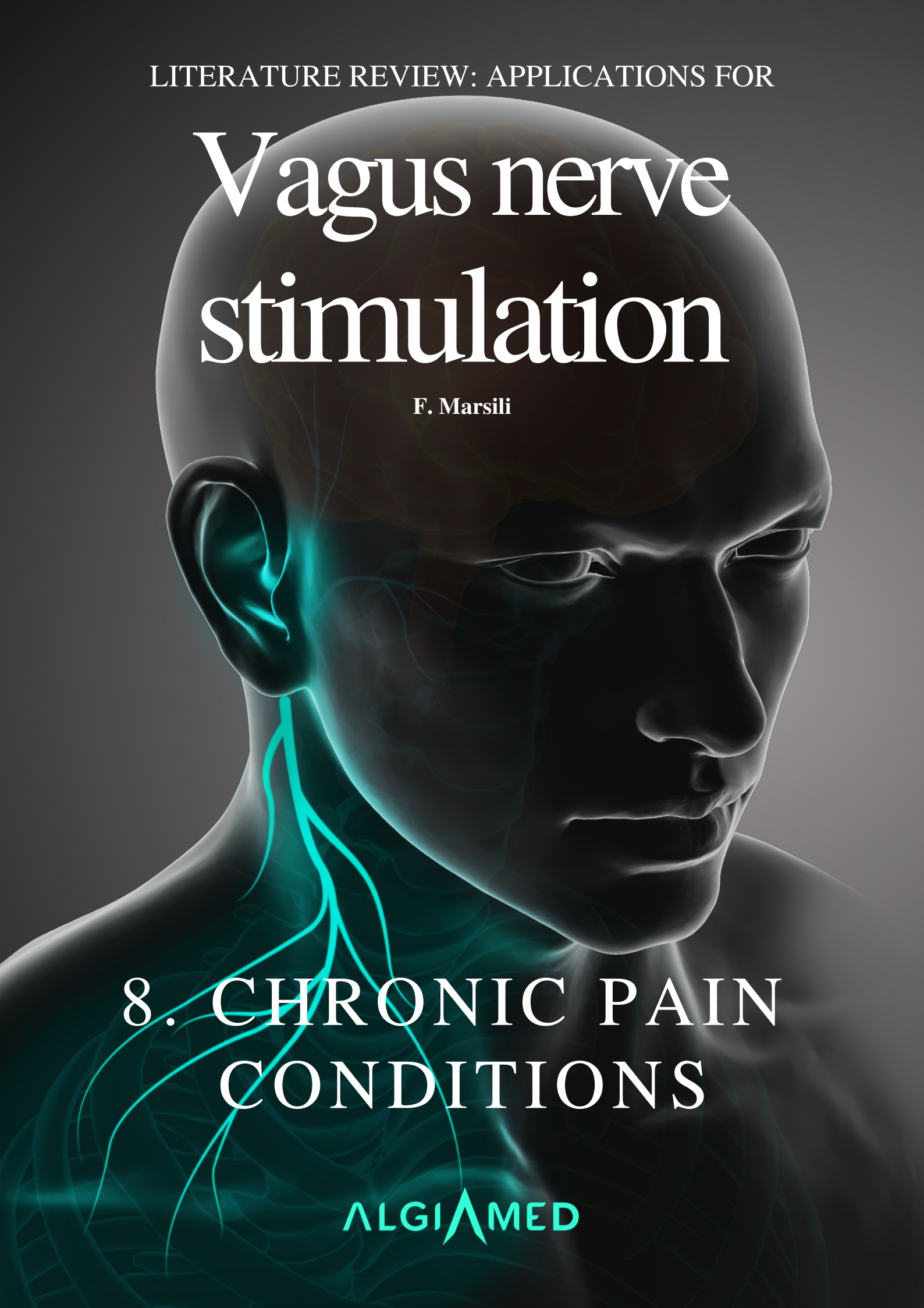
LITERATURE REVIEW: APPLICATIONS FOR

# Vagus nerve stimulation

F. Marsili



## 8. CHRONIC PAIN CONDITIONS

ALGIAMED

# Author's choice

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

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

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

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

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

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

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# 8. VNS and chronic pain conditions

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SHORT REPORT Open Access

# Non-invasive vagus nerve stimulation for acute treatment of high-frequency and chronic migraine: an open-label study

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## Abstract

**Background:** The treatment of migraine headache is challenging given the lack of a standardized approach to care, unsatisfactory response rates, and medication overuse. Neuromodulation therapy has gained interest as an alternative to pharmacologic therapy for primary headache disorders. This study investigated the effects of non-invasive vagus nerve stimulation (nVNS) in patients with high-frequency episodic migraine (HFEM) and chronic migraine (CM).

**Findings:** In this open-label, single-arm, multicenter study, patients with HFEM or CM self-treated up to 3 consecutive mild or moderate migraine attacks that occurred during a 2-week period by delivering two 120-s doses of nVNS at 3-min intervals to the right cervical branch of the vagus nerve. Of the 50 migraineurs enrolled (CM/HFEM: 36/14), 48 treated 131 attacks. The proportion of patients reporting *pain relief*, defined as a ≥50 % reduction in visual analog scale (VAS) score, was 56.3 % at 1 h and 64.6 % at 2 h. Of these patients, 35.4 % and 39.6 % achieved *pain-free status* (VAS = 0) at 1 and 2 h, respectively. When all attacks ($N = 131$) were considered, the pain-relief rate was 38.2 % at 1 h and 51.1 % at 2 h, whereas the pain-free rate was 17.6 % at 1 h and 22.9 % at 2 h. Treatment with nVNS was safe and well tolerated.

**Conclusion:** Non-invasive vagus nerve stimulation may be effective as acute treatment for HFEM or CM and may help to reduce medication overuse and medication-associated adverse events.

**Keywords:** Migraine; Neuromodulation; Vagus nerve; Acute treatment; Patient preference; Disability

## Findings

### Introduction

Migraine, a highly disabling neurological disorder, is characterized by recurrent moderate to severe attacks associated with vegetative symptoms [1]. Patients with frequent attacks may overuse medications, leading to migraine chronification and medication-overuse headache. During the last decade, neuromodulatory approaches have been developed for the management of headaches that do not respond adequately to therapy [2]. Invasive neurostimulation targeting the hypothalamus, sphenopalatine ganglia, and occipital, supraorbital, or auriculotemporal nerves has yielded encouraging results [2]. Vagus nerve stimulation (VNS), an invasive procedure, is approved for medically refractory epilepsy and depression [3, 4] and has demonstrated clinical benefit in intractable migraine with comorbid depression [5]. Experimentally, VNS has modulated neurotransmitters, influenced cerebral metabolism [6] and blood flow [7] in the limbic system and pain matrix regions, and exerted antinociceptive effects in acute and inflammatory pain models [8, 9]. Proposed mechanisms of VNS in pain pathways may involve modulation of excess glutamate levels in the trigeminal nucleus caudalis, effects on pain control centers, and modulation of cortical excitability [9–11].

A non-invasive VNS device (nVNS; gammaCore®) has been developed and is CE-marked for acute and prophylactic treatment of primary headache disorders including migraine and cluster headache [12]. In a recent open-label study of 30 episodic migraineurs, nVNS was effective in the acute treatment of migraine attacks and resulted in a 2-h pain-free rate of 22 % [11]. To further examine the clinical benefit of nVNS reported in the

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aforementioned study, we evaluated the acute effects of nVNS on migraine attacks at 1 and 2 h in a larger patient population with high-frequency episodic migraine (HFEM; ≥8 headache days per month, with or without aura) or chronic migraine (CM; ≥15 headache days per month) [1, 13].

