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Non-Invasive Vagal Nerve Stimulation Decreases Brain Activity During Trauma Scripts

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Abstract

**Background:** Traumatic stress can have lasting effects on neurobiology and result in psychiatric conditions such as posttraumatic stress disorder (PTSD). We hypothesize that non-invasive cervical vagal nerve stimulation (nVNS) may alleviate trauma symptoms by reducing stress sympathetic reactivity. This study examined how nVNS alters neural responses to personalized traumatic scripts. **Methods:** Nineteen participants who had experienced trauma but did not have the diagnosis of PTSD completed this double-blind sham-controlled study. In three sequential time blocks, personalized traumatic scripts were presented to participants immediately followed by either sham stimulation (n = 8; 0-14 V, 0.2 Hz, pulse width = 5s) or active nVNS (n = 11; 0-30 V, 25 Hz, pulse width = 40 ms). Brain activity during traumatic scripts was assessed using High Resolution Positron Emission Tomography (HR-PET) with radiolabeled water to measure brain blood flow. **Results:** Traumatic scripts resulted in significant activations within the bilateral medial and orbital prefrontal cortex, premotor cortex, anterior cingulate, thalamus, insula, hippocampus, right amygdala, and right putamen. Greater activation was observed during sham stimulation compared to nVNS within the bilateral prefrontal and orbitofrontal cortex, premotor cortex, temporal lobe, parahippocampal gyrus, insula, and left anterior cingulate. During the first exposure to the trauma scripts, greater activations were found in the motor cortices and ventral visual stream whereas prefrontal cortex and anterior cingulate activations were more predominant with later script presentations for those subjects receiving sham stimulation. **Conclusion:** nVNS decreases neural reactivity to an emotional stressor in limbic and other brain areas involved in stress, with changes over repeated exposures suggesting a shift from scene appraisal to cognitively processing the emotional event.
Introduction

Traumatic stress is known to have lasting effects on neurobiology [1, 2] and, in at risk individuals, can result in psychiatric conditions such as posttraumatic stress disorder (PTSD) [3]. During acute traumatic stress exposure, sympathetic nervous system (SNS) activity increases [1, 4, 5], resulting in a neuroendocrinological cascade with potentially deleterious neural consequences [6-8]. Heightened sympathetic response either during an acute traumatic event as well as chronic hyperarousal is a hallmark of PTSD [6], and therefore minimizing SNS reactivity to stress may be an effective avenue for treatment. One such approach, neuromodulation, has been previously effective in mitigating the acute stress response [5, 9], enhancing the anti-inflammatory response [10], providing neuroprotection [11], improving depression symptoms [12-15], aiding recovery following traumatic injury [16, 17], and enhancing cognitive recovery or fear extinction [18-20].

One neuromodulation technique with great promise to mitigate exacerbated SNS reactivity is the electrical stimulation of the vagus nerve (i.e., vagal nerve stimulation or VNS). VNS, administered using an implantable device delivering current to the vagus through a cuff electrode, has previously been observed to mitigate the stress response through lessened peripheral inflammatory and cardiovascular responses in addition to increasing activity in brain areas with functional connections to the vagus nerve [5, 21-26]. Lessened sympathetic response appears to results from stimulating the afferent fibers of the vagus nerve which activates the nucleus tractus solitarius (NTS) in the medulla oblongata, activating and the locus coeruleus in the pons [27], and eventually relays to emotional and stress-responsive areas in the brain such as the insula [28]. Activation of the locus coeruleus stimulates the brain noradrenergic system [29], which appears necessary for the therapeutic benefits of VNS [30]. Secondly, the decreased inflammatory response with VNS appears to result from NTS mediated activation of the dorsal motor nucleus and subsequent efferent pathway activation to the celiac ganglion, inhibiting cytokine production from the spleen [31]. In addition, VNS appears to
improve memory when applied during learning [32-34], potentially through increased sensory

gating or effects on neuroplasticity [35].

New non-invasive transcutaneous VNS (nVNS) devices have been developed that are
safe, cost-effective, and portable, potentially facilitating broader adoption (e.g., at-home) and
thus eliminates some of the previous limitations of implantable VNS such as high cost, surgery,
and introduction of a foreign object into the patient [36, 37]. Recent modeling [38] and
electrophysiological [39] studies have observed that cervical (neck) and auricular nVNS
stimulates afferent fibers of the vagus nerve similar to implantable VNS [40] along with the NTS
[41-44]. Despite these theoretical benefits and proof-of-concept studies, little evidence exists
regarding the mechanism of action [24, 37] of nVNS. Resting neuroimaging studies in healthy
adults receiving cymba conchae stimulation in the ear (auricular vagal branch) found increased
activations within the insula, paracentral lobule, somatosensory cortex, thalamus, contralateral
amygdala, and medulla with deactivations observed in the hippocampus and hypothalamus [42,
44]. A subsequent resting auricular nVNS study [43] observed similar findings, although nVNS
activated rather than deactivated the hypothalamus. Studies employing cervical nVNS have
observed activation within a cortical network of the insula, caudate, postcentral gyrus, anterior
cingulate, prefrontal cortex, cerebellum, basal ganglia, thalamus, and the primary sensory
cortex [41], while deactivating visual areas and the hippocampus.

