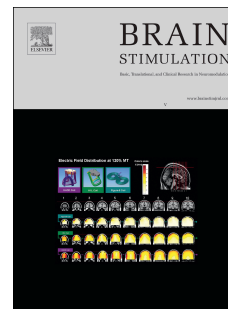# Journal Pre-proof

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

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Running Head: Non-Invasive Vagal Nerve Stimulation Lowers Activation

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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.

1 **Introduction**

2   Traumatic stress is known to have lasting effects on neurobiology [1, 2] and, in at risk

3 individuals, can result in psychiatric conditions such as posttraumatic stress disorder (PTSD) [3].

4 During acute traumatic stress exposure, sympathetic nervous system (SNS) activity increases

5 [1, 4, 5], resulting in a neuroendocrinological cascade with potentially deleterious neural

6 consequences [6-8]. Heightened sympathetic response either during an acute traumatic event

7 as well as chronic hyperarousal is a hallmark of PTSD [6], and therefore minimizing SNS

8 reactivity to stress may be an effective avenue for treatment. One such approach,

9 neuromodulation, has been previously effective in mitigating the acute stress response [5, 9],

10 enhancing the anti-inflammatory response [10], providing neuroprotection [11], improving

11 depression symptoms [12-15], aiding recovery following traumatic injury [16, 17], and enhancing

12 cognitive recovery or fear extinction [18-20].

13   One neuromodulation technique with great promise to mitigate exacerbated SNS

14 reactivity is the electrical stimulation of the vagus nerve (i.e., vagal nerve stimulation or VNS).

15 VNS, administered using an implantable device delivering current to the vagus through a cuff

16 electrode, has previously been observed to mitigate the stress response through lessened

17 peripheral inflammatory and cardiovascular responses in addition to increasing activity in brain

18 areas with functional connections to the vagus nerve [5, 21-26]. Lessened sympathetic

19 response appears to results from stimulating the afferent fibers of the vagus nerve which

20 activates the nucleus tractus solitarius (NTS) in the medulla oblongata, activating and the locus

21 coeruleus in the pons [27], and eventually relays to emotional and stress-responsive areas in

22 the brain such as the insula [28]. Activation of the locus coeruleus stimulates the brain

23 noradrenergic system [29], which appears necessary for the therapeutic benefits of VNS [30].

24 Secondly, the decreased inflammatory response with VNS appears to result from NTS mediated

25 activation of the dorsal motor nucleus and subsequent efferent pathway activation to the celiac

26 ganglion, inhibiting cytokine production from the spleen [31]. In addition, VNS appears to

27 improve memory when applied during learning [32-34], potentially through increased sensory

28 gating or effects on neuroplasticity [35].

29 New non-invasive transcutaneous VNS (nVNS) devices have been developed that are

30 safe, cost-effective, and portable, potentially facilitating broader adoption (e.g., at-home) and

31 thus eliminates some of the previous limitations of implantable VNS such as high cost, surgery,

32 and introduction of a foreign object into the patient [36, 37]. Recent modeling [38] and

33 electrophysiological [39] studies have observed that cervical (neck) and auricular nVNS

34 stimulates afferent fibers of the vagus nerve similar to implantable VNS [40] along with the NTS

35 [41-44]. Despite these theoretical benefits and proof-of-concept studies, little evidence exists

36 regarding the mechanism of action [24, 37] of nVNS. Resting neuroimaging studies in healthy

37 adults receiving cymba conchae stimulation in the ear (auricular vagal branch) found increased

38 activations within the insula, paracentral lobule, somatosensory cortex, thalamus, contralateral

39 amygdala, and medulla with deactivations observed in the hippocampus and hypothalamus [42,

40 44]. A subsequent resting auricular nVNS study [43] observed similar findings, although nVNS

41 activated rather than deactivated the hypothalamus. Studies employing cervical nVNS have

42 observed activation within a cortical network of the insula, caudate, postcentral gyrus, anterior

43 cingulate, prefrontal cortex, cerebellum, basal ganglia, thalamus, and the primary sensory

44 cortex [41], while deactivating visual areas and the hippocampus.

45 However, little is known about how cervical nVNS alters neural activation patterns in

46 response to an emotional stressor such as listening to scripts of previous traumatic events.

47 Traumatic scripts are known to produce an acute stress response and increase SNS activity in

48 previously traumatized individuals with [6, 45, 46] and without [47, 48] PTSD. Exposure to

49 traumatic scripts activates neural areas repeatedly found to be altered with PTSD and related

50 psychiatric disorders [6, 45, 49]: the amygdala, prefrontal cortex, anterior cingulate,

51 hippocampus, and insula. Since, as described above, cervical nVNS is thought to decrease

52 activity of SNS neural circuits [41-43], this study was designed to examine how cervical nVNS

4

53 alters neural responses to traumatic scripts in trauma-exposed individuals without PTSD. We

54 hypothesized that nVNS will decrease neural activity compared to a sham device in brain areas

55 involved in the stress response, specifically within the amygdala, prefrontal cortex, anterior

56 cingulate, hippocampus, and insula during exposure to personalized traumatic scripts.

57

58 **Materials and Methods**

59 <u>Participants</u>

60   This study was approved by the institutional review boards of Emory University

61 (#IRB00091171), Georgia Institute of Technology (#H17126), SPAWAR Systems Center

62 Pacific, and the Department of Navy Human Research Protection Program (ClinicalTrials.gov #

63 NCT02992899). It should be noted that, although the ClinicalTrials.gov study description

64 included the neuroimaging methods presented in this study, brain activity findings were not

65 listed as primary or secondary outcomes. All participants provided written and verbal informed

66 consent before enrolling in the study. Figure 1A presents the Consolidated Standards of

67 Reporting Trials (CONSORT) for this study. For the 46 individuals screened for eligibility, six

68 declined to participate and 13 did not meet the inclusion criteria. The 27 remaining participants

69 were randomized using an online tool into either sham stimulation or nVNS groups. Eight

70 participants were excluded from analysis due to equipment malfunction or withdrawals. The

71 remaining 19 participants were included in the analysis with n = 11 receiving nVNS and n = 8

72 receiving sham stimulation (Table 1).

73   Participants were healthy adults between 18 – 65 y with a history of psychological

74 trauma (as defined by the Diagnostic and Statistical Manual (DSM-5) [50]; details of trauma

75 presented in Supplementary Table 1) but no current diagnosis of PTSD or major psychiatric

76 disorder (e.g., schizophrenia, schizoaffective disorder, bipolar disorder, severe major

77 depression, bulimia or anorexia). The traumatic memory details of one participant was lost due

78 to technical error. Among the remaining eighteen traumatic memories, the most common

79   traumas were related to molestation or sexual abuse (28%), illness, injury, or death of a family

80   member (22%), personal injury including victim of assault and/or robbery (22%), car accidents

81   or house fire (22%), illness, injury, or death of non-family individuals (17%), and emotional

82   abuse from family or peers (17%). The Clinician Administered PTSD Scale (CAPS-5) was

83   administered by a trained member of the research staff to confirm absence of current PTSD

84   using previously validated scoring [51]. The Early Childhood Trauma Inventory Self-Report [52,

85   53] (64 total items) and a preliminary shortened version of the Adulthood Trauma Inventory [54]

86   (16 total items) were administered to examine trauma load throughout the lifespan. The

87   Structured Clinical Interview for DSM-IV (SCID) was also used to determine the presence of

88   psychiatric disorders [55]. Three participants (17%) met criteria for a past history of major

89   depression, one participant (6%) reported past PTSD, and one (6%) experienced past panic

90   disorder. No subjects met criteria for current or past alcohol or substance use disorder.

91   Exclusion criteria included: pregnancy, traumatic brain injury, meningitis, active implanted

92   devices, evidence or history of serious medical or neurological illness, post-menopausal status,

93   positive toxicology screen, and carotid atherosclerosis.

94

95   <u>Study Design</u>

96   Data collection occurred between May 2017 and October 2018 at Emory University

97   School of Medicine. nVNS and Sham devices were delivered to the research staff pre-

98   numbered by the manufacturer (*ElectroCore, Basking Ridge, NJ*) and they were distributed by

99   an individual not involved in any other aspect of the protocol. Research staff conducting

100   enrollment and data collection were masked to stimulus condition. Only the research staff

101   conducting data analysis, but not involved in data collection, knew the stimulus assignment.

102   During an initial screening, participants completed the psychiatric interview and gave a

103   written history of traumatic experiences. A 60-second personalized traumatic script was

104   developed and recorded by a member of the research team for each subject similar to previous

105   methods [45]. On the second visit, subjects participated in a high-resolution positron emission

106   tomography (HR-PET) scan session during which they listened to traumatic and neutral scripts

107   (Figure 1B). Neutral scripts were employed to induce neutral-to-positive affective responses in

108   participants. Scripts were delivered using headphones to participants who were supine in HR-

109   PET scanner. nVNS or sham was applied immediately following the personalized script to the

110   left side of the neck for two minutes by research personal. The length of stimulation was

111   automatically controlled by the device. Two HR-PET scans were also completed using

112   nVNS/sham stimulation with the subject at rest with eyes open, and not performing a task or

113   listening to a script.

114

115   <u>Non-Invasive Vagus Nerve Stimulation</u>

116   All stimulation (nVNS or sham) was administered with hand-held devices (*GammaCore,*

117   *ElectroCore, Basking Ridge, NJ*) using collar electrodes applied to the left side of the neck for

118   120 seconds. The appearance and operation of the nVNS and sham devices were identical.