## Methods

In this open-label, single-arm, multicenter study, 50 patients aged 18 to 65 years who were experiencing HFEM or CM [1, 13] were consecutively enrolled between February 1, 2013, and October 1, 2013, at the Headache and Pain Unit of the IRCCS San Raffaele Pisana in Rome, Italy, and the Headache Center of the Carlo Besta Neurological Institute and Foundation in Milan. The study protocol was approved by the **San Raffaele Pisana** institutional review board **(10/2013)**, and all patients who were enrolled in the study provided written informed consent. The study population excluded patients with a history of cerebrovascular, cardiovascular, or atherosclerotic disease (including carotid artery disease, heart arrhythmias, or syncope) or any significant neurological or systemic disorder and patients with an implanted electrical device.

At monthly educational meetings involving groups of 3 to 6 patients as well as neurologists and counselors, patients were instructed on how to use the nVNS device and were invited to describe their experiences with migraine and how they usually managed migraine attacks. Patients received basic information on vagus nerve physiology and vagal neurostimulation and watched a video demonstrating how nVNS is believed to work. Prior to study initiation, patients were actively encouraged to use nVNS and received training on the proper use of the device from a physician and via an instructional video.

Patients were instructed to use nVNS to self-treat up to three consecutive migraine attacks that occurred over a 2-week period. For each migraine attack, patients delivered two 120-s doses of electrical stimulation at 3-min intervals to the right cervical branch of the vagus nerve within 20 min of the onset of mild or moderate pain.

Patients were allowed to take a rescue medication if they perceived no reduction in pain 2 h after nVNS treatment. Pain severity was rated using a 0- to 10-cm visual analog scale (VAS) score (0 cm, *no pain*; 1-3 cm, *mild*; 4-6 cm, *moderate*; 7-10 cm, *severe*) at baseline, 1 h, and 2 h. Patients recorded pain severity in a headache diary, along with symptoms of nausea, photophobia, phonophobia, and functional disability (at baseline and 2 h); the use of rescue medications and adverse events were also recorded.

*Pain relief* was defined as a ≥50 % reduction in VAS score. *Pain-free* status was defined as a VAS score of 0. The primary end point was *pain-free* status at 2 h. Secondary end points were *pain relief* at 1 and 2 h; *pain-free* status at 1 h; absence of nausea, photophobia, and phonophobia at 2 h; complete recovery from functional disability at 2 h; use of rescue medication; safety; tolerability; and end-of-study assessment of patients' satisfaction (5-point scale: 1, *very dissatisfied*, to 5, *very satisfied*) with treatment, their willingness to use the device in the future, and their perceptions regarding the safety of nVNS. Descriptive statistics (ie, mean [standard deviation]) were used to describe categorical data; no other statistical analyses were performed.

## Results

We enrolled 50 patients (female/male: 40/10) affected by CM ($n = 36$) and HFEM ($n = 14$) (Table 1). Two patients with CM did not treat any migraine attacks; the remaining 48 patients treated a total of 131 attacks. Specifically, 30 patients with CM and 6 with HFEM treated 3 attacks each; 4 patients with CM and 7 with HFEM treated 2 attacks each; and 1 patient with HFEM treated 1 attack. After nVNS, 27 of 48 patients (56.3 %) reported *pain relief* at 1 h; of these patients, 35.4 % (n = 17) were pain free. Thirty-one patients (64.6 %) reported *pain relief* at 2 h, of which 39.6 % (n = 19) were pain free (Fig. 1). For all 131 migraine attacks, *pain relief* was reported for 38.2 % (50 of 131) of attacks at 1 h and for 51.1 % (67 of 131) at 2 h; *pain-free status* was reported for 17.6 % (23 of 131) of attacks at 1 h and for 22.9 % (30 of 131) of attacks at 2 h (Fig. 2). Achievement of *pain-free* status at 1 and 2 h for at least 1 attack was experienced in 33.3 % (11 of 33) of patients treating 3 attacks and 41.7 % (5 of 12) of patients treating 2 attacks (5 of 12).

**When comparing efficacy of nVNS among patients with CM versus HFEM, we found a consistent trend toward greater efficacy in patients with HFEM. The proportion of patients reporting *pain relief* after nVNS was greater in HFEM at 1 h (HFEM, 71.4 % [10 of 14]; CM, 50.0 % [17 of 34]) and at 2 h (HFEM, 78.6 % [11 of 14]; CM, 58.8 % [20 of 34]); achievement of *pain-free status* was also greater in HFEM at 1 h (HFEM, 50.0 % [7 of 14]; CM, 29.4 % [10 of 34]) and at 2 h (HFEM, 50.0 % [7 of 14]; CM, 35.5 % [12 of 34]) (Fig. 3). A similar trend was seen for all 131 attacks. A greater proportion of HFEM attacks achieved *pain relief* at 1 h (HFEM, 45.5 % [15 of 33]; CM, 35.7 % [35 of 98]) and 2 h (HFEM, 60.6 % [20 of 33]; CM, 48.0 % [47of 98]); more attacks achieved *pain-free status* at 1 h (HFEM, 30.3 % [10 of 33]; CM, 13.3 % [13 of 98]) and at 2 h (HFEM, 33.3 % [11 of 33]; CM, 19.4 % [19 of 98]) (Fig. 4).**

**The proportion of patients who responded to nVNS in ≥ 50 % of the migraine attacks at 2 h was 62.5 % for pain relief (78.6 % in HFEM, 55.9 % in CM) and 33.3 % for pain free (50 % in HFEM, 26.5 % in CM).**

**Table 1** Demographic and baseline characteristics of study population

| | All | HFEM | CM |
|---|---|---|---|
| | *N* = 50 | *n* = 14 | *n* = 36 |
| Mean (SD) age, y | 43.2 (11.3) | 43.2 (12.3) | 43.3 (10.8) |
| Female, n (%) | 40 (80) | 11 (78.6) | 29 (80.5) |
| Mean (SD) disease duration, y | 29.7 (11.2) | 30.4 (13.5) | 29.5 (10.2) |
| Mean (SD) number of migraine days per month | 15.4 (5.6) | 7.9 (2.3) | 18.3 (3.3) |
| Allodynia[a], n (%) | 18 (36) | 4 (28.6) | 14 (38.9) |
| Concomitant prophylaxis, n (%) | 39 (78) | 10 (71.4) | 29 (80.6) |
| Migraine Type, n (%) | | | |
| Migraine without aura | 14 (28) | 14 (100) | 0 |
| Medication overuse headache | 5 (10) | 0 | 5 (13.9) |
| Chronic migraine | 36 (72) | 0 | 36 (100) |
| Migraine Pain Location, n (%) | | | |
| Unilateral | 28 (56) | 10 (71.4) | 18 (50) |
| Bilateral | 18 (36) | 3 (21.4) | 15 (41.7) |
| Unilateral/bilateral | 4 (8) | 1 (7.2) | 3 (8.3) |
| Duration of Migraine Attacks, n (%) | | | |
| ≤24 h | 17 (34) | 5 (35.7) | 12 (33.3) |
| 25-48 h | 8 (16) | 2 (14.3) | 6 (16.7) |
| >48 h | 25 (50) | 7 (50) | 18 (50) |

*CM* chronic migraine, *HFEM* high-frequency episodic migraine; *SD* standard deviation
[a]Allodynia was assessed using the Allodynia Symptom Checklist

At 2 h, freedom from nausea was reported in 66.4 % (87 of 131) of attacks; freedom from photophobia and phonophobia was reported in 76.3 % (100 of 131) and 77.1 % (101 of 131) of attacks, respectively. Complete recovery from functional disability at 2 h was reported in 35.1 % of attacks. Rescue medications were taken in 53.4 % (70 of 131) of the attacks.

No major adverse events were reported. Mild tingling or pricking sensations at the stimulation site, reported by 67 % (32 of 48) of patients, was the only adverse event associated with nVNS. Nearly half of the patients (45.8 %; 22 of 48) reported satisfaction (ie, *satisfied* or *very satisfied*) with treatment and were willing to use the device in the future. All patients considered nVNS treatment to be safe.