However, little is known about how cervical nVNS alters neural activation patterns in
response to an emotional stressor such as listening to scripts of previous traumatic events.
Traumatic scripts are known to produce an acute stress response and increase SNS activity in
previously traumatized individuals with [6, 45, 46] and without [47, 48] PTSD. Exposure to
traumatic scripts activates neural areas repeatedly found to be altered with PTSD and related
psychiatric disorders [6, 45, 49]: the amygdala, prefrontal cortex, anterior cingulate,
hippocampus, and insula. Since, as described above, cervical nVNS is thought to decrease
activity of SNS neural circuits [41-43], this study was designed to examine how cervical nVNS
alters neural responses to traumatic scripts in trauma-exposed individuals without PTSD. We hypothesized that nVNS will decrease neural activity compared to a sham device in brain areas involved in the stress response, specifically within the amygdala, prefrontal cortex, anterior cingulate, hippocampus, and insula during exposure to personalized traumatic scripts.

**Materials and Methods**

**Participants**

This study was 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 (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. All participants provided written and verbal informed consent before enrolling in the study. Figure 1A presents the Consolidated Standards of Reporting Trials (CONSORT) for this study. For the 46 individuals screened for eligibility, six declined to participate and 13 did not meet the inclusion criteria. The 27 remaining participants were randomized using an online tool into either sham stimulation or nVNS groups. Eight participants were excluded from analysis due to equipment malfunction or withdrawals. The remaining 19 participants were included in the analysis with n = 11 receiving nVNS and n = 8 receiving sham stimulation (Table 1).

Participants were healthy adults between 18 – 65 y with a history of psychological trauma (as defined by the Diagnostic and Statistical Manual (DSM-5) [50]; details of trauma presented in Supplementary Table 1) but no current diagnosis of PTSD or major psychiatric disorder (e.g., schizophrenia, schizoaffective disorder, bipolar disorder, severe major depression, bulimia or anorexia). The traumatic memory details of one participant was lost due to technical error. Among the remaining eighteen traumatic memories, the most common
Traumas were related to molestation or sexual abuse (28%), illness, injury, or death of a family member (22%), personal injury including victim of assault and/or robbery (22%), car accidents or house fire (22%), illness, injury, or death of non-family individuals (17%), and emotional abuse from family or peers (17%). The Clinician Administered PTSD Scale (CAPS-5) was administered by a trained member of the research staff to confirm absence of current PTSD using previously validated scoring [51]. The Early Childhood Trauma Inventory Self-Report [52, 53] (64 total items) and a preliminary shortened version of the Adulthood Trauma Inventory [54] (16 total items) were administered to examine trauma load throughout the lifespan. The Structured Clinical Interview for DSM-IV (SCID) was also used to determine the presence of psychiatric disorders [55]. Three participants (17%) met criteria for a past history of major depression, one participant (6%) reported past PTSD, and one (6%) experienced past panic disorder. No subjects met criteria for current or past alcohol or substance use disorder. Exclusion criteria included: pregnancy, traumatic brain injury, meningitis, active implanted devices, evidence or history of serious medical or neurological illness, post-menopausal status, positive toxicology screen, and carotid atherosclerosis.

Study Design

Data collection occurred between May 2017 and October 2018 at Emory University School of Medicine. nVNS and Sham devices were delivered to the research staff pre-numbered by the manufacturer (ElectroCore, Basking Ridge, NJ) and they were distributed by an individual not involved in any other aspect of the protocol. Research staff conducting enrollment and data collection were masked to stimulus condition. Only the research staff conducting data analysis, but not involved in data collection, knew the stimulus assignment. During an initial screening, participants completed the psychiatric interview and gave a written history of traumatic experiences. A 60-second personalized traumatic script was developed and recorded by a member of the research team for each subject similar to previous
methods [45]. On the second visit, subjects participated in a high-resolution positron emission
tomography (HR-PET) scan session during which they listened to traumatic and neutral scripts
(Figure 1B). Neutral scripts were employed to induce neutral-to-positive affective responses in
participants. Scripts were delivered using headphones to participants who were supine in HR-
PET scanner. nVNS or sham was applied immediately following the personalized script to the
left side of the neck for two minutes by research personal. The length of stimulation was
automatically controlled by the device. Two HR-PET scans were also completed using
nVNS/sham stimulation with the subject at rest with eyes open, and not performing a task or
listening to a script.

