibitor), and ustekinumab (IL-12/23 inhibitor) reduced symptoms of depression and anxiety in individuals with psoriasis [189–192]. However, Raison et al. [193] showed that infliximab (TNF inhibitor) was only effective in treatment-resistant depressed patients with higher baseline inflammation. Further, a clinical trial in patients with bipolar depression reported that baseline inflammation moderated the effect of infliximab on reducing anhedonia symptoms [194].

Non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase 2 (COX-2) inhibitors negatively regulate proinflammatory cytokine production, and thus reduce inflammation. The COX-2 inhibitor celecoxib was shown to improve depression symptoms in patients with the major depressive disorder [195–197], potentially decreasing IL-6 levels [197]. Although no clinical studies evaluated NSAIDs and COX-2 inhibitors for the treatment of PTSD, COX-2 inhibitors were shown to reduce anxiety in mice exposed to stress [198]. In addition, ibuprofen (NSAID) treatment reduced anxiety symptoms in a rat model of PTSD while decreasing expression of *TNF-α* and *IL-1β* and increasing *BDNF* expression in the hippocampus, suggesting that the therapeutic effect of ibuprofen on PTSD was mediated by decreased anti-inflammatory activity and increased BDNF levels in the brain [199].

NACHT domain- leucine-rich repeat- and pyrin domain-containing protein 3 (NLRP3) inflammasome inhibitors block inflammatory cytokine production. Beta-hydroxybutyrate (BHB), an endogenic NLRP3 inflammasome inhibitor, was shown to reduce depressive and anxiety behaviors in rodent models of depression and stress, potentially though decreasing hippocampal TNF-α concentrations [200, 201]. BHB was also effective in reducing anxiety behaviors in rodent models of PTSD and restoring serum TNF-α levels that were elevated in response to single prolonged stress [202].

Glucocorticoids suppress the inflammatory response following stress exposure by promoting the production of anti-inflammatory cytokines and by inhibiting the synthesis of proinflammatory cytokines [18] (Fig. 2). In addition, glucocorticoids participate in transporting protective T cells to the brain during acute trauma [203]. Given the low cortisol concentrations in individuals with PTSD [18], clinical studies examined the effectiveness of synthetic glucocorticoids for treatment of PTSD or preventing PTSD development following acute traumatic stress. Clinical trials showed that stand-alone glucocorticoid treatment or glucocorticoid augmentation combined with psychotherapy improved PTSD symptoms [204–207]. However, a recent clinical trial testing the effectiveness of augmentation of prolonged exposure (PE) with glucocorticoid reported that glucocorticoid augmentation did not significantly ameliorate PTSD symptoms [208]. Still, their exploratory analyses showed that glucocorticoid augmentation improved hyperarousal symptoms in veterans who experienced mild traumatic brain injury and reduced avoidance symptoms in veterans with increased baseline glucocorticoid sensitivity [208]. Moreover, studies investigating the preventative effect of glucocorticoids following exposure to trauma showed that glucocorticoid treatment following acute trauma significantly reduced stress symptoms [209, 210] and decreased the incidence of PTSD [211, 212]. Recently, a large meta-analysis of randomized controlled trials of glucocorticoid treatment reported that, although glucocorticoid treatment alleviated PTSD symptoms, preventative glucocorticoid administration following acute trauma was more effective [213].

Noradrenergic beta-receptor blockers (e.g., propranolol) inhibit norepinephrine signaling that promotes the production of pro-inflammatory cytokines [53, 54]. Studies of animal models showed that propranolol administration following stress exposure reduced pro-inflammatory cytokine levels and abrogated stress-induced changes in the immune-cell composition [214, 215]. Importantly, blockage of noradrenergic beta-receptors has been shown to inhibit reconsolidation of fear memory [216]. Indeed, clinical trials reported that propanol treatment with memory reactivation therapy reduced PTSD symptoms [217, 218]. Beta-blocker administration for the suspected acute coronary syndrome was also shown to reduce PTSD symptoms at 1-month follow-up [219]. However, research on the preventative effects of propranolol is contradictory. Initial studies reported that propanol treatment initiated within 20 hours of the trauma lowered PTSD incidence 2-months after trauma exposure and reduced physiological reactivity to trauma cues after 3 months [220, 221]. In contrast, Stein et al. [222] reported that propanol administration within 48 hours of trauma had no benefits on PTSD symptoms at 1, 4, and 8 months follow-up. Since propanol appears to act by reducing the effect of SNS arousal on trauma memory consolidation, early initiation of propanol treatment seems to be crucial.

Angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARBs) have been evaluated as possible anti-inflammatory therapeutic strategies, considering they are effective in the treatment of cardiometabolic disorders that are highly co-morbid with PTSD [17]. ACE-I and ARBs exert their anti-inflammatory activity in the brain by reducing the expression and secretion of pro-inflammatory cytokines and decreasing microglial activation [223]. Candesartan (ACE-I) was shown to ameliorate impairment of fear extinction in response to inflammatory activity induced by lipopolysaccharide administration [224]. A cross-sectional clinical observation study reported that individuals with PTSD on ACE-I or ARB treatment have lower hyperarousal symptoms compared to patients not taking these medications [225]. This study also showed that ACE-I or ARB use was associated with lower PTSD symptoms in trauma-exposed individuals [225]. However, a recent clinical trial reported that losartan (ARB) did not ameliorate PTSD symptoms [226].

Cannabinoids are also considered for PTSD treatment due to their anti-inflammatory effects. Endocannabinoid (eCB) signaling from macrophage and monocyte cells, including microglia in the brain, participates in inflammatory processes related to PTSD. While deficient eCB signaling promotes inflammation, augmented eCB signaling suppresses inflammation by reducing the secretion of pro-inflammatory cytokines, inhibiting NF-kB-mediated inflammatory gene transcription, decreasing microglial activation, and promoting the release of anti-inflammatory cytokines [227, 228]. Multiple studies showed that nabilone, a synthetic cannabinoid, reduced PTSD-related nightmares and insomnia and improved PTSD symptoms [229–231].

FTY720 (Fingolimod), approved for the treatment of MS, has drawn researchers' interest for PTSD treatment. Fingolimod is a synthetic analog of sphingosine that non-selectively binds to sphingosine-1-phosphate receptors (S1PRs). Decreased S1PRs activity in response to fingolimod prevents leukocyte migration from lymphocytes to the CNS, thereby suppressing the immune response [232]. Fingolimod was shown to promote neurogenesis, which correlated with improved contextual fear memory in mouse models [233, 234]. Notably, Fingolimod decreased despair and social anxiety-like behavior and reduced blood lymphocyte counts in a rat model of stress by reducing vascular remodeling in the brain [235].

Psychotherapy and behavioral interventions are effective treatments for PTSD and may reduce inflammation by reducing

perceived stress and increasing emotion regulation [236]. Currently, there are no studies evaluating the effect of gold-standard PTSD psychotherapies, including PE, on inflammation. Nevertheless, other forms of psychotherapy, including eye movement desensitization, were associated with alterations in TNF-α in soldiers with PTSD [237]. A recent clinical trial of reminder-focused positive psychiatry on attention-deficit hyperactive disorder and PTSD reported a decrease in CRP levels at 6 weeks follow-up [238]. Moreover, other behavioral interventions, including yoga and mindfulness, are associated with decreases in inflammation [236, 239].

CONCLUDING REMARKS

A growing body of evidence indicates an inflammatory environment in individuals with PTSD. However, many of the epidemiologic studies were limited by the fact that they only investigated specific peripheral cytokines and potentially missed key regulators in the process. Animal models deficient in key inflammatory genes may help identify mechanisms underlying the relationship between inflammation and anxiety-like or social behaviors relevant to PTSD. Similarly, longitudinal epidemiologic studies will be necessary to understand the directionality between PTSD and inflammation as well as immune-related conditions co-morbid with PTSD. For instance, since the gut-brain axis plays an important role in both PTSD and IBD, microbiome studies of PTSD coupled with metabolomics may advance our understanding of this comorbidity.

It is also unclear how peripheral immune alterations are indicative of neuroinflammation. Findings from imaging studies support a link between peripheral inflammation and both cognitive and emotional problems associated with PTSD through alterations in neurocircuitry relevant to fear and anxiety (reviewed in [123]). While this is encouraging, many of the reports of changes in neurotransmitter function in response to inflammation were from studies of depression, and additional research targeting PTSD is required to fully understand how the alterations in neurotransmitter function are relevant to PTSD symptoms.

Finally, the link between PTSD, inflammation, and the brain paves the way for potential anti-inflammatory treatment or preventative therapies. Although multiple anti-inflammatory treatment strategies showed promising results in pre-clinical settings and clinical trials, clinical studies with larger sample sizes and more diverse populations are warranted to fully understand the therapeutic mechanism of action, effectiveness, and possible side effects of these anti-inflammatory treatments.

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AUTHOR CONTRIBUTIONS

SK conceived the idea, designed the review structure, and wrote the manuscript. NCSO contributed to sections on HPA axis and ANS. JCF contributed to sections on neuroimaging and treatment, and reviewed and edited other sections of the paper. VM contributed to sections on immune biomarkers, other drivers of inflammation in PTSD, and treatment, and reviewed and edited other sections of the paper. AKS conceptualized the paper and its organization, comprehensively reviewed all sections, and provided critical edits and revisions on all drafts.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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