119   The location for placement of collar electrodes on the carotid and application of conductive gel

120   to maintain adequate skin-electrode contact has been previously described in detail [39]. nVNS

121   devices produced an AC voltage signal consisting of five 5kHz sine bursts (1 ms of five sine

122   waves; pulse width = 40 ms) repeating at a rate of 25 Hz. The sham devices produce an AC

123   biphasic voltage signal consisting of 0.2 Hz square pulses (pulse width = 5 s) eliciting a mild

124   sensation similar to nVNS but without stimulating the vagus nerve. nVNS stimulation intensity

125   was adjustable and ranged from 0 to 5 au (arbitrary units) with a corresponding peak output

126   ranging from 0-30 V (~0-60 mA) for active nVNS and 0-14 V (~0-60 mA) for sham. Stimulation

127   periods started with gradually increasing intensity until participants instructed to stop; stimulation

128   intensity was therefore the maximum tolerable without pain. The maximum tolerable amplitude

129   level was 3 ± 0.9 au for nVNS and all participants with sham used an intensity of 5. In our larger

130   sample not restricted by HR-PET availability [5], the mean sham stimulation (12.8 V) was below

131 the 14 V maximum for the sham device, indicating the unanimous selection of 5 au in this cohort

132 was a sampling artifact. The research staff, who were blinded to stimulation type, confirmed all

133 participants perceived the stimulation.

134

135 <u>Neuroimaging and Analysis</u>

136   HR-PET imaging was completed using a High Resolution Research Tomograph (HR-

137 PET) (*CTI, Knoxville, TN*) [56]. During each scan, participants lay supine in the scanner and

138 were instructed to be as still as possible. Brain perfusion was assessed to quantify regional

139 voxel-wise activity responses (2 mm spatial resolution) to the traumatic and neutral scripts. Five

140 seconds ~~into~~ before each script presentation, 20 mCi of radio-labeled water ($H_2[O^{15}]$), produced

141 in an on-site cyclotron, was intravenously administered before a two-minute HR-PET scan was

142 collected following to measure brain blood perfusion. Script presentation began concurrently

143 with HR-PET scan initiation. Radio-labeled water was injected five seconds before the

144 stimulation period for the nVNS-only scans (scan numbers five and six, Figure 1B).

145   HR-PET images were analyzed similarly to previous research [57] using statistical

146 parametrical mapping (SPM12; *www.fil.ion.ucl.ac.uk/spm*). First, using the fourteen individual

147 scans, a mean intensity image was generated. The individual scans were spatially normalized to

148 the mean intensity image, transformed into a common anatomical space (SPM PET Template),

149 smoothed using a three-dimensional Gaussian filter at 5-mm full width half maximum, and lastly

150 normalized to whole brain activity. First level models were computed using the neutral script

151 scan and traumatic script conditions. This model was grand mean scaled, estimated, and

152 contrasts computed for activation (traumatic − neutral scripts) and deactivation (neutral −

153 traumatic scripts). For stimulation only scans (scan numbers five and six, Figure 1), a one

154 sample t-test was completed to examine areas of elevated brain activity during the scan.

155 Second level (between-participant) analyses were completed using contrast images from the

156 first level analysis and grouped by stimulation type. In addition, an analysis was completed

157 across three trauma script blocks (TSB1-3; Figure 1). Each TSB consisted of two trauma and

158 neutral scripts except for TSB1 where the first trauma script was omitted because it preceded

159 any stimulation.

160

161 <u>Statistical Analysis</u>

162   Normality of data was assessed using the Shapiro-Wilk test within R (*v3.4.0; www.r-*

163 *project.org*). Comparisons between nVNS and sham groups were completed using a two-

164 sample t-test or Mann-Whitney-Wilcoxon test for continuous and Fisher's exact test for discrete

165 variables, respectively. For regional brain blood flow analyses (HR-PET), comparison contrasts

166 examining areas of greater activation and deactivation between nVNS and sham were encoded

167 according to previous guidelines [58] resulting in between-group t-statistic brain maps.

168 Significant voxel clusters for main brain analyses were identified using a threshold of $p < 0.005$

169 (uncorrected) and minimum voxel cluster size of eleven to minimize Type I and Type II errors in

170 neuroimaging research [59, 60]. For trauma script block analysis, p values were Bonferroni

171 corrected for multiple comparisons, yielding an alpha of 0.0025 (n = 2) across time points and

172 0.0017 (n = 3) between stimulation types at each trauma script block. In addition, the trauma

173 script block analyses were masked with the overall activity maps. Significant cluster peaks were

174 identified using the distance from the anterior commissure with x, y, and z coordinates

175 transformed from Montreal Neurological Institute (MNI) space to those of the Talairach

176 stereotaxic atlas [61]. Cluster peaks were also identified using Brodmann Areas (BA) sourced

177 from the Talairach daemon (*www.talairach.org*). The *a priori* α level for non-brain imaging data

178 was chosen at 0.05. All data are presented as mean ± SD.

179

180 **Results**

181 Demographics

182   There were no significant differences between participants receiving nVNS or sham on

183 any demographic, descriptive, or clinical variable (Table 1). nVNS and sham also demonstrated

184 similar PTSD checklist scores which were below the threshold for current PTSD (Table 1).

185

186 Brain Activity During the Trauma Script Periods

187   Figure 2 presents the overall patterns of activation and deactivation, collapsed across

188 stimulation type, during exposure to the traumatic scripts. In general, trauma scripts elevated

189 brain activity within the medial and orbital frontal lobe and limbic areas and decreased activity

190 within the occipital lobe and cerebellum. Specifically, across the entire sample, trauma scripts

191 resulted in significant ($p < 0.005$) activations within the bilateral medial and orbital prefrontal

192 cortex, premotor cortex, anterior cingulate, thalamus, insula, hippocampus, pallidum, temporal

193 lobe, parietal lobe, cerebellum, right amygdala, and right putamen. In addition, across the whole

194 sample trauma scripts elicited significant deactivations ($p < 0.005$) within the bilateral

195 cerebellum, parietal precuneus, occipital lobe, subgenual anterior cingulate, temporal lobe,

196 superior and middle frontal gyrus, and left caudate.

197   The application of nVNS throughout the traumatic script presentation mitigated brain

198 activity compared to sham (Figure 3 and Tables 2-3), specifically within the medial and orbital

199 frontal lobe. Compared to nVNS, sham elicited significantly ($p < 0.005$) greater brain activations

200 during trauma scripts were observed within the bilateral frontal lobe (BA 10, 11, 45, 47;

201 prefrontal and orbitofrontal cortices, Broca's area), premotor cortex (BA 6), temporal lobe (BA

202 20-22, 42; fusiform, inferior, middle, superior gyrus), parietal precuneus and angular gyrus (BA

203 7, 19, 39), parahippocampal gyrus, thalamus, insula, cerebellum, right occipital lobe (BA 18),

204 and left anterior cingulate (BA 32). Listening to the trauma scripts elicited one cluster (voxel size

205 = 20, peak Z = 3.38, Talairach Coordinates = -24, -38, -18) of significant deactivation during

206   nVNS compared to sham stimulation within the left cerebellum. Compared to nVNS, sham

207   elicited greater deactivations during the trauma scripts within the bilateral posterior cingulate

208   (BA 29-31), bilateral occipital lobe (BA 17-19, 31; cuneus and lingual, inferior, middle gyri),

209   bilateral prefrontal cortex (BA 5, 6, 9, 10, 46), bilateral temporal lobe (BA 21, 22, 39; middle,

210   transverse, superior gyri), bilateral parietal lobe (BA 7, 40, 43; postcentral gyrus, precuneus,

211   inferior lobule), right anterior cingulate (BA 10), and right cerebellum.

212

213   <u>Trauma Script Block Analysis</u>

214   The overall timeseries analysis found greater activations only during TSB3 compared to

215   TSB2 within the frontoparietal areas and the putamen while greater deactivations successively

216   appeared within occipital and temporal areas (Figure 4, Table 4). Compared to TSB1, listening

217   to the trauma scripts elicited greater ($p < 0.0025$) deactivation within the left inferior temporal

218   gyrus (BA 20) and occipital cuneus (BA 18) during TSB2. Compared to TSB2, trauma scripts

219   elicited greater ($p < 0.0025$) activations within the right postcentral parietal lobe (BA 1), right

220   putamen, right superior temporal gyrus (BA 22), and left precentral gyrus (BA) and greater

221   deactivations within the right superior temporal gyrus (BA 22), left middle temporal gyrus (BA

222   21), and left occipital cuneus (BA 31) during TSB3. Supplementary Table 2 and Supplementary

223   Figure 1 describe the trauma script block changes with sham. No changes were observed from

224   TSB1 to TSB2 ($p > 0.0025$). Compared to TSB2, hearing the trauma scripts elicited greater

225   activations within right postcentral gyrus (BA 1), right caudate, bilateral claustrum, lateral

226   prefrontal cortex (BA 40, 44, 46), and left posterior cingulate (BA 31) and deactivations within

227   the right cerebellum and left occipital precuneus (BA 31) with sham stimulation. nVNS did not

228   exhibit any changes in activation ($p > 0.0025$) or deactivations between TSB1 and TSB2.

229   Compared to TSB2, being exposed to the trauma scripts elicited significant ($p < 0.0025$)

230   deactivation within the left parietal precuneus (BA 7), right posterior cingulate (BA 30), right

231   hippocampus, and left middle temporal gyrus (BA 21) during TSB3.