Non-Invasive Vagus Nerve Stimulation

All stimulation (nVNS or sham) was administered with hand-held devices (GammaCore,
ElectroCore, Basking Ridge, NJ) using collar electrodes applied to the left side of the neck for
120 seconds. The appearance and operation of the nVNS and sham devices were identical.
The location for placement of collar electrodes on the carotid and application of conductive gel
to maintain adequate skin-electrode contact has been previously described in detail [39]. nVNS
devices produced an AC voltage signal consisting of five 5kHz sine bursts (1 ms of five sine
waves; pulse width = 40 ms) repeating at a rate of 25 Hz. The sham devices produce an AC
biphasic voltage signal consisting of 0.2 Hz square pulses (pulse width = 5 s) eliciting a mild
sensation similar to nVNS but without stimulating the vagus nerve. nVNS stimulation intensity
was adjustable and ranged from 0 to 5 au (arbitrary units) with a corresponding peak output
ranging from 0-30 V (~0-60 mA) for active nVNS and 0-14 V (~0-60 mA) for sham. Stimulation
periods started with gradually increasing intensity until participants instructed to stop; stimulation
intensity was therefore the maximum tolerable without pain. The maximum tolerable amplitude
level was 3 ± 0.9 au for nVNS and all participants with sham used an intensity of 5. In our larger
sample not restricted by HR-PET availability [5], the mean sham stimulation (12.8 V) was below
the 14 V maximum for the sham device, indicating the unanimous selection of 5 au in this cohort
was a sampling artifact. The research staff, who were blinded to stimulation type, confirmed all
participants perceived the stimulation.

Neuroimaging and Analysis

HR-PET imaging was completed using a High Resolution Research Tomograph (HR-PET) ([CTI, Knoxville, TN] [56]). During each scan, participants lay supine in the scanner and
were instructed to be as still as possible. Brain perfusion was assessed to quantify regional
voxel-wise activity responses (2 mm spatial resolution) to the traumatic and neutral scripts. Five
seconds into before each script presentation, 20 mCi of radio-labeled water (H$_2$[O$^{15}$]), produced
in an on-site cyclotron, was intravenously administrated before a two-minute HR-PET scan was
collected following to measure brain blood perfusion. Script presentation began concurrently
with HR-PET scan initiation. Radio-labeled water was injected five seconds before the
stimulation period for the nVNS-only scans (scan numbers five and six, Figure 1B).

HR-PET images were analyzed similarly to previous research [57] using statistical
parametrical mapping (SPM12; [www.fil.ion.ucl.ac.uk/spm]). First, using the fourteen individual
scans, a mean intensity image was generated. The individual scans were spatially normalized to
the mean intensity image, transformed into a common anatomical space (SPM PET Template),
smoothed using a three-dimensional Gaussian filter at 5-mm full width half maximum, and finally
normalized to whole brain activity. First level models were computed using the neutral script
scan and traumatic script conditions. This model was grand mean scaled, estimated, and
contrasts computed for activation (traumatic – neutral scripts) and deactivation (neutral –
traumatic scripts). For stimulation only scans (scan numbers five and six, Figure 1), a one
sample t-test was completed to examine areas of elevated brain activity during the scan.
Second level (between-participant) analyses were completed using contrast images from the
first level analysis and grouped by stimulation type. In addition, an analysis was completed
across three trauma script blocks (TSB1-3; Figure 1). Each TSB consisted of two trauma and
neutral scripts except for TSB1 where the first trauma script was omitted because it preceded
any stimulation.

Statistical Analysis

Normality of data was assessed using the Shapiro-Wilk test within R (v3.4.0; www.r-
project.org). Comparisons between nVNS and sham groups were completed using a two-
sample t-test or Mann-Whitney-Wilcoxon test for continuous and Fisher’s exact test for discrete
variables, respectively. For regional brain blood flow analyses (HR-PET), comparison contrasts
examining areas of greater activation and deactivation between nVNS and sham were encoded
according to previous guidelines [58] resulting in between-group t-statistic brain maps.
Significant voxel clusters for main brain analyses were identified using a threshold of p < 0.005
(uncorrected) and minimum voxel cluster size of eleven to minimize Type I and Type II errors in
neuroimaging research [59, 60]. For trauma script block analysis, p values were Bonferroni
corrected for multiple comparisons, yielding an alpha of 0.0025 (n = 2) across time points and
0.0017 (n = 3) between stimulation types at each trauma script block. In addition, the trauma
script block analyses were masked with the overall activity maps. 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 [61]. Cluster peaks were also identified using Brodmann Areas (BA) sourced
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

Demographics

There were no significant differences between participants receiving nVNS or sham on any demographic, descriptive, or clinical variable (Table 1). nVNS and sham also demonstrated similar PTSD checklist scores which were below the threshold for current PTSD (Table 1).