## Discussion

Results from the present study validate prior evidence that shows nVNS is effective for the acute treatment of migraine attacks in patients with HFEM or CM [11]. With enrollment of a larger (N = 50), more severely affected population who experienced more migraine attacks (N = 131), our research extends data from previous studies that showed a 2-h pain-free response of 22 % [11]. More than half of the patients (64.6 %) in our study experienced *pain relief* at 2 h, and 39.6 % were *pain free*

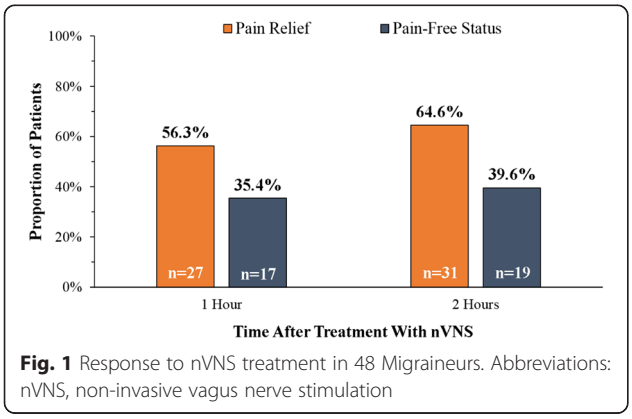


**Fig. 1** Response to nVNS treatment in 48 Migraineurs. Abbreviations: nVNS, non-invasive vagus nerve stimulation

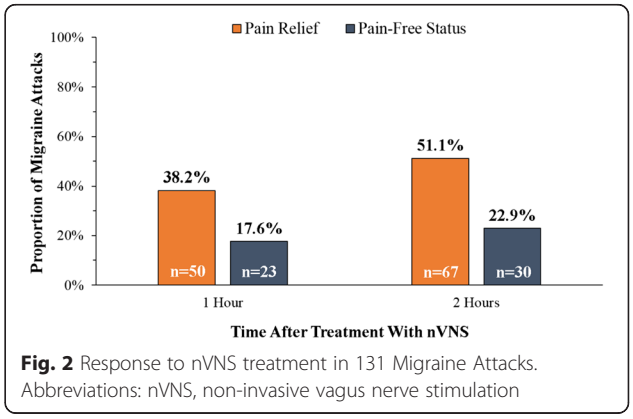


**Fig. 2** Response to nVNS treatment in 131 Migraine Attacks. Abbreviations: nVNS, non-invasive vagus nerve stimulation

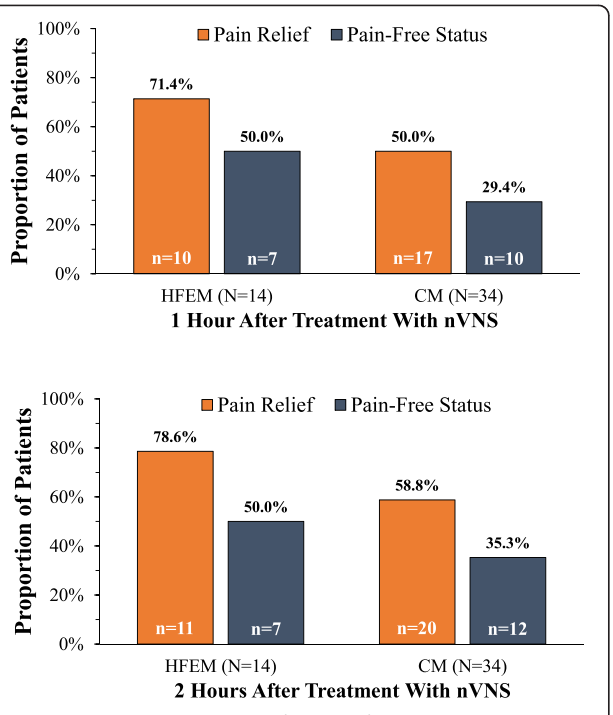


**Fig. 3** Response to nVNSat 1 and 2 Hours by Patient Type

at 2 h; a novel finding is the response to nVNS at 1 h, with 56.3 % of patients experiencing *pain relief*, including 35.4 % of patients who were pain free. **Additionally, we discovered that patients with a lower frequency of attacks (ie, HFEM; 8-14 headache days per month) appeared to achieve a better response than those with a higher frequency of attacks (CM; ≥15 headache days per month).** This finding represents an early treatment paradigm in which nVNS was administered when migraine pain was mild or moderate rather than severe. Although this paradigm may increase the placebo effect, it was selected because headaches in CM are typically reported to be mild or moderate compared with more severe headaches in episodic migraine [14, 15]. Moreover, persistent activity of pain-processing regions within the brain and low expectation of success in patients with CM may mitigate any placebo effect [16]. Other limitations of this study are the open-label design, lack of control group, and short duration. **Moreover, larger studies are required.** However, studies of nVNS in migraine [11] and cluster headache [17] have also implemented a short-term, single-arm, open-label design to demonstrate the feasibility of nVNS in real-world clinical practice. Preliminary data from large-scale, multicenter, randomized, controlled studies of nVNS in CM [18] and chronic cluster headache [19] have further corroborated its clinical benefit.

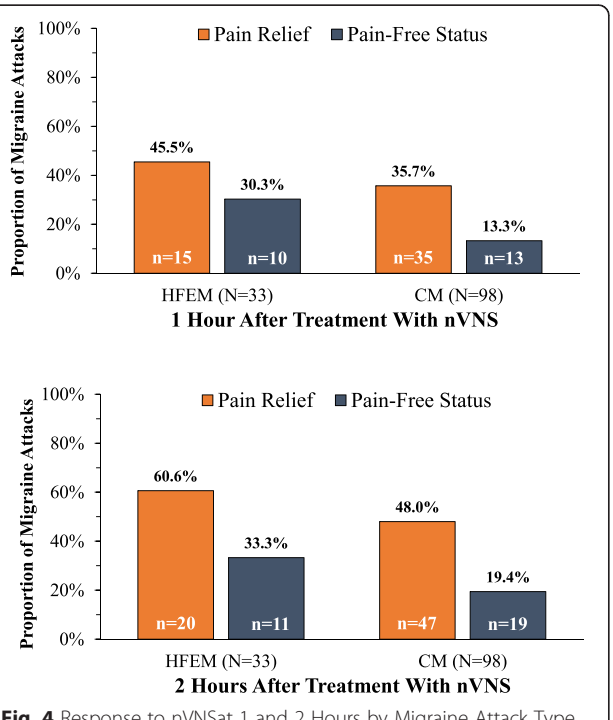


**Fig. 4** Response to nVNSat 1 and 2 Hours by Migraine Attack Type

We investigated the benefit of nVNS in a real-world clinical setting; findings from this study will expand the body of clinical evidence on nVNS to the HFEM/CM population whose pain is difficult to manage. Furthermore, we implemented intensive educational training to ensure treatment adherence, assessed headache response at a short interval (ie, 1 h), and evaluated treatment satisfaction. Our data confirm that nVNS is well tolerated and safe and is associated with treatment satisfaction and therapeutic adherence. From a risk-benefit perspective, nVNS therapy achieved pain relief without serious side effects, which may decrease patients' reliance on migraine medications and, in turn, lower the risk of medication overuse.

#### Competing interests

Dr. Piero Barbanti has served as a consultant or scientific advisor for Merck, Janssen Pharmaceuticals, Lusofarmaco, Abbott, Allergan, and electroCore. Dr. Licia Grazzi has also served as a consultant and scientific advisor for Allergan and electroCore. Eric Liebler is an employee of electroCore. Dr. Gabriella Egeo, Dr. Gennaro Bussone, and Ms. Anna Maria Padovan have no competing interests to declare.

#### Authors' contributions

PB and LG developed the study concept and design, analyzed the data, and are responsible for all the content in this manuscript. GE was the study coordinator, and AMP trained the study participants to use the nVNS device. GB provided study guidance and critique of the manuscript. EL provided study guidance, critique, and editorial support for this manuscript. All authors read and approved the final manuscript.