232   During TSB1, sham elicited a general upregulation of stress-related brain areas

233   compared to active nVNS (Figures 5-6, Tables 5-6) while differences in TSB2 and TSB3 were

234   more focal to the frontal and parietal lobes, respectively. Listening to trauma scripts caused

235   greater activation for subjects undergoing sham stimulation compared to nVNS within the

236   bilateral cerebellum, bilateral temporal lobe (inferior and fusiform gyrus; BA 20), bilateral

237   prefrontal cortex (BA 10, 11, 46), right secondary motor cortex (BA 6), right parietal precuneus

238   (BA 7), right insula, right anterior cingulate (BA 24), and bilateral parahippocampal gyrus during

239   TSB1 (Figure 5, Table 5). In addition, listening to the trauma scripts caused greater deactivation

240   for subjects undergoing sham stimulation compared to nVNS within the right insula, left

241   precentral gyrus (BA 9), bilateral inferior frontal gyrus (BA 45, 46), bilateral middle temporal lobe

242   (BA 37), left anterior cingulate (BA 32), bilateral posterior cingulate (BA 30, 31), and right

243   parahippocampal gyrus during TSB1. Listening to trauma scripts caused greater activation for

244   subjects undergoing sham stimulation compared to nVNS within the right cerebellum, left

245   inferior temporal cortex (BA 37), right medial temporal cortex (BA 21), Broca's area (BA 45), left

246   visual eye field (BA 8), right dorsomedial perfrontal cortex (BA 9), right paracentral lobule (BA

247   5), and left dorsal anterior cingulate (BA 24) during TSB2. Additionally, listening to trauma

248   scripts caused greater deactivation for subjects undergoing sham stimulation compared to

249   nVNS in the bilateral insula, right parahippocampal gyrus, right thalamus, left cerebellum, left

250   postcentral gyrus and precuneus (BA 7), bilateral premotor cortex (BA 6), right prefrontal cortex

251   (BA 9, 10, 44), and left superior temporal gyrus (BA 39) during TSB2. Listening to trauma scripts

252   caused greater activation for subjects undergoing sham stimulation compared to nVNS within

253   the left prefrontal cortex (BA 10, 46), right precentral gyus (BA 44), right inferior, superior, and

254   postcentral parietal lobe (BA 7, 19, 40), left somatosensory cortex (BA 5), right fusiform gyrus

255   (BA 37), bilateral claustrum, and right caudate along with greater deactivations within the

256   bilateral prefrontal cortex (BA 9, 10, 47), right anterior cingulate (BA 32), right superior temporal

257   gyrus (BA 38), left occipital precuneus (BA 31), and right cerebellum during TSB3. Listening to

258 trauma scripts caused greater activation for subjects undergoing nVNS compared to sham

259 stimulation in the right precentral gyrus (16 voxels, BA 4) during TSB2; no differences (p >

260 0.0017) were observed during TSB1 and TSB2.

261

262 <u>Sham Stimulation vs. nVNS</u>

263 Without trauma scripts, brain activity with sham remained elevated within the frontal lobe

264 and limbic system (Figure 7, Table 7) while nVNS resulted in greater activity within the occipital

265 lobe and inferior frontal lobe. Participants receiving sham stimulation had significantly (p <

266 0.005) greater brain activity compared to nVNS within the bilateral anterior cingulate (BA 32),

267 bilateral rostromedial and dorsomedial prefrontal cortex (BA 10; BA 8, 9), bilateral lateral

268 oribitofrontal frontal cortex (BA 11, 47), bilateral parietal precuneus (BA 7), left paracentral

269 lobule (BA 5), right somatosensory cortex (BA 2), left inferior parietal lobe (BA 40), bilateral

270 middle temporal lobe (BA 20, 21), bilateral cerebellum, left insula, and right occipital cuneus (BA

271 17). In contrast, participants receiving nVNS exhibited significantly (p < 0.005) greater brain

272 activity compared to sham stimulation within the bilateral inferior frontal gyrus (BA 47), bilateral

273 middle temporal gyrus (BA 21, 37), and left inferior occipital gyrus (BA 17; Figure 7, Table 7).

274

275 **Discussion**

276 This study showed that nVNS compared to sham stimulation decreased activation in

277 multiple limbic and stress-related brain areas associated with exposure to personalized

278 traumatic scripts in traumatized individuals without current PTSD. Our trauma script block

279 analysis suggests the blunted neural responses occur immediately upon traumatic script

280 exposure and persist throughout the duration of emotional stress. During resting conditions (no

281 traumatic script), nVNS also lowered brain activity within stress-response areas such as the

282 anterior cingulate and frontal lobe. Taken together, the results of this study provide evidence

283 that nVNS applied to the cervical branch of the vagus nerve is effective in lowering neural

284   reactivity to personalized emotional stress and therefore may have utility as a treatment for

285   many psychiatric disorders such as PTSD.

286   The primary finding of this study was that the application of nVNS following exposure to

287   the personalize trauma scripts significantly reduced activity in select brain regions compared to

288   a sham device. This is the first study to observe a dynamic change in neural responses during

289   an emotional stressor. Listening to the personalized trauma scripts elicited similar brain

290   activation patterns to what was previously reported in healthy traumatized individuals and

291   patients with PTSD [46, 62-68]: the bilateral prefrontal cortex, premotor cortex, anterior

292   cingulate, insula, hippocampus, cerebellum, right amygdala, and right putamen. We believe

293   these activations represent increased prefrontal lobe activity with additional upregulation of

294   associated cortical networks [69]. In this study nVNS reduced brain activation in a multitude of

295   brain areas that are similar to those reported in brain activity studies with auricular and cervical

296   nVNS and seem to specifically activate parasympathetic networks leading to reduced reactivity

297   with stress [41, 43].

298   In addition to the large-scale effects, this study found evidence that emotion-specific

299   neural networks were downregulated by nVNS. The largest area of lower activation while

300   listening to the trauma scripts paired with nVNS compared to sham stimulation was located

301   within the left rostromedial prefrontal cortex (BA 10) extending into the left dorsolateral

302   prefrontal cortex (BA 46). Rostromedial prefrontal cortex activity increases when listening to

303   trauma scripts and, in trauma-exposed individuals, appears to have functional connections to

304   visual and autobiographical recall areas (hippocampus, temporal lobe, occipital lobe) [70]. The

305   rostromedial prefrontal cortex also mediates changes in affect [71] and emotional appraisal (as

306   opposed to cognitive appraisal) during autobiographical recall of personal traumatic events [72].

307   Dorsolateral prefrontal cortex (BA 46) is also active during traumatic autobiographical event

308   recall [72] and is likely involved in appraisal of the emotional state [69], and is functionally

309   connected to other areas (parietal cortex, middle temporal gyrus, motor areas, insula) with less

14

310 activation when listening to trauma scripts paired with nVNS. Furthermore, the less rostromedial

311 prefrontal cortex activity with concomitant pregenual anterior cingulate (BA 24) deactivation was

312 found during autobiographical recall followed by nVNS which suggests there is active inhibition

313 of the emotional and cognitive centers of the anterior cingulate (BA 24) [70, 73, 74]. The left

314 insula, which is associated with negative affect and is functionally connected to the prefrontal

315 cortex was less active with nVNS compared to sham stimulation [69, 71]. The insula and medial

316 prefrontal cortex comprise are the areas of output to peripheral cardiovascular and autonomic

317 responses and thus play an important role in regulation of the stress response [75]. Taken

318 together, these results suggest personalized trauma scripts elicited the canonical pattern of

319 emotionally responsive brain areas and the application of nVNS significantly diminished this

320 response.

321   The second major finding of the present study was that nVNS suppresses emotionally

322 responsive brain areas at different time points. This is important given the importance of

323 understanding the temporal specificity of nVNS in the context of neuromodulation treatment for

324 psychiatric disorders [76]. In animal models, invasive VNS produces a rapid excitation/inhibitory

325 response within the forebrain [77] along with increased brain-derived neurotrophic factor in the

326 hippocampus and norepinephrine concentration in prefrontal areas [78]. In humans, we have

327 added to this literature by: identifying sensitive physiological biomarkers indicating a lower

328 stress response with nVNS [5] along with classifying sham/active stimulation [79] and ii)

329 observing evidence of stimulation in humans, as measured at the periphery, within 16 and 21 s

330 with and without traumatic stress, respectively [80]. These combined efforts are supportive

331 efforts to eventually allow for closed-loop stimulation via monitoring of peripheral signals.

332   The time-series data contained within this manuscript align with the sustained changes

333 observed in with VNS such as neuroplasticity [81] and improved memory performance [32]. The

334 first traumatic script exposure with nVNS resulted in decreased brain activation in a pattern

335 appearing to indicate scene reconstruction as evidenced by upregulations of the ventral visual

336 stream and motor areas compared to sham stimulation. While the trauma scripts did not include

337 a visual stimulus, the left fusiform gyrus and parahippocampal gyrus were less activated with

338 nVNS and thus may represent the reconstruction of subjects and environment during the

339 traumatic event [82, 83]. In addition, right posterior cingulate (BA 30) and other parietal areas

340 were deactivated less with nVNS compared to sham stimulation which may indicate a decrease

341 in the left-lateralized visual network which is related to visualizing autobiographical events [84,

342 85]. Greater activation in motor areas has been found previously employing the trauma script

343 paradigm in individuals with PTSD and may indicate poor fear response extinction [66].