Brain Activity During the Trauma Script Periods

Figure 2 presents the overall patterns of activation and deactivation, collapsed across stimulation type, during exposure to the traumatic scripts. In general, trauma scripts elevated brain activity within the medial and orbital frontal lobe and limbic areas and decreased activity within the occipital lobe and cerebellum. Specifically, across the entire sample, trauma scripts resulted in significant (p < 0.005) activations within the bilateral medial and orbital prefrontal cortex, premotor cortex, anterior cingulate, thalamus, insula, hippocampus, pallidum, temporal lobe, parietal lobe, cerebellum, right amygdala, and right putamen. In addition, across the whole sample trauma scripts elicited significant deactivations (p < 0.005) within the bilateral cerebellum, parietal precuneus, occipital lobe, subgenual anterior cingulate, temporal lobe, superior and middle frontal gyrus, and left caudate.

The application of nVNS throughout the traumatic script presentation mitigated brain activity compared to sham (Figure 3 and Tables 2-3), specifically within the medial and orbital frontal lobe. Compared to nVNS, sham elicited significantly (p < 0.005) greater brain activations during trauma scripts were observed within the bilateral frontal lobe (BA 10, 11, 45, 47; prefrontal and orbitofrontal cortices, Broca’s area), premotor cortex (BA 6), temporal lobe (BA 20-22, 42; fusiform, inferior, middle, superior gyrus), parietal precuneus and angular gyrus (BA 7, 19, 39), parahippocampal gyrus, thalamus, insula, cerebelum, right occipital lobe (BA 18), and left anterior cingulate (BA 32). Listening to the trauma scripts elicited one cluster (voxel size = 20, peak Z = 3.38, Talairach Coordinates = -24, -38, -18) of significant deactivation during...
nVNS compared to sham stimulation within the left cerebellum. Compared to nVNS, sham elicited greater deactivations during the trauma scripts within the bilateral posterior cingulate (BA 29-31), bilateral occipital lobe (BA 17-19, 31; cuneus and lingual, inferior, middle gyri), bilateral prefrontal cortex (BA 5, 6, 9, 10, 46), bilateral temporal lobe (BA 21, 22, 39; middle, transverse, superior gyri), bilateral parietal lobe (BA 7, 40, 43; postcentral gyrus, precuneus, inferior lobule), right anterior cingulate (BA 10), and right cerebellum.

**Trauma Script Block Analysis**

The overall timeseries analysis found greater activations only during TSB3 compared to TSB2 within the frontoparietal areas and the putamen while greater deactivations successively appeared within occipital and temporal areas (Figure 4, Table 4). Compared to TSB1, listening to the trauma scripts elicited greater (p < 0.0025) deactivation within the left inferior temporal gyrus (BA 20) and occipital cuneus (BA 18) during TSB2. Compared to TSB2, trauma scripts elicited greater (p < 0.0025) activations within the right postcentral parietal lobe (BA 1), right putamen, right superior temporal gyrus (BA 22), and left precentral gyrus (BA) and greater deactivations within the right superior temporal gyrus (BA 22), left middle temporal gyrus (BA 21), and left occipital cuneus (BA 31) during TSB3. Supplementary Table 2 and Supplementary Figure 1 describe the trauma script block changes with sham. No changes were observed from TSB1 to TSB2 (p > 0.0025). Compared to TSB2, hearing the trauma scripts elicited greater activations within right postcentral gyrus (BA 1), right caudate, bilateral claustrum, lateral prefrontal cortex (BA 40, 44, 46), and left posterior cingulate (BA 31) and deactivations within the right cerebellum and left occipital precuneus (BA 31) with sham stimulation. nVNS did not exhibit any changes in activation (p > 0.0025) or deactivations between TSB1 and TSB2. Compared to TSB2, being exposed to the trauma scripts elicited significant (p < 0.0025) deactivation within the left parietal precuneus (BA 7), right posterior cingulate (BA 30), right hippocampus, and left middle temporal gyrus (BA 21) during TSB3.
During TSB1, sham elicited a general upregulation of stress-related brain areas compared to active nVNS (Figures 5-6, Tables 5-6) while differences in TSB2 and TSB3 were more focal to the frontal and parietal lobes, respectively. Listening to trauma scripts caused greater activation for subjects undergoing sham stimulation compared to nVNS within the bilateral cerebellum, bilateral temporal lobe (inferior and fusiform gyrus; BA 20), bilateral prefrontal cortex (BA 10, 11, 46), right secondary motor cortex (BA 6), right parietal precuneus (BA 7), right insula, right anterior cingulate (BA 24), and bilateral parahippocampal gyrus during TSB1 (Figure 5, Table 5). In addition, listening to the trauma scripts caused greater deactivation for subjects undergoing sham stimulation compared to nVNS within the right insula, left precentral gyrus (BA 9), bilateral inferior frontal gyrus (BA 45, 46), bilateral middle temporal lobe (BA 37), left anterior cingulate (BA 32), bilateral posterior cingulate (BA 30, 31), and right parahippocampal gyrus during TSB1. Listening to trauma scripts caused greater activation for subjects undergoing sham stimulation compared to nVNS within the right cerebellum, left inferior temporal cortex (BA 37), right medial temporal cortex (BA 21), Broca’s area (BA 45), left visual eye field (BA 8), right dorsomedial perfrontal cortex (BA 9), right paracentral lobule (BA 5), and left dorsal anterior cingulate (BA 24) during TSB2. Additionally, listening to trauma scripts caused greater deactivation for subjects undergoing sham stimulation compared to nVNS in the bilateral insula, right parahippocampal gyrus, right thalamus, left cerebellum, left postcentral gyrus and precuneus (BA 7), bilateral premotor cortex (BA 6), right prefrontal cortex (BA 9, 10, 44), and left superior temporal gyrus (BA 39) during TSB2. Listening to trauma scripts caused greater activation for subjects undergoing sham stimulation compared to nVNS within the left prefrontal cortex (BA 10, 46), right precentral gyrus (BA 44), right inferior, superior, and postcentral parietal lobe (BA 7, 19, 40), left somatosensory cortex (BA 5), right fusiform gyrus (BA 37), bilateral claustrum, and right caudate along with greater deactivations within the bilateral prefrontal cortex (BA 9, 10, 47), right anterior cingulate (BA 32), right superior temporal gyrus (BA 38), left occipital precuneus (BA 31), and right cerebellum during TSB3. Listening to
trauma scripts caused greater activation for subjects undergoing nVNS compared to sham stimulation in the right precentral gyrus (16 voxels, BA 4) during TSB2; no differences (p > 0.0017) were observed during TSB1 and TSB2.