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PLOS ONE

RESEARCH ARTICLE

# Noninvasive vagus nerve stimulation alters neural response and physiological autonomic tone to noxious thermal challenge

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**Data Availability Statement:** All data sources, demographics, GSR, pain rating and fMRI data are available as Supporting Information files.

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**Competing interests:** Bruce Simon PhD has stock/shares in Electrocore LLC. Imanuel Lerman MD MSc carried out prior investigator initiated research that was funded by Electrocore LLC including investigating anti-inflammatory effects of nVNS

## Abstract

The mechanisms by which noninvasive vagal nerve stimulation (nVNS) affect central and peripheral neural circuits that subserve pain and autonomic physiology are not clear, and thus remain an area of intense investigation. Effects of nVNS vs sham stimulation on subject responses to five noxious thermal stimuli (applied to left lower extremity), were measured in 30 healthy subjects (n = 15 sham and n = 15 nVNS), with fMRI and physiological galvanic skin response (GSR). With repeated noxious thermal stimuli a group × time analysis showed a significantly ($p < .001$) decreased response with nVNS in bilateral primary and secondary somatosensory cortices (SI and SII), left dorsoposterior insular cortex, bilateral paracentral lobule, bilateral medial dorsal thalamus, right anterior cingulate cortex, and right orbitofrontal cortex. A group × time × GSR analysis showed a significantly decreased response in the nVNS group ($p < .0005$) bilaterally in SI, lower and mid medullary brainstem, and inferior occipital cortex. Finally, nVNS treatment showed decreased activity in pronociceptive brainstem nuclei (e.g. the reticular nucleus and rostral ventromedial medulla) and key autonomic integration nuclei (e.g. the rostroventrolateral medulla, nucleus ambiguous, and dorsal motor nucleus of the vagus nerve). In aggregate, noninvasive vagal nerve stimulation reduced the physiological response to noxious thermal stimuli and impacted neural circuits important for pain processing and autonomic output.

and neural effects of nVNS measured with MEG. These prior studies do not overlap with this study and Electrocore had no part in this study funding or design. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

## Introduction

### Noninvasive vagus nerve stimulation

Afferent and efferent vagus nerve signaling are critical mediators of physiological homeostasis, modulating heart rate, gastrointestinal tract motility and secretion, pancreatic endocrine and exocrine secretion, hepatic glucose production, and other skeletal and visceral functions that together make the vagus nerve the principle nerve of the parasympathetic nervous system [1]. Vagal fibers can be activated with exogenous electrical stimulation carried out with surgically implanted vagus nerve stimulation (sVNS) devices (implanted around the vagus nerve in the carotid sheath). Surgically implanted vagus nerve stimulation is approved by the United States Food and Drug Administration (FDA) for the treatment of epilepsy [2] and for treatment-resistant major depression (TRMD); [3–5]. However, cervical sVNS can result in complications, including hoarseness, dyspnea, nausea, and postoperative pain [6, 7].

Noninvasive techniques for VNS have beneficial effects in treating epilepsy, depression, and pain. Treatment includes the use of devices that activate the auricular branch (termed Arnold's nerve) of the vagus nerve [8–10] and the cervical vagus nerve (found within the carotid sheath) [11]. Cervical transcutaneous noninvasive vagus nerve stimulation (nVNS) has shown promising therapeutic effects in the treatment of acute and chronic migraine headaches [12–14], and acute and chronic cluster headaches [15], and is now FDA-approved to treat both episodic cluster [14] and acute migraine headaches [7, 16, 17]. Recent work has shown that, with finite element modeling of cervical nVNS, the electrical field significantly penetrates the human neck and is sufficient to activate the cervical vagus nerve [11]. Moreover cervical nVNS is known to result in characteristic evoked potentials when measured with EEG that match evoked potentials produced by implanted vagal nerve stimulators [18]. Collectively, transcutaneous cervical nVNS results in vagal activation that affects pain transmission and experience.

### Pain autonomic responses and vagus nerve stimulation

Pain is a multimodal experience represented by a broad network of cortical and subcortical structures, including the primary (SI) and secondary somatosensory (SII) cortices, bilateral insular cortex (IC), anterior cingulate cortex (ACC), prefrontal cortex (PFC), thalamus, and brainstem nuclei [19, 20]. Noxious thermal (painful) stimulation activates a sympathetic response, as measured by an increase in galvanic skin response (GSR); [21–23], with a dose response relationship to increasing thermal stimulus magnitude [24]. Prior work has identified pain-mediated increased activation of the IC, amygdala, ACC, and PFC that correlates with pain-evoked sympathetic activity (i.e. GSR), and together offer a baseline construct for the neural basis of this autonomic pain dimension [25–29]. In the present study, we used functional magnetic resonance imaging (fMRI) and primary physiological outcomes (GSR) to test the hypothesis that nVNS may alter typical cortical and subcortical neural and physiological autonomic responses to aversive noxious thermal stimuli more than to sham treatment. Prior literature supports antinociceptive effects of vagal nerve stimulation in preclinical pain models [30–35]. The antinociceptive effects of VNS are postulated to depend on afferent signaling to the nucleus tractus solitarius (NTS), nucleus raphe magnus (NRM), and locus coeruleus (LC) [32]. Based on this work, it has been proposed that vagal afferent inputs to NTS, NRM, and LC result in a summative signal (including activation of descending noradrenergic, serotonergic, and spinal opiodergic tracts) that inhibits dorsal horn neurons [34] [32] [35]. Adding to preclinical work, multiple translational clinical studies also show similar antinociceptive effects of acute [10, 36–39] and chronic VNS [40].

Recent fMRI studies have revealed that nVNS affects brain areas important in pain processing (e.g. the medial thalamus, dorsal ACC, IC, and PFC; [41–44], thus highlighting a potential supraspinal vagal influence on pain perception. Only a single small pilot study ($n$ = 20) has evaluated the neural effects of transcutaneous VNS using auricular "Arnold's nerve" stimulation on experimental pain [37]. The results did not show a difference between groups, but a post-hoc analysis of "responders", i.e. subjects ($n$ = 12) with increased pain threshold post-nVNS, showed decreased activation during the application of pain stimuli in the left dorsoposterior insula, ACC, ventromedial PFC, caudate nucleus, and hypothalamus [37]. Notably, this study performed continuous transcutaneous auricular VNS during the noxious thermal challenge, possibly confounding the results as emerging literature shows pronociceptive effects during actual VNS, while the antinociceptive effects occur post-VNS [45, 46]. Taken together, the evidence accumulated to date suggests that VNS alters clinical pain perception, but that VNS must be carefully timed to produce antinociceptive effects.

### Study objectives

The objective of this study was to gain a richer understanding of post-nVNS effects on sensory discriminative neurocircuits, affective pain neurocircuits, and the peripheral autonomic response to noxious thermal stimuli. Our goal was to determine the extent of post-nVNS neural effects on pain-related brain activation and autonomic tone. Taken together, this knowledge could guide and improve the efficacious use of nVNS in pain-disease states.

## Materials and methods

### Participants

Thirty male and female subjects (age range, 18–54) were recruited through the Altman Clinical and Translation Research Institute at the University of California, San Diego Health System. Screening, exclusion, and inclusion criteria are found in Supplementary Information (S1 File). All participants were right-handed and provided written, informed consent to participate in the study. The Institutional Review Board at the University of California, San Diego Health Systems approved this study (UCSD IRB project # 150202).