344   During the second period of listening to the trauma script, the largest cluster of

345 decreased activity with nVNS compared to sham stimulation occurred within the left pregenual

346 anterior cingulate (BA 24) and in the left inferior temporal gyrus (BA 37). The pregenual anterior

347 cingulate processes viscerosensory signals and activity is related to feelings of unpleasantness

348 [69, 86]. The pregenual anterior cingulate is also functionally connected to the rostromedial

349 prefrontal cortex [69] which was also downregulated in the nVNS compared to sham stimulation

350 group. We speculate that lower left inferior temporal gyrus activation following nVNS is

351 independent from the rostromedial prefrontal cortex-pregenual anterior cingulate and, together

352 with the third time point, indicate a prolonged period of lower emotional processing and feelings

353 of unpleasantness compared to sham stimulation [87]. This is further supported by lower brain

354 activity with nVNS during the third trauma stimulation block occurring within the left lateral (BA

355 46) and rostromedial (BA 10) prefrontal cortex as well as functionally connected areas of the left

356 inferior parietal lobe which process emotional regulation and autobiographical events [69]. In

357 summary, the findings suggest that traumatic script exposure paired with nVNS initially was

358 associated with an lower activation of brain areas that were likely involved in visualization of the

359 stressful scene and mentally acting out motor responses to threat, with a later shift to less

360 emotional processing of the event.

361   Taken together, the two major findings of this study indicate the potential for nVNS use a

362   therapeutic tool. The current study showed that nVNS blocks the effects of reminders of

363   traumatic stress on activation of the medial prefrontal cortex/anterior cingulate in traumatized

364   individuals without PTSD. We have previously shown that traumatic reminders in non-PTSD

365   trauma exposed individuals (e.g., combat veterans without PTSD) exposed to combat-related

366   slides and sounds, results in an activation of medial prefrontal cortex/anterior cingulate [63],

367   which we suggested was a normal brain response to emotionally arousing stimuli in which

368   activation in this area led to suppression of amygdala function and hence excessive reactivity to

369   stimuli that did not in fact represent a real threat, while PTSD patients in contrast failed to

370   activate this area to the same degree and therefore had increased fear and physiological

371   reactivity to what were in fact innocuous stimuli. The current findings suggest that nVNS blunts

372   the responsivity of medial prefrontal cortex/anterior cingulate to traumatic reminders, suggesting

373   that it modulates emotional responsivity of the brain, but how that translates to a treatment

374   effect for PTSD, in which one in theory might hope for an enhancement of medial prefrontal

375   cortex/anterior cingulate to promote inhibition of the amygdala [95-97], is not clear. While

376   invasive VNS has repeatedly demonstrated the capacity to improve extinction in rodent models

377   [18, 88, 89], the evidence supporting similar benefits with transcutaneous VNS in humans is

378   less clear [90-93]. Understanding these discrepancies is an important tenant of future nVNS

379   research, and therefore more studies are needed to understand the efficacy of nVNS in

380   extinction paradigms including optimizing stimulation parameters. One reported limitation with

381   transcutaneous VNS investigations has been the lack of a distinct physiological signal to

382   demonstrate its efficacy [24]. Our recent work [5, 79, 80] has utilized novel cardiovascular

383   biomarkers to identify candidate signals to demonstrate efficacy of nVNS. These findings,

384   combined with the current results, could help provide stimulation feedback with prognostic value

385   to improve efficacy of extinction therapy. nVNS has the potential to improve treatment strategies

386   utilizing repeated exposure or other behaviors such as cognitive-behavioral therapy or

387 mindfulness-based stress reduction which have shown to be effective in improving traumatic

388 stress-related disorders such as PTSD [94]. Further work utilizing these sensitive novel

389 physiological markers coupled with a therapeutic intervention are needed to better understand

390 the potential clinical utility as nVNS.

391   Consistent with other studies, we found nVNS increased brain activity within the left

392 occipital lobe [41] and bilateral inferior frontal gyrus [15, 28, 41-43] without the presence of

393 personalized trauma scripts. We did not find increases in brain activity with nVNS in other areas

394 previously reported under resting conditions [15, 28, 41-44, 98] such as the insula and

395 hippocampus, but the current study is not directly comparable given the previous exposure to

396 trauma scripts. The effects of trauma scripts can be persistent [68] and previous exposure likely

397 precluded the measurement of a true resting state similar to the previous studies. Therefore, we

398 believe our findings without trauma scripts are a function of the trauma script paradigm.

399   While this study presents novel findings regarding the influence of nVNS as a

400 mechanism for reducing neural responses to emotional stress, we recognize there are some

401 limitations to our work. First, we do not have a direct measurement of vagus nerve activity. A

402 number of studies utilizing the same cervical nVNS device have found neural evidence

403 supporting stimulation of the vagus with similar stimulation-specific sensory evoked potentials

404 as implantable devices [39, 99, 100] and changes in power spectrum consistent with inhibitory

405 electrical activity [35, 101]. Regarding the sham device, participants were not asked what type

406 of stimulation they received. These prompts should be asked in future investigations to

407 understand potential biases in response patterns. Secondly, as alluded to above, the resting

408 HR-PET scans without hearing neutral or personalized traumatic scripts were likely influenced

409 by preceding trauma scripts and we cannot discern the isolate effects of nVNS alone similar to

410 previous studies [41, 42]. Third, the cross-sectional nature of this study precludes evidence of

411 longer-term effects of transcutaneous cervical nVNS. We also acknowledge that this work

412 needs to be replicated with a larger sample of subjects. Future studies should also assess the

413   associations between neural responses during traumatic scripts, with and without nVNS, to

414   changes in behavioral and physiological changes.

415   <u>Conclusion</u>

416   In conclusion, this study has provided novel findings about neural responses to nVNS

417   while listening to personalized, emotionally stressful trauma scripts. We have demonstrated

418   profound effects of nVNS in blunting the upregulation of neural responses elicited by trauma

419   scripts. These effects were observed during all three exposures to trauma scripts in a pattern

420   which may suggest that cervical nVNS decreases activity during both scene reconstruction and

421   subsequent adverse emotional responses. We believe that future studies employing nVNS to

422   enhance fear extinction as a treatment for PTSD or other emotional affective disorders might be

423   fruitful.

19

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.

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

Measure	nVNS (n = 11)	Sham (n = 8)	p value
Age (y)	28.7 ± 8.4	25.3 ± 3.4	0.90
Sex	4 F, 7 M	5 F, 3 M	0.37
BMI (kg·m^{-2}),	26.2 ± 5.7	28.5 ± 5.3	0.38
PTSD Checklist Score	29.3 ± 9.5	29.4 ± 10.3	0.65
Early Trauma Inventory	11.0 ± 7.1	8.8 ± 6.9	0.53
Adulthood Trauma Inventory	4.1 ± 1.6	3.5 ± 2.9	0.68
Race/Ethnicity			0.14
White/Caucasian	5 (45.5%)	1 (12.5%)	
Black/African American	3 (27.3%)	2 (25.0%)	
Asian	1 (9.1%)	4 (50.0%)	
Multiracial	-	1 (12.5%)	
Other	2 (18.2%)	-	
Marital Status			1.0
Never Married	8 (72.7%)	7 (87.5%)	
Married / Civil Partnership	2 (18.2%)	1 (14.3%)	
Divorced / Separated	1 (9.1%)	-	

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.

| Voxel Number | Brain Region | Brodmann Area | Talairach | | | Z Score |
			X	Y	Z	
594	L Frontal Lobe, Middle Gyrus	10	-44	51	6	4.98
	L Frontal Lobe, Inferior Gyrus	46	-44	43	8	4.62
	L Frontal Lobe, Superior Gyrus	10	-30	62	0	3.74
19	L Frontal Lobe, Inferior Gyrus	45	-48	26	17	4.40
37	R Temporal Lobe, Superior Gyrus	42	67	-21	13	4.35
36	R Frontal Lobe, Inferior Gyrus	45	51	24	16	4.35
339	R Parahippocampal Gyrus	36	22	-37	-5	4.16
	R Parahippocampal Gyrus	35	18	-32	-10	3.91
	R Cerebellum		26	-50	-21	3.79
177	L Claustrum		-38	-15	6	4.14
	L Temporal Lobe, Superior Gyrus	22	-46	-17	6	3.99
60	R Frontal Lobe, Middle Gyrus	8	26	20	41	4.07
	R Frontal Lobe, Middle Gyrus	8	32	27	41	3.22
121	R Claustrum		38	-4	5	4.02
	R Temporal Lobe, Superior Gyrus	22	46	-18	-1	3.70
	R Temporal Lobe, Superior Gyrus	22	51	-12	2	3.47
58	L Cerebellum		-20	-38	-18	4.02
75	L Anterior Cingulate Gyrus	32	-2	25	29	4.01
921	L Frontal Lobe, Inferior Gyrus	11	-14	34	-17	3.99
	L Frontal Lobe, Rectal Gyrus	11	-6	38	-22	3.98
	L Frontal Lobe, Rectal Gyrus	11	-6	22	-21	3.83
12	L Parahippocampal Gyrus		-34	-20	-11	3.86
97	L Insula	13	-42	8	1	3.69
	L Claustrum		-38	-2	2	3.56
	L Frontal Lobe, Precentral Gyrus	6	-48	-2	5	3.12
69	R Frontal Lobe, Inferior Gyrus	47	42	21	-9	3.62
23	L Anterior Cingulate	32	-8	41	8	3.62
38	R Parietal Lobe, Inferior Lobule	40	48	-35	37	3.61
103	R Temporal Lobe, Fusiform Gyrus	20	55	-42	-21	3.58
	R Temporal Lobe, Inferior Gyrus	20	61	-42	-16	3.04
47	R Frontal Lobe, Middle Gyrus	11	30	48	-12	3.57
254	R Temporal Lobe, Middle Gyrus	21	59	-43	-3	3.55
	R Temporal Lobe, Middle Gyrus	21	61	-54	3	3.53
	R Temporal Lobe, Middle Gyrus	22	55	-41	4	3.33
120	L Frontal Lobe, Precentral Gyrus	44	-59	10	13	3.53
	L Frontal Lobe, Precentral Gyrus	44	-55	10	6	3.18
	L Temporal Lobe, Superior Gyrus	22	-61	2	5	3.00
106	R Frontal Lobe, Medial Gyrus	6	14	-5	57	3.53
	R Frontal Lobe, Precentral Gyrus	6	28	-9	49	2.84
31	L Thalamus		-12	-16	-1	3.52
105	L Insula	13	-42	5	-7	3.49
	L Frontal Lobe, Inferior Gyrus	13	-32	13	-11	3.27
	L Temporal Lobe, Superior Gyrus	38	-40	13	-19	3.01
32	L Parietal Lobe, Postcentral Gyrus	3	-18	-32	54	3.44
154	L Temporal Lobe, Middle Gyrus	21	-65	-45	-3	3.39
	L Temporal Lobe, Inferior Gyrus	37	-57	-55	-7	3.19