Sham Stimulation vs. nVNS

Without trauma scripts, brain activity with sham remained elevated within the frontal lobe and limbic system (Figure 7, Table 7) while nVNS resulted in greater activity within the occipital lobe and inferior frontal lobe. Participants receiving sham stimulation had significantly (p < 0.005) greater brain activity compared to nVNS within the bilateral anterior cingulate (BA 32), bilateral rostromedial and dorsomedial prefrontal cortex (BA 10; BA 8, 9), bilateral lateral orbitofrontal frontal cortex (BA 11, 47), bilateral parietal precuneus (BA 7), left paracentral lobule (BA 5), right somatosensory cortex (BA 2), left inferior parietal lobe (BA 40), bilateral middle temporal lobe (BA 20, 21), bilateral cerebellum, left insula, and right occipital cuneus (BA 17). In contrast, participants receiving nVNS exhibited significantly (p < 0.005) greater brain activity compared to sham stimulation within the bilateral inferior frontal gyrus (BA 47), bilateral middle temporal gyrus (BA 21, 37), and left inferior occipital gyrus (BA 17; Figure 7, Table 7).

Discussion

This study showed that nVNS compared to sham stimulation decreased activation in multiple limbic and stress-related brain areas associated with exposure to personalized traumatic scripts in traumatized individuals without current PTSD. Our trauma script block analysis suggests the blunted neural responses occur immediately upon traumatic script exposure and persist throughout the duration of emotional stress. During resting conditions (no traumatic script), nVNS also lowered brain activity within stress-response areas such as the anterior cingulate and frontal lobe. Taken together, the results of this study provide evidence that nVNS applied to the cervical branch of the vagus nerve is effective in lowering neural
reactivity to personalized emotional stress and therefore may have utility as a treatment for many psychiatric disorders such as PTSD.

The primary finding of this study was that the application of nVNS following exposure to the personalize trauma scripts significantly reduced activity in select brain regions compared to a sham device. This is the first study to observe a dynamic change in neural responses during an emotional stressor. Listening to the personalized trauma scripts elicited similar brain activation patterns to what was previously reported in healthy traumatized individuals and patients with PTSD [46, 62-68]: the bilateral prefrontal cortex, premotor cortex, anterior cingulate, insula, hippocampus, cerebellum, right amygdala, and right putamen. We believe these activations represent increased prefrontal lobe activity with additional upregulation of associated cortical networks [69]. In this study nVNS reduced brain activation in a multitude of brain areas that are similar to those reported in brain activity studies with auricular and cervical nVNS and seem to specifically activate parasympathetic networks leading to reduced reactivity with stress [41, 43].