### Intervention

Subjects were randomized to receive either nVNS (n = 15) or sham (n = 15) treatment (Fig 1A). A pair of nonferromagnetic stainless-steel surface electrodes (1-cm diameter) were placed on the subject and secured with an adjustable Velcro strap collar. The 2 devices were identical in appearance and subjects were blinded to specific intervention. Application of the device was made to either the right anterior cervical area (overlying the carotid artery) for active nVNS, or the right lateral cervical area (posterior to sternocleidomastoid) for the sham treatment. Surface electrodes were connected to the battery-powered stimulation unit by a 6-m shielded, grounded cable. Both the sham and nVNS devices delivered 1-ms duration bursts of 5 sinusoidal wave pulses at 5000 Hz with a repetition rate of 25 Hz, and a continuous train duration of 2 minutes. In both the nVNS and sham treatments, a computational fixed, initial 30-second ramp-up period was followed by 90 seconds of peak stimulation. In the nVNS treatment, the voltage was increased to 24 V, whereas in the sham stimulation it was increased to 4.5 V. With sham stimulation, subjects generally experience greater discomfort (because of stimulation of neck muscles), with maximal tolerable amplitude typically only 2–8 V[44, 47]. Therefore, we employed a maximum sham voltage of 4.5 V to the far lateral neck position [44, 47]. Prior work suggests that use of the far lateral neck position with low voltage does not result in

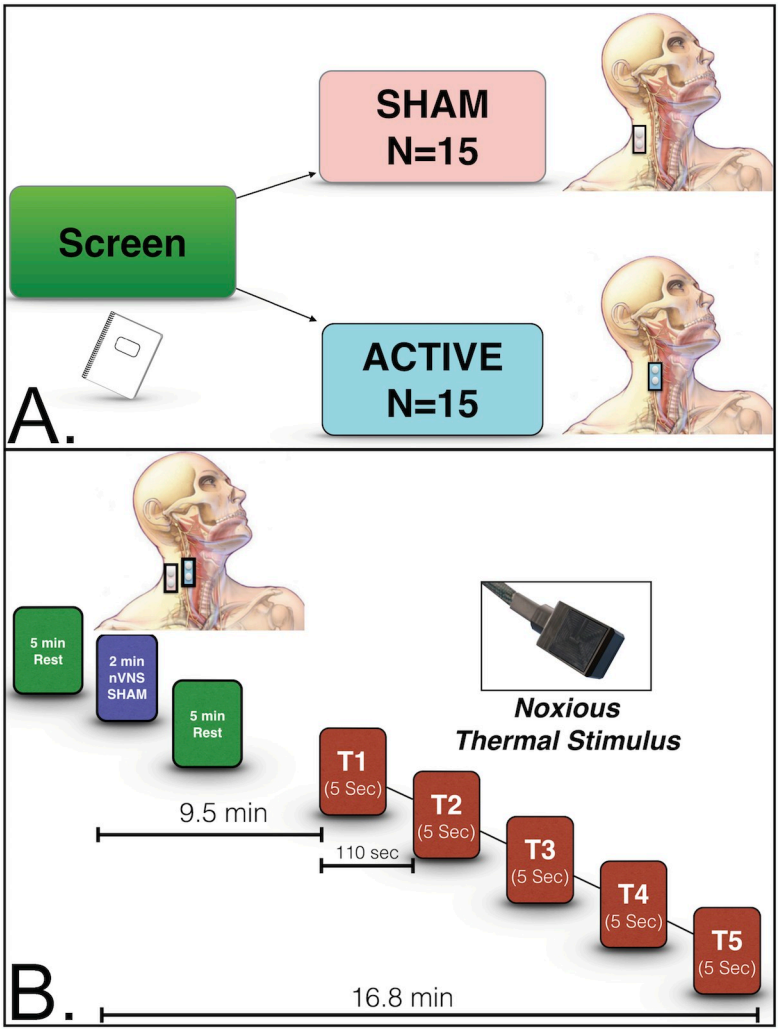


**Fig 1. Noninvasive vagus nerve stimulation study design.** (a) Subjects were screened and randomized to either the sham treatment or nVNS group. Sham stimulation was carried out posteriolateral to the sternocleidomastoid. In the nVNS group, stimulation occurred anteromedial to the sternocleidomastoid and lateral to the trachea. In both the nVNS and sham treatments, a computational fixed, initial 30-second ramp-up period was followed by 90 seconds of peak stimulation. **(b)** Subjects were allowed to rest for 5 minutes before undergoing 2 minutes of nVNS (electrodes placed over carotid) or sham stimulation (electrodes placed far lateral to the sternocleidomastoid). Subjects then rested for an additional 5 minutes. Nine and a half minutes after either nVNS or sham stimulation, 5 successive noxious thermal stimuli were applied in bouts of 5 seconds each, up to 49.8˚C. Each heat stimulus began 110 seconds after the start of the previous one. fMRI, and GSR acquisition were taken 9.5 to 16.8 minutes after nVNS or sham treatment.

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stimulation of the vagus nerve[44, 47]. Mourdoukoutas and colleague's [11], recent work shows (with finite element modeling) that active 20 V nVNS positioned directly over the carotid artery results in electric field penetrance that activates the vagus nerve. Based on this modeling we chose the maximum setting of 24 V known to activate the vagus nerve in the cervical neck. Both nVNS and sham stimulation were carried out 9.5 minutes prior to the noxious thermal stimulus paradigm (Fig 1B).

## Thermal stimulus task

The thermal heat threshold and thermal heat tolerance were obtained prior to the MRI scan, as previously described [48] (S1 File). During the MRI scan, noxious thermal stimulation up to a

temperature of 49.8˚C was applied for 5 seconds via a fMRI-compatible thermode (probe size 3 x 3 cm; TSA-II, NeuroSensory Analyzer, MEDOC Advanced Medical Systems, Rimat Yishai, Israel) attached via Velcro strap, to the left lower extremity (left anteromedial lower leg, anterior to the medial gastrocnemius) in all participants. Five noxious thermal stimuli were successively applied for 5 seconds each, with a 105-second interval between each application. The total duration of the task was 9 minutes and 15 seconds (Fig 1B). To tabulate the subjective pain report, we used the numerical pain rating scale (NPRS), a validated pain-intensity score, with a test-retest reliability of 0.71 to 0.99 that is highly correlated with the numerical pain rating scale and McGill Pain Questionnaire [49]. Ten seconds after each noxious thermal stimulus ended(noxious thermal stimulus application from 1.5–6.5 sec), subjects visualized a projector screen that displayed NPRS, at which point they were asked to rate their pain intensity) numbered 0 to 10 (where 0 = no pain, and 10 = most intense pain possible). On the visualized screen a cursor was slowly moved across the NPRS scale from 0-10(left to right over approximately a 10 second time interval). Prior to scanning, the subject was instructed to raise the right thumb when the cursor indicated the pain level experienced. A video camera visualized the subject's right thumb as it was raised (when the cursor passed under the specific number indicating their (i.e., #5/ 10) numerical pain rating). The subject's pain report (number when thumb was raised) was then recorded in the source document. Pain ratings were provided 10 seconds after termination of the pain stimulus and thus could be separated in the slow event related design.

## Galvanic skin response

We used the BioPac MP150 Psychophysiological Monitoring System (BioPac System Inc., Santa Barbara, CA) to measure psychophysiological reactivity at rest and during the noxious thermal stimulus pain paradigm. The GSR was recorded using 2 electrodes positioned on the volar pads of the distal phalanx of the middle and ring fingers of the right hand, and GSR was sampled with a frequency of 1000 Hz. The mean GSR (in microsiemens) prior to the application of each (#1-#5) noxious thermal heat stimulus (baseline GSR) was compared to the peak GSR response after the application of noxious thermal stimulus for each trial (#1-#5). The slope of GSR from baseline to peak was calculated (microsiemens/s). Additionally, the time (in seconds) from baseline (prior to each noxious thermal stimulus) to the peak GSR response (each post-noxious thermal stimulus) was measured and compared within and between groups. The mean GSR response was defined as the average GSR (over 25 seconds) obtained after the peak GSR was reached. Data analysis, including sample selection and artifact removal, was carried out with AcqKnowledge software (version 4.42, BioPac System Inc.) and the R statistical programming language, version 3.4.3 [50].