	L Temporal Lobe, Middle Gyrus	21	-55	-47	-1	2.98
27	L Cerebellum		-24	-62	-31	3.39
	L Cerebellum		-24	-69	-27	2.89
12	L Cerebellum		-32	-46	-28	3.38
23	L Insula	13	-32	-25	10	3.38
	L Insula	13	-36	-21	14	3.14
89	L Frontal Lobe, Middle Gyrus	8	-26	18	49	3.37
	L Frontal Lobe, Superior Gyrus	8	-18	24	46	3.18
	L Frontal Lobe, Middle Gyrus	6	-22	6	47	3.01
138	R Frontal Lobe, Middle Gyrus	11	28	36	-15	3.37
	R Temporal Lobe, Superior Gyrus	38	36	20	-24	3.09
	R Frontal Lobe, Inferior Gyrus	47	30	20	-19	2.73
48	L Parietal Lobe, Precuneus	19	-28	-76	37	3.34
	L Parietal Lobe, Precuneus	19	-34	-66	37	2.98
34	R Cerebellum		42	-50	-33	3.32
	R Cerebellum		40	-52	-26	2.75
30	L Parahippocampal Gyrus	30	-16	-35	-5	3.32
	L Parahippocampal Gyrus	27	-18	-37	2	2.76
75	R Temporal Lobe, Superior Gyrus	22	55	-4	-7	3.31
	R Temporal Lobe, Superior Gyrus	22	61	-2	3	3.07
50	R Temporal Lobe, Middle Gyrus	21	55	-29	-7	3.30
54	L Parietal Lobe, Angular Gyrus	39	-40	-58	36	3.29
171	R Cerebellum		30	-69	-22	3.29
	R Cerebellum		32	-77	-23	3.27
	R Cerebellum		20	-79	-16	2.88
48	R Parahippocampal Gyrus	36	42	-22	-17	3.26
32	R Frontal Lobe, Middle Gyrus	6	28	4	47	3.26
36	L Cerebellum		-20	-87	-26	3.25
28	L Frontal Lobe, Inferior Gyrus	47	-42	25	-13	3.25
11	L Parahippocampal Gyrus	37	-34	-41	-8	3.18
16	R Frontal Lobe, Inferior Gyrus	9	50	11	28	3.17
15	R Cerebellum		0	-38	-17	3.15
53	R Parietal Lobe, Angular Gyrus	39	40	-62	33	3.14
11	L Cerebellum		-24	-34	-27	3.13
14	L Temporal Lobe, Superior Gyrus		-69	-23	-2	3.13
17	L Parietal Lobe, Precuneus	7	-24	-50	48	3.09
	L Parietal Lobe, Precuneus	7	-30	-44	51	2.67
13	R Cerebellum		12	-79	-30	3.09
14	L Cerebellum		-18	-69	-22	3.08
24	R Temporal Lobe, Transverse Gyrus	41	53	-23	10	3.08
	R Temporal Lobe, Superior Gyrus	41	50	-31	8	2.97
39	R Parietal Lobe, Superior Lobule	7	28	-64	42	3.05
37	R Thalamus		20	-21	-1	3.04
47	L Frontal Lobe, Middle Gyrus	11	-30	48	-11	3.03
14	R Cerebellum		36	-30	-27	3.02
13	R Temporal Lobe, Inferior Gyrus	20	63	-26	-19	3.00
12	R Occipital Lobe, Middle Gyrus	18	44	-85	1	2.99
14	L Temporal Lobe, Inferior Gyrus	20	-48	-26	-10	2.97
15	L Frontal Lobe, Medial Gyrus	11	-2	50	-11	2.97
49	L Frontal Lobe, Precentral Gyrus	6	-22	-17	56	2.96
	L Frontal Lobe, Sub-Gyral	6	-24	-5	52	2.92
	L Frontal Lobe, Middle Gyrus	6	-16	-7	59	2.74
33	L Cerebellum		-38	-75	-23	2.96
24	R Cerebellum		22	-88	-21	2.96
24	L Temporal Lobe, Superior Gyrus	38	-50	13	-9	2.95
	L Temporal Lobe, Superior Gyrus	38	-53	7	-5	2.94

14	R Parietal Lobe, Supramarginal Gyrus	40	40	-49	34	2.93
18	R Temporal Lobe, Middle Gyrus	39	48	-75	22	2.92
24	R Parietal Lobe, Precuneus	7	20	-50	56	2.90
14	R Parietal Lobe, Inferior Lobule	40	34	-54	45	2.89
44	R Cerebellum		48	-69	-27	2.88
15	R Parietal Lobe, Postcentral Gyrus	3	40	-25	53	2.87
12	R Parietal Lobe, Postcentral Gyrus	3	28	-36	50	2.86
15	R Parahippocampal Gyrus	36	34	-35	-10	2.85
	R Temporal Lobe, Fusiform Gyrus	20	36	-42	-15	2.69
16	R Parietal Lobe, Supramarginal Gyrus	40	55	-41	36	2.84
21	L Cerebellum		-28	-83	-19	2.79
13	L Temporal Lobe, Inferior Gyrus	20	-40	-9	-33	2.79

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.

Voxel Number	Brain Region	Brodmann Area	Talairach			Z Score
			X	Y	Z	
136	R Posterior Cingulate	29	16	-48	11	4.64
	R Occipital Lobe, Precuneus		26	-51	21	2.68
23	R Temporal Lobe, Middle Gyrus	21	63	3	-9	4.49
129	R Temporal Lobe, Transverse Gyrus	42	61	-13	16	4.26
	R Parietal Lobe, Postcentral Gyrus	43	67	-7	15	4.12
	R Temporal Lobe, Superior Gyrus	22	69	-3	9	3.44
99	L Occipital Lobe, Lingual Gyrus	17	-20	-80	2	3.90
	L Occipital Lobe, Cuneus	17	-16	-89	6	3.76
	L Occipital Lobe, Cuneus	17	-12	-79	7	3.40
212	R Occipital Lobe, Inferior Gyrus	19	38	-68	-2	3.69
	R Occipital Lobe, Inferior Gyrus	19	44	-74	-5	3.58
	R Occipital Lobe, Middle Gyrus	37	40	-64	6	3.42
41	R Anterior Cingulate	10	16	47	-1	3.62
51	L Occipital Lobe, Middle Gyrus	19	-34	-83	14	3.56
	L Occipital Lobe, Middle Gyrus		-32	-71	15	3.36
38	L Frontal Lobe, Medial Gyrus	10	-20	49	13	3.55
44	L Posterior Cingulate Gyrus	31	-14	-27	42	3.54
	L Posterior Cingulate Gyrus	31	-8	-23	45	2.98
27	L Frontal Lobe, Precentral Gyrus	6	-32	2	35	3.39
46	L Temporal Lobe, Middle Gyrus		-44	-46	9	3.36
	L Temporal Lobe, Superior Gyrus	22	-40	-52	13	3.26
	L Temporal Lobe, Superior Gyrus	22	-55	-44	11	3.22
12	R Frontal Lobe, Paracentral Lobule	5	4	-32	54	3.35
81	R Occipital Lobe, Middle Gyrus	18	26	-85	2	3.35
	R Occipital Lobe, Cuneus	30	22	-70	8	3.14
12	L Frontal Lobe, Paracentral Lobule	5	-4	-40	57	3.31
26	L Frontal Lobe, Precentral Gyrus	6	-44	-14	26	3.31
28	L Frontal Lobe, Middle Frontal Gyrus	46	-40	14	18	3.29
15	R Anterior Cingulate	24	10	35	3	3.28
14	R Cerebellum		30	-36	-22	3.27
19	L Frontal Lobe, Medial Gyrus	9	-2	51	14	3.26
45	R Frontal Lobe, Precentral Gyrus	9	42	4	32	3.25
16	R Posterior Cingulate	30	8	-69	13	3.22
17	L Frontal Lobe, Precentral Gyrus	6	-53	-6	30	3.21
45	R Occipital Lobe, Cuneus	18	24	-77	17	3.21
	R Parietal Lobe, Precuneus	31	18	-73	22	3.14
16	R Anterior Cingulate	32	12	25	36	3.21
13	L Parietal Lobe, Inferior Lobule	40	-46	-37	29	3.16
24	L Occipital Lobe, Precuneus	31	-20	-63	25	3.16
	L Temporal Lobe, Middle Gyrus	39	-28	-63	27	2.78
12	L Parietal Lobe, Precuneus	7	-14	-58	36	3.14
11	L Posterior Cingulate	30	-26	-64	11	3.03
20	R Temporal Lobe, Middle Gyrus	21	59	10	-27	3.00
	R Temporal Lobe, Superior Gyrus	38	59	11	-19	2.71
11	L Occipital Lobe, Cuneus	18	-20	-79	17	2.98
12	R Frontal Lobe, Superior Frontal Gyrus	9	6	48	33	2.90