In addition to the large-scale effects, this study found evidence that emotion-specific neural networks were downregulated by nVNS. The largest area of lower activation while listening to the trauma scripts paired with nVNS compared to sham stimulation was located within the left rostromedial prefrontal cortex (BA 10) extending into the left dorsolateral prefrontal cortex (BA 46). Rostromedial prefrontal cortex activity increases when listening to trauma scripts and, in trauma-exposed individuals, appears to have functional connections to visual and autobiographical recall areas (hippocampus, temporal lobe, occipital lobe) [70]. The rostromedial prefrontal cortex also mediates changes in affect [71] and emotional appraisal (as opposed to cognitive appraisal) during autobiographical recall of personal traumatic events [72]. Dorsolateral prefrontal cortex (BA 46) is also active during traumatic autobiographical event recall [72] and is likely involved in appraisal of the emotional state [69], and is functionally connected to other areas (parietal cortex, middle temporal gyrus, motor areas, insula) with less
activation when listening to trauma scripts paired with nVNS. Furthermore, the less rostromedial
prefrontal cortex activity with concomitant pregenual anterior cingulate (BA 24) deactivation was
found during autobiographical recall followed by nVNS which suggests there is active inhibition
of the emotional and cognitive centers of the anterior cingulate (BA 24) [70, 73, 74]. The left
insula, which is associated with negative affect and is functionally connected to the prefrontal
cortex was less active with nVNS compared to sham stimulation [69, 71]. The insula and medial
prefrontal cortex comprise are the areas of output to peripheral cardiovascular and autonomic
responses and thus play an important role in regulation of the stress response [75]. Taken
together, these results suggest personalized trauma scripts elicited the canonical pattern of
emotionally responsive brain areas and the application of nVNS significantly diminished this
response.

The second major finding of the present study was that nVNS suppresses emotionally
responsive brain areas at different time points. This is important given the importance of
understanding the temporal specificity of nVNS in the context of neuromodulation treatment for
psychiatric disorders [76]. In animal models, invasive VNS produces a rapid excitation/inhibitory
response within the forebrain [77] along with increased brain-derived neurotrophic factor in the
hippocampus and norepinephrine concentration in prefrontal areas [78]. In humans, we have
added to this literature by: identifying sensitive physiological biomarkers indicating a lower
stress response with nVNS [5] along with classifying sham/active stimulation [79] and ii)
observing evidence of stimulation in humans, as measured at the periphery, within 16 and 21 s
with and without traumatic stress, respectively [80]. These combined efforts are supportive
efforts to eventually allow for closed-loop stimulation via monitoring of peripheral signals.

The time-series data contained within this manuscript align with the sustained changes
observed in with VNS such as neuroplasticity [81] and improved memory performance [32]. The
first traumatic script exposure with nVNS resulted in decreased brain activation in a pattern
appearing to indicate scene reconstruction as evidenced by upregulations of the ventral visual
stream and motor areas compared to sham stimulation. While the trauma scripts did not include a visual stimulus, the left fusiform gyrus and parahippocampal gyrus were less activated with nVNS and thus may represent the reconstruction of subjects and environment during the traumatic event [82, 83]. In addition, right posterior cingulate (BA 30) and other parietal areas were deactivated less with nVNS compared to sham stimulation which may indicate a decrease in the left-lateralized visual network which is related to visualizing autobiographical events [84, 85]. Greater activation in motor areas has been found previously employing the trauma script paradigm in individuals with PTSD and may indicate poor fear response extinction [66].

During the second period of listening to the trauma script, the largest cluster of decreased activity with nVNS compared to sham stimulation occurred within the left pregenual anterior cingulate (BA 24) and in the left inferior temporal gyrus (BA 37). The pregenual anterior cingulate processes viscerosensory signals and activity is related to feelings of unpleasantness [69, 86]. The pregenual anterior cingulate is also functionally connected to the rostromedial prefrontal cortex [69] which was also downregulated in the nVNS compared to sham stimulation group. We speculate that lower left inferior temporal gyrus activation following nVNS is independent from the rostromedial prefrontal cortex-pregenual anterior cingulate and, together with the third time point, indicate a prolonged period of lower emotional processing and feelings of unpleasantness compared to sham stimulation [87]. This is further supported by lower brain activity with nVNS during the third trauma stimulation block occurring within the left lateral (BA 46) and rostromedial (BA 10) prefrontal cortex as well as functionally connected areas of the left inferior parietal lobe which process emotional regulation and autobiographical events [69]. In summary, the findings suggest that traumatic script exposure paired with nVNS initially was associated with an lower activation of brain areas that were likely involved in visualization of the stressful scene and mentally acting out motor responses to threat, with a later shift to less emotional processing of the event.
Taken together, the two major findings of this study indicate the potential for nVNS use as a therapeutic tool. The current study showed that nVNS blocks the effects of reminders of traumatic stress on activation of the medial prefrontal cortex/anterior cingulate in traumatized individuals without PTSD. We have previously shown that traumatic reminders in non-PTSD trauma exposed individuals (e.g., combat veterans without PTSD) exposed to combat-related slides and sounds, results in an activation of medial prefrontal cortex/anterior cingulate [63], which we suggested was a normal brain response to emotionally arousing stimuli in which activation in this area led to suppression of amygdala function and hence excessive reactivity to stimuli that did not in fact represent a real threat, while PTSD patients in contrast failed to activate this area to the same degree and therefore had increased fear and physiological reactivity to what were in fact innocuous stimuli. The current findings suggest that nVNS blunts the responsivity of medial prefrontal cortex/anterior cingulate to traumatic reminders, suggesting that it modulates emotional responsivity of the brain, but how that translates to a treatment effect for PTSD, in which one in theory might hope for an enhancement of medial prefrontal cortex/anterior cingulate to promote inhibition of the amygdala [95-97], is not clear. While invasive VNS has repeatedly demonstrated the capacity to improve extinction in rodent models [18, 88, 89], the evidence supporting similar benefits with transcutaneous VNS in humans is less clear [90-93]. Understanding these discrepancies is an important tenant of future nVNS research, and therefore more studies are needed to understand the efficacy of nVNS in extinction paradigms including optimizing stimulation parameters. One reported limitation with transcutaneous VNS investigations has been the lack of a distinct physiological signal to demonstrate its efficacy [24]. Our recent work [5, 79, 80] has utilized novel cardiovascular biomarkers to identify candidate signals to demonstrate efficacy of nVNS. These findings, combined with the current results, could help provide stimulation feedback with prognostic value to improve efficacy of extinction therapy. nVNS has the potential to improve treatment strategies utilizing repeated exposure or other behaviors such as cognitive-behavioral therapy or
mindfulness-based stress reduction which have shown to be effective in improving traumatic stress-related disorders such as PTSD [94]. Further work utilizing these sensitive novel physiological markers coupled with a therapeutic intervention are needed to better understand the potential clinical utility as nVNS.