## Image acquisition

T2*-weighted echo-planar images were acquired on a 3T General Electric Discovery MR 750 [Milwaukee, WI; 360 volumes, TR = 1.5 s, TE = 30 ms, flip angle = 80˚, FOV 24 cm, 64 × 64 matrix, 3.75 × 3.75-mm in-plane resolution, 30 3.0 mm (1-mm gap) ascending interleaved axial slices] using an 8-channel brain array coil. High-resolution T1-weighted FSPGR anatomical images (flip angle = 8˚, 256 × 256 matrix, 172 1-mm sagittal slices, TR = 8.1 s, TE = 3.17 ms, 1 × 1-mm in-plane resolution) were acquired to permit activation localization and spatial normalization.

## Statistical analysis

**Group demographics of GSR analyses.** Group differences in questionnaires and demographic analyses were calculated with Mann-Whitney U tests. BIOPAC system measurements

of GSR were incorporated into a mixed-model regression to evaluate within- and between-group (nVNS vs sham) changes in GSR with each noxious thermal stimulus (from baseline, i.e. prior to each (#1-#5) noxious thermal stimulus to after the noxious thermal stimulus has been applied (#1-#5). The within- and between-group GSR post-thermal noxious stimulus mean value (microsiemens), time to peak (seconds), and slope from the baseline GSR to the peak (microsiemens/seconds) were compared. All statistical calculations were performed using the R statistical programming language, version 3.4.3 [50].

**MRI preprocessing.** Structural and functional image processing and analysis were completed using analysis of functional neuroimages (AFNI) software [51] and R statistical packages. Echo planar images were slice-time and motion-corrected and aligned to high-resolution anatomic images in AFNI. Volumes with >20% voxels marked as outliers using 3dToutcount were censored and dropped from the analysis. For all group data points in the LME analyses 1.5% data censor were identified as outlier. Percentage Outlier voxels in the time series were interpolated using 3dDespike. Functional data were aligned to standard space, resampled to 4-mm isotropic voxels, and smoothed with a Gaussian spatial filter (to 6 mm full width at half-maximum). Hemodynamics of the pain experience were modeled using line interpolation (3dDeconvolve/3dREMLfit modeled with TENT) for the span from the initiation of thermal heat stimulus and the following 15 seconds as modeled by 5 regressors overtime. These regressors were reconstructed to form a time series with 11 data points 1.5 seconds apart, which was used in subsequent analysis.

Group differences in the time course of Blood Oxygen Level-Dependent (BOLD) responses over the entire course of the pain experience were measured over the 5 noxious thermal applications. Time-course data were modeled using AFNI's 3dDeconvolve TENT function. The TENT function is a linear interpolation of the hemodynamic response function over time described as piecewise linear splines. A group (nVNS or sham) × time, and (nVNS or sham) × time × GSR linear mixed-effects analysis (LME) using AFNI's 3dLME was conducted to compare time-course data from nVNS vs sham. Effects of interest included (group × time) and (group × time × GSR) interactions, in which all were fixed effects without covariates. Group and GSR were handled as between subject factors and time was a within subject factor. Multivoxel multiple comparisons were performed by Monte Carlo simulations (using AFNI 3dClustSim modeled with 3-perameter modeling noise) to reduce the potential for false positive results. A per-voxel threshold of $p < .001$, a cluster-wise threshold of $p < .001$, and a minimum number of 14 voxels per cluster were used. The Montreal Neurological Institute (MNI) atlas was used to identify clusters. Brainstem nuclei localizations in the group × time × GSR LME were compared with graphical representations of brainstem nuclei from the Duvernoy atlas [52] and compared to prior grey and white matter brainstem maps by Beissner and colleagues [53].

## Results

### Participant demographics and psychiatric assessments

The mean age between the nVNS (24.7 ± 3.7 years) and sham group (30.7 ± 10.3 year) was not statistically different, as determined by a Mann-Whitney U test ($p = .349$). Subjects did not report having elevated anxiety, depression, or posttraumatic stress disorder (PTSD), as measured by the Beck Anxiety Index (BAI), Beck Depression Inventory 2 (BDI-2), or the PTSD Check List–Civilian version (PCL-C). Accordingly, no significant difference in mean scores between groups was noted for these measures. There were no significant differences in gender or race between the sham and nVNS groups. Two subjects failed the initial screen and were excluded from the study; one had a preexisting arrhythmia disorder (Wolf-Parkinson-White

syndrome) and the other had braces (Table 1). The total sample used for analysis (after exclusion of the 2 subjects who failed screening) was 15 subjects in each of the VNS and sham groups.

## Pain and physiologic measures

**Baseline pain measures.** Subject responses to the baseline MPQ, measured at rest prior to thermal threshold or tolerance testing, were not different between the groups (Table 2). Heat thresholds, measured using the method of limits, were similar across groups (nVNS, 41.2˚-C ± 2.8˚C; vs sham, 41.9˚C ± 2.0˚C; $p$ = .935), as was heat tolerance, also, measured using the method of limits,(nVNS, 49.0˚C ± 1.4˚C; vs sham, 48.71˚C ± 1.2˚C; $p$ = 0.467; Table 2).

## Pain reports during the fMRI task as measured by the NPRS

During the MRI task, 5 successive noxious thermal stimuli were administered based on thermal tolerance measures, up to 49.8˚C (Fig 1B). The pain intensity score, measured as the mean NPRS score reported during the noxious thermal stimulus paradigm, was similar between the groups for each application of thermal stimulus (S1 Fig). Both groups reported NPRS scores that were lower with the fifth thermal stimulus (decrease in NPRS, -0.678, ± 0.209; $t$ = -3.241; $p$ = .002) compared with the first stimulus. We then compared the change in mean pain report (NPRS) across each of the successive noxious thermal stimuli (T1-T5) between groups. In contrast to the nVNS group, subjects who underwent sham stimulation showed an increase in NPRS with each of the successive noxious thermal stimuli from the second to the fourth (T2-T4) (this change in pain score for each of the successive noxious thermal stimuli (T2-T4) was calculated as a slope, i.e. sham slope; 0.150 ± 0.122) vs the decrease in NPRS with each of the successive noxious thermal stimuli observed for nVNS (T2-4), (nVNS slope; -0.233 ± 0.122; p = .0301) (S1 Fig). One subject in the sham group was unable to complete the fifth 5-second noxious thermal stimulus due to discomfort. No other adverse events occurred during the study.

**Table 1. Subject demographics and psychiatric measures.**

| | Sham ($n$ = 15) | nVNS ($n$ = 15) | Significance |
|---|---|---|---|
| | Mean (min, max) [%] | Mean (min, max) [%] | $p$ |
| Age (years) | 27.0 (18.0, 54.0) | 25.0 (18.0, 31.0) | 0.349[a] |
| Sex | 8M:7F [53%: 47%] | 11M:4F [73%: 27%] | 0.256 [b] |
| Race | | | 0.460 [a] |
| Asian | 5 [33%] | 7 [46%] | |
| Black | 1 [7%] | 0 [0%] | |
| White | 9 [60%] | 7 [46%] | |
| Other | 0 [0%] | 1 [7%] | |
| Excluded | 0 [0%] | 2 [14%] | |
| BAI | 1.0 (0.0, 12.0) | 1.0 (0.0, 13.0) | 0.577 [a] |
| BDI-2 | 1.0 (0.0, 14.0) | 2.0 (0.0, 17.0) | 0.538 [a] |
| PCL-C | 18.3 (17.0, 28.0) | 18.3 (17.0, 28.0) | 0.469 [a] |

BAI = Beck Anxiety Inventory; BDI-2 = Beck Depression Inventory 2; PCL-C = Posttraumatic Stress Disorder Check List–Civilian version.

[a] = Mann Whitney U statistical test.

[b] = Fishers exact test.