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

Voxel Number	Brain Region	Brodmann Area	Talairach			Z Score
			X	Y	Z	
Activation						
TSB2 > TSB1: ND						
TSB3 > TSB2						
11	R Parietal Lobe, Postcentral Gyrus	1	65	-21	38	4.05
12	L Frontal Lobe, Precentral Gyrus	6	-57	6	11	3.86
18	R Putamen		20	4	1	3.63
11	R Temporal Lobe, Superior Gyrus	22	59	4	1	3.25
Deactivation						
TSB2 > TSB1						
28	L Temporal Lobe, Inferior Gyrus	20	-51	-51	-11	3.75
11	L Occipital Lobe, Cuneus	18	0	-85	11	3.31
TSB3 > TSB2						
11	L Occipital Lobe, Precuneus	31	-4	-72	27	3.60
12	R Temporal Lobe, Superior Gyrus	22	53	-48	16	3.52
25	L Temporal Lobe, Middle Gyrus	21	-63	-12	-9	3.47
16	L Occipital Lobe, Precuneus	31	0	-65	20	3.43

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.

Voxel Number	Brain Region	Brodmann Area	Talairach X	Y	Z	Z Score
TSB1						
21	L Cerebellum		-46	-49	-40	4.37
15	L Cerebellum		-34	-46	-30	4.21
23	L Cerebellum		-26	-36	-17	4.17
23	R Parahippocampal Gyrus		28	-33	-7	3.81
13	L Occipital Lobe, Fusiform Gyrus	37	-36	-41	-8	3.80
58	L Parahippocampal Gyrus		-36	-16	-16	3.77
	L Temporal Lobe, Fusiform Gyrus	20	-44	-19	-23	3.07
	L Parahippocampal Gyrus		-28	-20	-16	3.06
21	R Cerebellum		14	-83	-35	3.76
36	R Temporal Lobe, Inferior Gyrus	20	67	-28	-20	3.58
13	R Limbic Lobe, Uncus	20	34	-9	-30	3.58
24	R Frontal Lobe, Middle Gyrus	10	32	47	-2	3.57
50	R Frontal Lobe, Medial Gyrus	6	12	8	52	3.54
	R Anterior Cingulate	24	4	0	45	3.38
11	R Parietal Lobe, Precuneus	7	8	-35	45	3.42
17	R Cerebellum		20	-40	-22	3.42
32	L Frontal Lobe, Superior Gyrus	10	-20	56	2	3.41
11	R Insula	13	42	-6	5	3.40
13	L Cerebellum		-28	-66	-29	3.27
17	L Frontal Lobe, Rectal Gyrus	11	-6	15	-19	3.23
12	L Frontal Lobe, Inferior Gyrus	46	-44	43	6	3.21
12	L Frontal Lobe, Middle Gyrus	11	-26	36	-12	3.04
	TSB2					
41	L Temporal Lobe, Inferior Gyrus	37	-57	-61	-7	5.12
11	L Frontal Lobe, Inferior Gyrus	45	-46	22	14	4.40
22	R Cerebellum		26	-50	-24	4.12
23	R Temporal Lobe, Sub-Gyral	21	46	-6	-13	4.06
49	L Limbic Lobe, Anterior Cingulate	24	-10	20	21	3.78
20	R Frontal Lobe, Middle Gyrus	9	28	28	25	3.66
	R Frontal Lobe, Middle Gyrus	9	36	29	30	3.52
17	L Frontal Lobe, Middle Gyrus	8	-26	25	34	3.59
14	R Cerebellum		12	-87	-31	3.58
14	R Temporal Lobe, Medial Gyrus	21	51	-43	0	3.45
	TSB3					
17	R Caudate		14	20	9	5.54
118	R Parietal Lobe, Postcentral Gyrus	1	65	-21	38	5.51
	R Parietal Lobe, Inferior Lobule	40	67	-29	33	4.32
	R Parietal Lobe, Inferior Lobule	40	53	-37	45	4.25
18	L Frontal Lobe, Paracentral Lobule	5	-12	-31	48	4.22
47	R Claustrum		26	23	2	4.18
	R Claustrum		30	17	-1	3.90
63	L Frontal Lobe, Middle Gyrus	46	-46	51	8	4.11

	L Frontal Lobe, Inferior Gyrus	46	-48	43	11	3.37
	L Frontal Lobe, Middle Gyrus	10	-38	59	7	3.07
11	R Parietal Lobe, Precuneus	19	8	-80	39	3.77
19	L Claustrum		-34	-9	11	3.72
17	R Parietal Lobe, Superior Lobule	7	34	-68	47	3.64
13	R Temporal Lobe, Fusiform Gyrus	37	51	-45	-13	3.59
12	R Parietal Lobe, Superior Lobule	7	20	-67	52	3.45

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

| Voxel Number | Brain Region | Brodmann Area | Talairach | | | Z Score |
			X	Y	Z	
TSB1						
64	R Temporal Lobe, Fusiform Gyrus	37	40	-57	-6	4.66
	R Temporal Lobe, Fusiform Gyrus	37	46	-51	-9	3.12
33	R Parahippocampal Gyrus	30	10	-39	0	4.52
60	R Frontal Lobe, Inferior Gyrus	46	34	17	24	4.44
32	R Parietal Lobe, Postcentral Gyrus	43	63	-11	16	4.27
37	R Limbic Lobe, Posterior Cingulate	30	14	-50	12	4.25
25	L Frontal Lobe, Precentral Gyrus	9	-32	4	35	4.25
25	L Anterior Cingulate	32	-10	32	10	4.14
16	R Posterior Cingulate	30	24	-70	8	3.99
17	R Occipital Lobe, Cuneus	18	12	-71	20	3.75
12	L Frontal Lobe, Inferior Gyrus	45	-53	11	23	3.72
28	L Posterior Cingulate	31	-14	-41	32	3.57
14	R Occipital Lobe, Cuneus	18	16	-84	21	3.56
14	L Temporal Lobe, Middle Gyrus	37	-42	-60	1	3.35
14	R Occipital Lobe, Inferior Gyrus	37	44	-66	-2	3.34
13	R Occipital Lobe, Inferior Gyrus	19	36	-78	-6	3.30
11	R Insula	13	38	-24	20	3.27
TSB2						
43	R Frontal Lobe, Precentral Gyrus	6	50	-2	7	4.58
15	L Parietal Lobe, Precuneus	7	-4	-33	45	4.58
13	R Occipital Lobe, Middle Gyrus	19	32	-90	18	4.55
25	R Insula	13	48	-24	21	4.45
	R Parietal Lobe, Postcentral Gyrus	40	57	-26	23	3.24
49	L Frontal Lobe, Middle Gyrus	6	-26	-9	63	4.29
	L Frontal Lobe, Superior Gyrus	6	-18	-6	66	4.00
11	R Insula	13	38	-9	16	4.18
16	L Cerebellum		-32	-45	-16	4.05
14	R Frontal Lobe, Medial Gyrus	10	16	51	6	4.02
14	R Frontal Lobe, Superior Gyrus	6	24	7	58	3.94
13	L Cerebellum		-2	-78	-11	3.91
14	L Frontal Lobe, Superior Gyrus	6	-18	14	56	3.91
14	R Frontal Lobe, Precentral Gyrus	44	63	8	11	3.89
	R Frontal Lobe, Inferior Gyrus	9	67	11	18	3.60
13	L Temporal Lobe, Superior Gyrus	39	-46	-48	11	3.59

11	R Frontal Lobe, Middle Gyrus	9	38	35	35	3.59
14	L Insula	13	-34	6	8	3.56
12	R Thalamus		8	-7	10	3.31
TSB3						
18	L Frontal Lobe, Superior Gyrus	10	-16	51	18	4.57
15	L Frontal Lobe, Middle Gyrus	9	-42	11	35	4.46
18	R Temporal Lobe, Superior Gyrus	38	48	18	-21	4.37
20	R Cerebellum		30	-30	-22	4.34
26	R Frontal Lobe, Medial Gyrus	10	14	45	3	4.21
22	L Occipital Lobe, Precuneus	31	-2	-67	20	3.99
14	R Anterior Cingulate	32	10	35	7	3.76
13	R Frontal Lobe, Inferior Gyrus	47	57	33	-5	3.44
	R Frontal Lobe, Inferior Gyrus	47	61	21	-3	3.18