Consistent with other studies, we found nVNS increased brain activity within the left occipital lobe [41] and bilateral inferior frontal gyrus [15, 28, 41-43] without the presence of personalized trauma scripts. We did not find increases in brain activity with nVNS in other areas previously reported under resting conditions [15, 28, 41-44, 98] such as the insula and hippocampus, but the current study is not directly comparable given the previous exposure to trauma scripts. The effects of trauma scripts can be persistent [68] and previous exposure likely precluded the measurement of a true resting state similar to the previous studies. Therefore, we believe our findings without trauma scripts are a function of the trauma script paradigm.

While this study presents novel findings regarding the influence of nVNS as a mechanism for reducing neural responses to emotional stress, we recognize there are some limitations to our work. First, we do not have a direct measurement of vagus nerve activity. A number of studies utilizing the same cervical nVNS device have found neural evidence supporting stimulation of the vagus with similar stimulation-specific sensory evoked potentials as implantable devices [39, 99, 100] and changes in power spectrum consistent with inhibitory electrical activity [35, 101]. Regarding the sham device, participants were not asked what type of stimulation they received. These prompts should be asked in future investigations to understand potential biases in response patterns. Secondly, as alluded to above, the resting HR-PET scans without hearing neutral or personalized traumatic scripts were likely influenced by preceding trauma scripts and we cannot discern the isolate effects of nVNS alone similar to previous studies [41, 42]. Third, the cross-sectional nature of this study precludes evidence of longer-term effects of transcutaneous cervical nVNS. We also acknowledge that this work needs to be replicated with a larger sample of subjects. Future studies should also assess the
associations between neural responses during traumatic scripts, with and without nVNS, to changes in behavioral and physiological changes.

**Conclusion**

In conclusion, this study has provided novel findings about neural responses to nVNS while listening to personalized, emotionally stressful trauma scripts. We have demonstrated profound effects of nVNS in blunting the upregulation of neural responses elicited by trauma scripts. These effects were observed during all three exposures to trauma scripts in a pattern which may suggest that cervical nVNS decreases activity during both scene reconstruction and subsequent adverse emotional responses. We believe that future studies employing nVNS to enhance fear extinction as a treatment for PTSD or other emotional affective disorders might be fruitful.

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Figure Captions

**Figure 1:** Participant recruitment and study timeline. A: Consolidated Standards of Reporting Trials (CONSORT) diagram of the study. HR-PET = High Resolution Positron Emission Tomography; nVNS = non-invasive vagal nerve stimulation. B: Protocol timeline for the High-Resolution Positron Emission Tomography (HR-PET) scanning session. All trauma scripts were delivered via headphones with non-invasive cervical vagal nerve stimulation (nVNS) or sham stimulation occurring after script completion. Total scanning session length was five hours, with each trauma script / HR-PET scan lasting two minutes and stimulation lasting two minutes. The rest period lasted for 90 minutes and time between scans was approximately five minutes.

**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. Talairach x coordinates below slices indicate location with negative and positive coordinates located in the left and right hemisphere, respectively. Color bars indicate z-values of cluster.