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**Table 2. Baseline pain measures.**

| | Sham ($n$ = 15) | nVNS ($n$ = 15) | Mann-Whitney U |
|---|---|---|---|
| | Mean (min, max) [%] | Mean (min, max) [%] | $p$ |
| Adverse events[a] | 1 [7%] | 0 [0%] | |
| MPQ | 0.0 (0.0, 11.0) | 5.0 (0.0, 59.0) | 0.096 |
| Heat threshold (˚C) | 42.2 (39.1, 46.0 | 42.4 (34.0, 48.2) | 0.935 |
| Heat tolerance (˚C) | 48.7 (47.1, 50.0) | 49.3 (44.7, 50.6) | 0.467 |

MPQ = McGill Pain Questionnaire.

[a]Unable to continue heat pain trial.

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## Galvanic skin response

The GSR was recorded with each noxious thermal stimulus. The time from the onset of the each of noxious thermal stimuli to the peak GSR was measured in seconds. Mixed-model regression analyses conducted across all noxious thermal stimuli (T1-5) and between groups (nVNS vs sham) showed a significantly shorter time to peak in the nVNS group ($p$ = .020; Fig 2A). Post-hoc comparisons between groups (with a 2-sample $t$ test) revealed that subjects who underwent nVNS had a shorter time to peak GSR compared with sham subjects during the application of noxious thermal stimuli T1 and T2 ($p < .05$). Similar trends also approached significance for T3 and T4 ($p < .09$; Fig 2A; S1 Table). We then measured the GSR slope (in microsiemens) from the baseline GSR (prior to the application of each noxious thermal stimulus) to the peak GSR (accompanying each noxious thermal stimulus) and compared how this slope changed with each of the noxious thermal stimuli (T1-5). This GSR slope decreased equally in both groups for T1 to T3 (Fig 2B). But in contrast to the nVNS group, which had an average decrease in slope (-0.0461 microsiemens/second) for T3 to T5, the sham group showed an increase in the average slope to peak GSR from T3 to T5 (0.049 microsiemens/seconds), with a significant between-group difference observed (group x time interaction, -0.09508; $p$ = .0412; Fig 2B).

Within-group analysis conducted using a Mann-Whitney U test showed that the mean GSR (measured for each of the successive noxious thermal stimuli) was successively lower in the sham group after the application of the noxious thermal stimulus for T1, compared with T4 and T5 ($p < .05$); T2 vs T3 ($p < .05$), T4, and T5, ($p < .001$); T3 vs T4 and T5 ($p < .001$); and T4 vs T5 ($p < .001$; S2 Table). In the nVNS group, the mean GSR was successively reduced after the application of the noxious thermal stimulus for T1 vs T3 ($p$ = .016), T1 vs T4, and T5 ($p < .005$); T2 vs T3, T4, T5, ($p < .001$); and T3 vs T4 and T5 ($p < .001$; S3 Table).

## Imaging results

**Group differences during the application of thermal stimuli.** During the application of a noxious thermal stimulus, 21 regions met cluster thresholds in group × time LME analyses (i.e., nVNS vs sham × time). Examination of this interaction indicated that regions in the left insula, right cerebellum/declive, and right cuneus had large clusters of greater activation (sham > nVNS). Additional regions important in the processing of thermal stimuli included the left somatosensory cortex, bilateral mediodorsal thalamus, right dorsal anterior cingulate gyrus, left supramarginal gyrus, and right medial frontal gyrus (orbitofrontal cortex [OFC]; Table 3). A TENT function analysis showed significantly greater activation during the

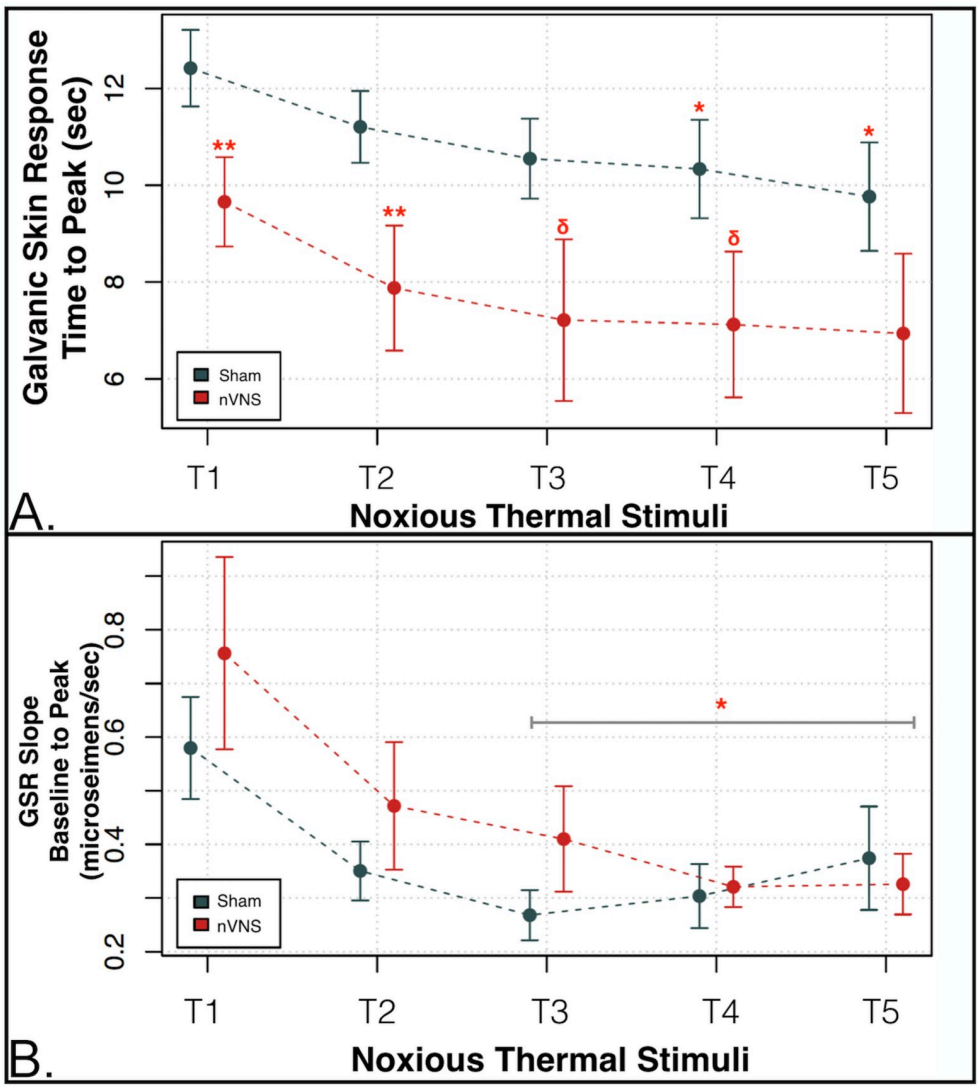


**Fig 2. nVNS vs sham autonomic measures of sympathetic tone galvanic skin response (GSR) with noxious thermal challenge.** (A) The time to peak galvanic skin response (GSR) measured in seconds after the application of each of the noxious thermal stimuli was significantly reduced in the nVNS group for noxious thermal stimuli 1 and 2 (T1 and T2) ($^{**}p < .05$) compared with the sham group, and approached significance for T3 and T4 ($^{\delta}p < .09$). Mixed-model regression showed that the combined (T1-T5) time to peak GSR in the nVNS group was significantly shorter compared with the sham group ($p < .02$). (B) The GSR slope (in microsiemens) from the baseline GSR (prior to the application of each noxious thermal stimulus) to the peak GSR (accompanying each noxious thermal stimulus) was measured in each group. The slope from the baseline GSR to the peak response decreased in both groups with each successively applied noxious thermal stimulus from T1 to T3. However, whereas the nVNS group showed a negative average slope to peak GSR of -0.0461 from T3 to T5, the sham group showed a positive average slope to peak GSR of 0.049 from T3 to T5. The between-group difference (group x time interaction = -0.09508) for T3 to T5 was significant at $^{*}p < .05$. Red circles = nVNS group. Blue circles = sham group.