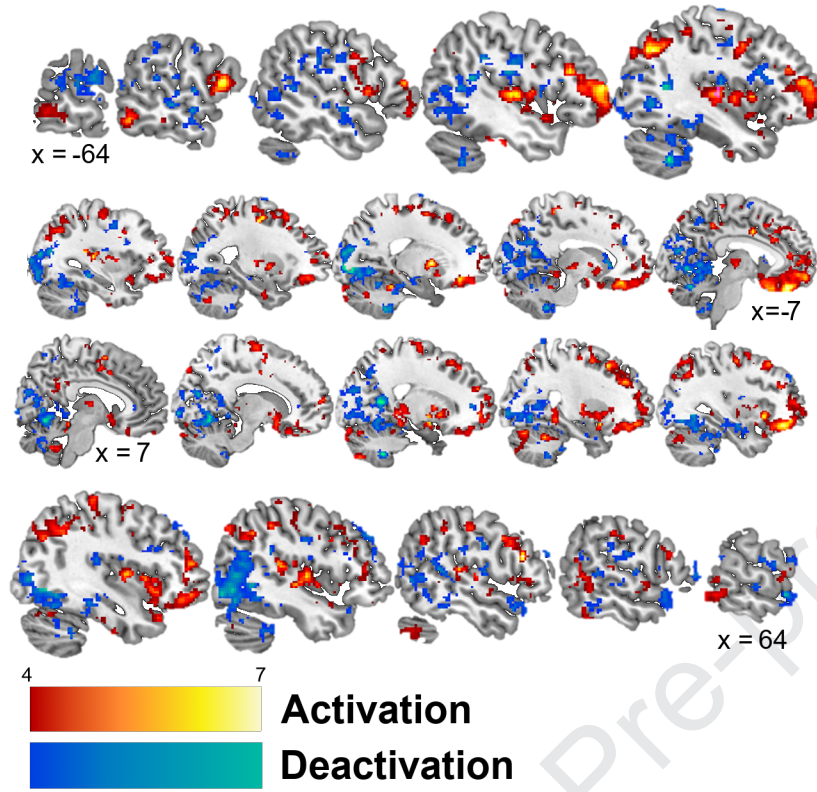**Table 7:** Brain areas with significantly (p < 0.005) greater activity during the application of sham non-invasive cervical vagal nerve stimulation (nVNS) and active nVNS during rest as measured with positron emission tomography. Significant clusters are presented by size (number of voxels) and location (Brodmann area, cluster peak Talairach coordinates). Sub-cluster peaks are also identified.
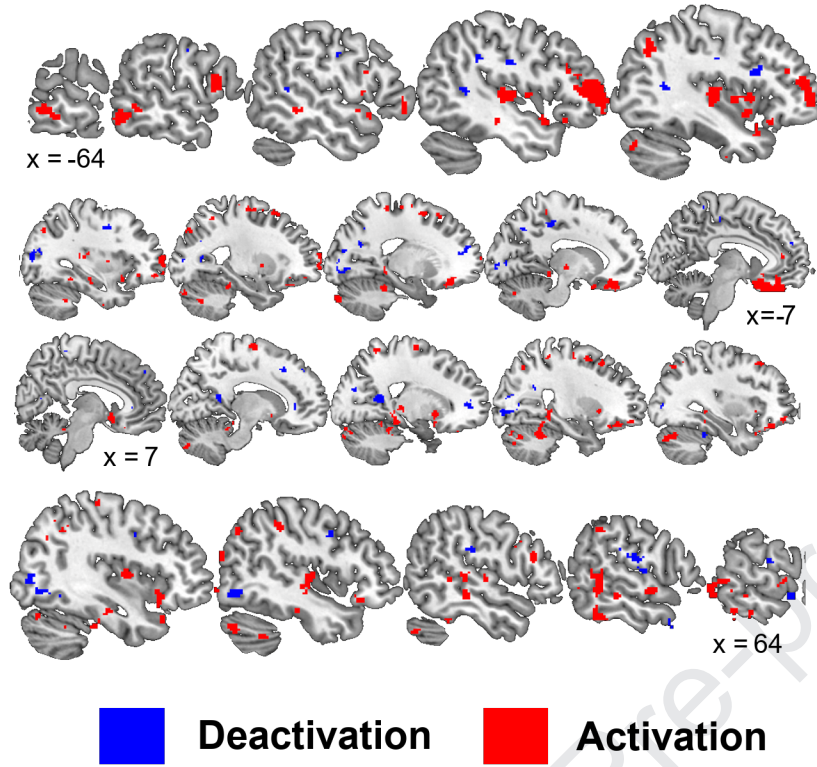
Voxel Number	Brain Region	Brodmann Area	Talairach			Z Score
			X	Y	Z	
Sham > nVNS						
138	L Parietal Lobe, Inferior Lobule	40	-53	-39	27	4.10
	L Insula	13	-44	-41	25	4.08
116	L Parietal Lobe, Precuneus	7	-14	-60	47	4.07
	L Parietal Lobe, Precuneus	7	-8	-72	45	3.47
109	L Temporal Lobe, Middle Gyrus	21	-44	3	-25	3.72
42	R Cerebellum		4	-61	-10	3.67
84	L Frontal Lobe, Medial Gyrus	8	-12	33	35	3.66
	L Frontal Lobe, Medial Gyrus	9	-10	40	32	3.63
68	L Cerebellum		-40	-54	-33	3.58
51	R Frontal Lobe, Medial Gyrus	9	20	44	20	3.49
60	L Frontal Lobe, Middle Gyrus	47	-40	35	-8	3.46
24	L Parietal Lobe, Precuneus	7	-2	-72	34	3.43
16	R Frontal Lobe, Middle Gyrus	46	51	21	22	3.40
23	L Frontal Lobe, Middle Gyrus	11	-22	46	-9	3.39
59	L Cerebellum		-26	-70	-37	3.39
110	L Anterior Cingulate	32	-6	43	5	3.37
	L Frontal Lobe, Medial Gyrus	10	-16	45	5	3.27
	L Anterior Cingulate	32	-16	39	12	2.76
23	R Parietal Lobe, Precuneus	19	30	-80	34	3.35
55	R Frontal Lobe, Medial Gyrus	10	20	51	6	3.27
	R Frontal Lobe, Medial Gyrus	10	12	51	4	2.93
47	L Temporal Lobe, Middle Gyrus	20	-55	-37	-7	3.22
	L Temporal Lobe, Middle Gyrus	20	-50	-34	-10	2.76
15	R Frontal Lobe, Medial Gyrus	10	8	46	-6	3.19
26	R Parietal Lobe, Postcentral Gyrus	2	69	-20	26	3.16
11	R Temporal Lobe, Supramarginal Gyrus	40	61	-47	27	3.13
18	L Temporal Lobe, Middle Gyrus	21	-59	-18	-14	3.13
12	R Occipital Lobe, Cuneus	17	16	-87	11	3.08
12	R Anterior Cingulate	32	20	17	36	3.06
35	L Frontal Lobe, Medial Gyrus	10	0	62	0	3.05
	R Frontal Lobe, Superior Gyrus	10	6	60	-6	2.70
47	R Parietal Lobe, Precuneus	7	10	-74	41	3.05
	R Parietal Lobe, Precuneus	7	6	-71	47	2.88
13	R Frontal Lobe, Superior Gyrus	9	8	52	21	3.04
30	R Cerebellum		28	-72	-35	3.03

20	L Insula	13	-40	4	-7	3.01
13	R Cerebellum		16	-58	-39	2.98
12	R Parietal Lobe, Angular Gyrus	39	44	-62	35	2.98
11	L Frontal Lobe, Paracentral Lobule	5	-4	-38	47	2.93
nVNS > Sham						
31	L Occipital Lobe, Inferior Gyrus	17	-14	-92	-6	3.36
63	R Temporal Lobe, Middle Gyrus	37	44	-66	7	3.33
31	L Frontal Lobe, Inferior Gyrus	47	-34	27	-5	3.24
18	R Frontal Lobe, Inferior Gyrus	47	50	42	10	3.17
21	L Temporal Lobe, Middle Gyrus	21	-53	9	-24	2.91