**Figure 3:** Sagittal slices presenting significant (p < 0.005) areas with greater activation (red) and deactivation (blue) while listening to personalized traumatic scripts and application of a sham compared to non-invasive cervical vagal nerve stimulation (nVNS). Talairach x coordinates below slices indicate location with negative and positive coordinates located in the left and right hemisphere, respectively.
Figure 4: Coronal slices of significant (p < 0.0025) areas with greater activation (red) and deactivation (blue) during the third compared to second personalized trauma script block (TSB). Color gradient indicates magnitude of activation/deactivation. Talairach y coordinates below slices indicate location with negative and positive coordinates located in the left and right hemisphere, respectively.

Figure 5: Sagittal slices presenting significant (p < 0.0017) areas with greater activation during the trauma scripts in participants receiving sham compared to non-invasive cervical vagal nerve stimulation (nVNS) during the first (TSB1), second (TSB2), and third (TSB3) iterations of trauma exposure. Talairach x coordinates below slices indicate location with negative and positive coordinates located in the left and right hemisphere, respectively.

Figure 6: Sagittal slices presenting significant (p < 0.0017) areas with greater deactivation during the trauma scripts in participants receiving sham compared to non-invasive cervical vagal nerve stimulation (nVNS) during the first (TSB1), second (TSB2), and third (TSB3) iterations of trauma exposure. Talairach x coordinates below slices indicate location with negative and positive coordinates located in the left and right hemisphere, respectively.

Figure 7: Sagittal slices presenting significant (p < 0.005) areas with greater activation (red) during sham compared to non-invasive cervical vagal nerve stimulation (nVNS) and during nVNS compared to sham (blue) during resting conditions. Talairach x coordinates below slices indicate location with negative and positive coordinates located in the left and right hemisphere, respectively.
References


Bremner JD, Vermetten E, Mazure CM. Development and preliminary psychometric properties of an instrument for the measurement of childhood trauma: the Early Trauma Inventory. Depress Anxiety 2000;12(1):1-12.


Gläscher J, Gitelman D. Contrast weights in flexible factorial design with multiple groups of subjects. SPM@ JISCMAIL AC UK) Sml, editor 2008:1-12.


Table 1: Participant Demographics for the active non-invasive vagal nerve stimulation (nVNS) and sham groups

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<th>Sham (n = 8)</th>
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Table 2: Brain areas with significantly (p < 0.005) greater activations in participants receiving sham compared with non-invasive cervical vagal nerve (nVNS) stimulation during exposure to personalized trauma scripts as measured with positron emission tomography. Significant clusters are presented by size (number of voxels) and location (Brodmann area, cluster peak Talairach coordinates). Sub-cluster peaks are also identified.

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Table 3: Brain areas with significantly (p < 0.005) greater deactivation in participants receiving sham compared to non-invasive cervical vagal nerve stimulation (nVNS) while listening to personalized traumatic scripts as measured with positron emission tomography. Significant clusters are presented by size (number of voxels) and location (Brodmann area, cluster peak Talairach coordinates). Sub-cluster peaks are also identified.

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Table 4: Brain areas with significantly (p < 0.0025) greater activity during the first (TSB1),
second (TSB2), and third (TSB3) applications of either sham or active non-invasive cervical
vagal nerve stimulation during trauma as measured with positron emission tomography.
Significant clusters are presented by size (number of voxels) and location (Brodmann area,
cluster peak Talairach coordinates). Sub-cluster peaks are also identified. ND = no difference

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Table 5: Brain areas with significantly (p < 0.0017) greater activity during the first (TSB1), second (TSB2), and third (TSB3) applications of either sham compared to active non-invasive cervical vagal nerve stimulation during trauma scripts as measured with positron emission tomography. Significant clusters are presented by size (number of voxels) and location (Brodmann area, cluster peak Talairach coordinates). Sub-cluster peaks are also identified.

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Table 6: Brain areas with significantly (p < 0.0017) greater deactivations with sham compared to non-invasive cervical vagal nerve stimulation nVNS during the first (TSB1), second (TSB2), and third (TSB3) exposures to personalized trauma as measured with positron emission tomography. Significant clusters are presented by size (number of voxels) and location (Brodmann area, cluster peak Talairach coordinates). Sub-cluster peaks are also identified. ND = no difference

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Table 7: Brain areas with significantly (p < 0.005) greater activity during the application of sham non-invasive cervical vagal nerve stimulation (nVNS) and active nVNS during rest as measured with positron emission tomography. Significant clusters are presented by size (number of voxels) and location (Brodmann area, cluster peak Talairach coordinates). Sub-cluster peaks are also identified.

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TSB3 > TSB2
Activation

TSB2 > TSB3
Deactivation
Highlights

- Non-invasive vagal nerve stimulation decreased brain activity during emotional stress.
- Stimulation attenuated the increased neural activity in the fear memory circuitry.
- Patterns of decreased activation suggest improved appraisal and emotional processing.
Author Declaration

We wish to draw the attention of the Editor to the following fact which may be considered as potential conflict of interest to this work: Author J. Douglas Bremner reported having funding support from ElectroCore LLC. For other authors, there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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* Corresponding author
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