x = -64

x=-7

x = 7

x = 64

4   7   Activation

Deactivation

x = -64

x=-7

x = 7

x = 64

Deactivation   Activation

TSB3 > TSB2
Activation

TSB2 > TSB3
Deactivation

x = -64

x=-7

x = 7

x = 64

TSB1   TSB2   TSB3

x = -64

x=-7

x = 7

x = 64

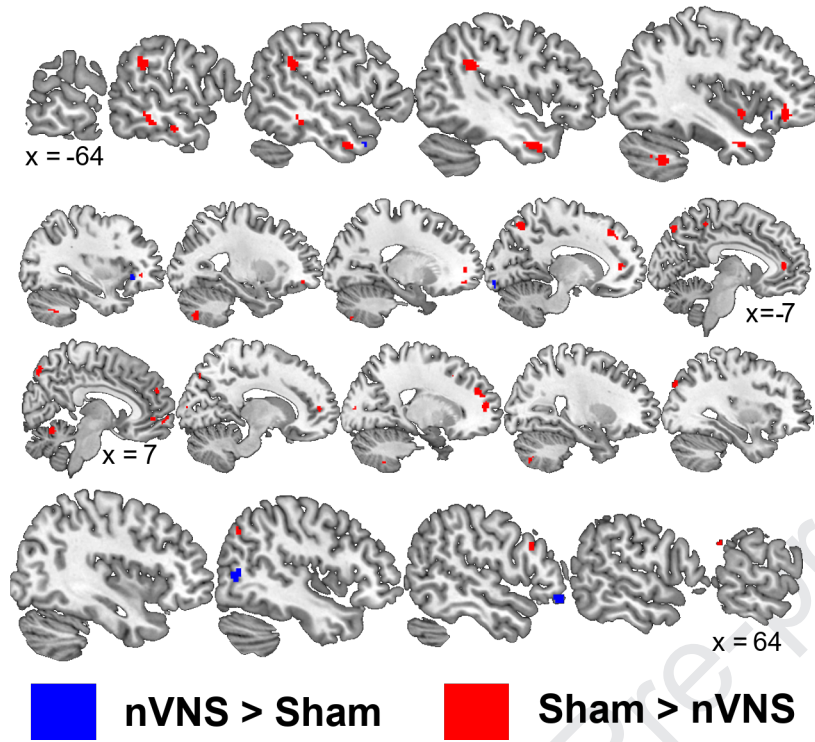**TSB1**   **TSB2**   **TSB3**

x = -64

x=-7

x = 7

x = 64

nVNS > Sham   Sham > nVNS
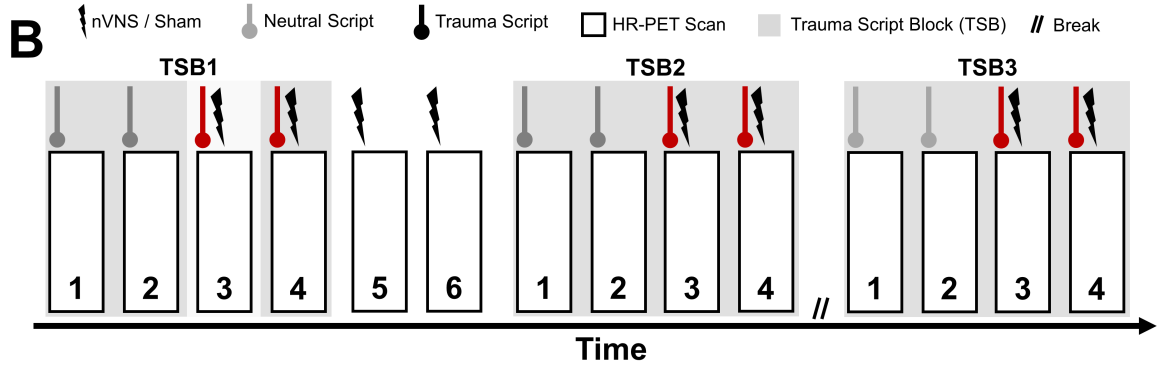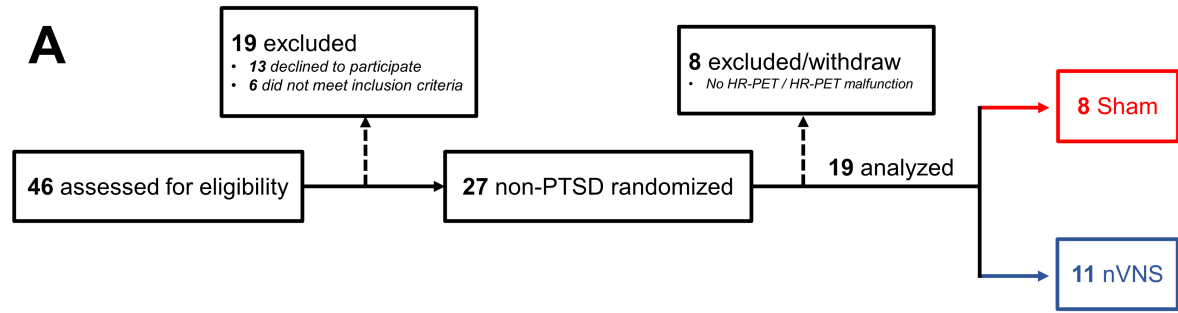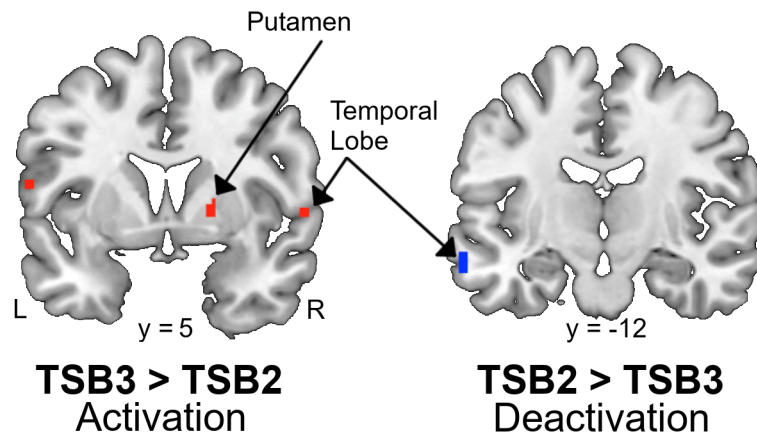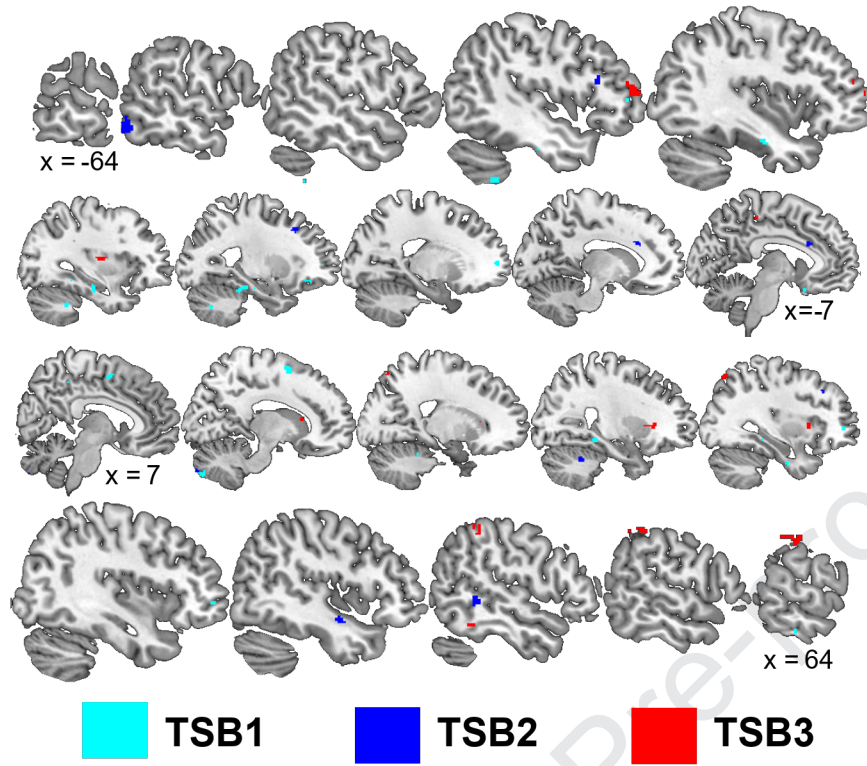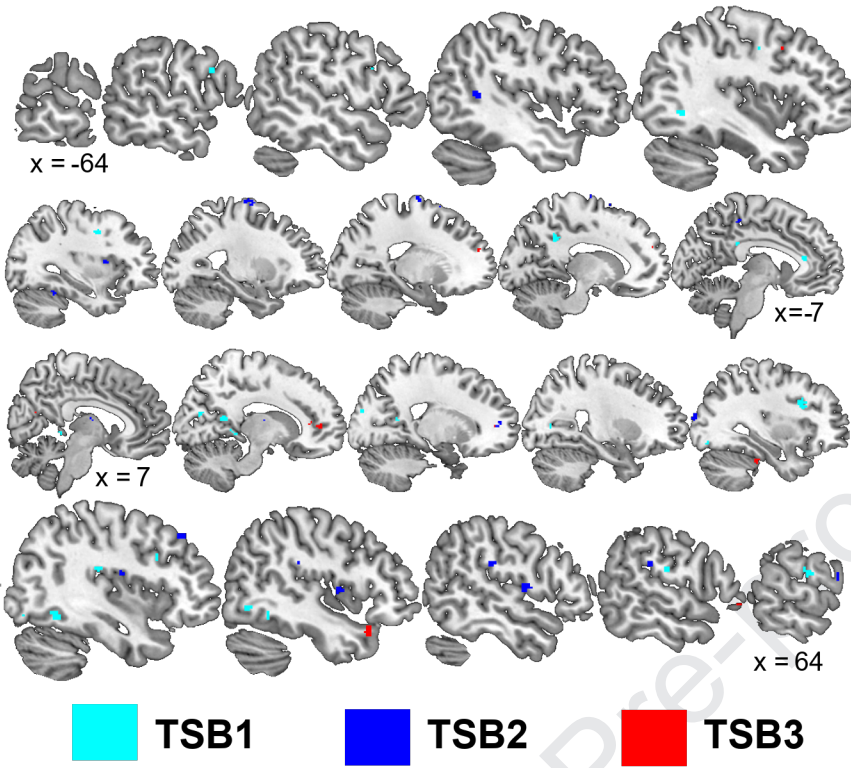
Highlights

- 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.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from mattwittbrodt@emory.edu.

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