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application of noxious thermal heat stimuli in the sham group in the SI (Fig 3A and Fig 3B), SII (Fig 3C–3E), left dorsoposterior insula (Fig 3F and Fig 3G), and bilateral mediodorsal thalamus, as well as in the dorsal anterior cingulate (area 24; Fig 3H and 3J), and right medial frontal gyrus (OFC; Fig 3K and Fig 3L).

**Table 3. Cluster results for group × time analysis of noxious thermal stimuli.**

| Voxels | x | y | z | Within | BA | *t*-test | *p*-value |
|---|---|---|---|---|---|---|---|
| 261 | 19 | -63 | -24 | Right Cerebellum | | 3.976308 | 0.0004 |
| 131 | -43 | -36 | 28 | Left Insula, Left Secondary Somatosensory | 13 | 4.032758 | 0.0004 |
| | | | | Cortex (SII), Left Dorsoposterior Insula | | | |
| 130 | 25 | -80 | 8 | Right Cuneus | 17 | 3.994926 | 0.0004 |
| 88 | 1 | -31 | 66 | Bilateral Primary Somatosensory Cortex (SI) | 3a | 3.899217 | 0.0006 |
| 56 | -18 | -72 | -24 | Left Cerebellum | 18 | 3.695176 | 0.0009 |
| 49 | 2 | -21 | -3 | Bilateral Mediodorsal Thalamus | | 4.020902 | 0.0004 |
| 35 | 1 | -31 | 36 | Right Cingulate Gyrus | 31 | 3.785605 | 0.0007 |
| 33 | 32 | 42 | -12 | Right Orbitofrontal Cortex | 47 | 4.373772 | 0.0002 |
| 33 | -21 | -87 | 2 | Left Lingual Gyrus | 17 | 3.664152 | 0.001 |
| 26 | 7 | 8 | 44 | Right Dorsal Anterior Cingulate Gyrus | 24 | 4.024239 | 0.0004 |
| 25 | 3 | -78 | 48 | Right Precuneus | 7 | 3.816214 | 0.0007 |
| 23 | -40 | -44 | 8 | Left Superior Temporal Gyrus | 41 | 4.816714 | <0.0001 |
| 23 | -19 | -48 | 61 | Left Precuneus | 5 | 3.791053 | 0.0007 |
| 21 | -36 | -70 | -31 | Left Cerebellum | | 4.120962 | 0.0003 |
| 21 | -21 | -38 | 12 | Left Caudate | 48 | 3.740923 | 0.0008 |
| 20 | -23 | -82 | 31 | Left Precuneus | 19 | 3.751421 | 0.0008 |
| 17 | -41 | -16 | 44 | Left Precentral Gyrus | 4 | 3.636735 | 0.001 |
| 16 | -21 | -31 | -2 | Left Parahippocampal Gyrus | 36 | 3.643388 | 0.001 |
| 16 | -18 | -1 | 15 | Left Caudate | 48 | 3.966382 | 0.0005 |
| 16 | 50 | -26 | 22 | Right Insula, Right Inferior Parietal Lobule | 13 | 3.732593 | 0.0009 |
| | | | | Right Secondary Somatosensory (SII) | | | |
| 16 | -17 | -48 | 24 | Left Cingulate Gyrus | 31 | 4.035837 | 0.0004 |

BA = Broadmann's Area

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## Imaging results with LME analysis

To better understand the relationships between neural and autonomic measures during thermal stimuli, the GSR mean, measured from the peak after thermal stimulus for 15 seconds, was incorporated into a group (nVNS vs sham) × linear time x GSR LME analysis using AFNI's 3dLME to compare time-course data from the nVNS and sham groups. The group × time × GSR interaction showed that 3 regions met cluster thresholds; the postcentral gyrus/somatosensory cortex (Fig 4A and Fig 4B), cerebellum/medullary brainstem (Fig 4C and Fig 4D), and left occipital gyrus (Table 4). At the medullary level (i.e., level of the olive from the lower pons, spanning to the lower medulla) multiple afferent fibers enter the brainstem, including vagus, glossopharyngeal, hypoglossal, and accessory nerves that synapse on multiple brainstem nuclei (i.e., nucleus ambiguous (NAmb), dorsal motor nucleus of the vagus nerve (DMNX) and nucleus tractus solitarius (NTS)). Other brainstem nuclei important for pain processing (i.e., the rostral ventromedial medulla (RVM), rostral ventrolateral medulla (RVLM), and nucleus reticularis (Rt)) are also found at this level. Brainstem nuclei localizations were compared with graphical representations of brainstem nuclei from the Duvernoy atlas [52] and compared with prior grey and white matter brainstem maps by Biessner and colleagues [53]. Subjects in the sham and nVNS groups were separated by median into high and low mean GSR categories, and the group × time × GSR interaction in the areas corresponding to the above nuclei (within medulla/brainstem) were examined. During the application of noxious thermal stimuli, subjects who underwent sham treatment and showed a high GSR

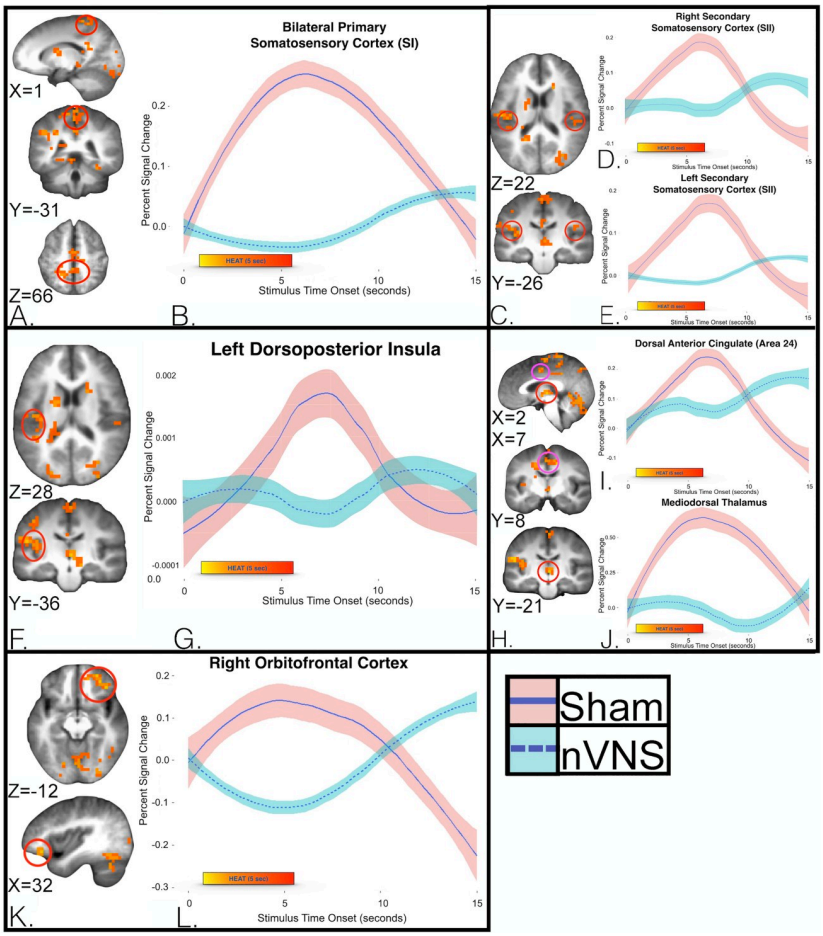


**Fig 3. Group differences in the time course of Blood Oxygen Level-Dependent (BOLD) responses over the entire course of the pain experience.** Imaging of (a) the bilateral somatosensory cortex (SI), and (c) SII, (f) left dorsoposterior insula, (h) bilateral mediodorsal thalamus and dorsal anterior cingulate (area 24), and (k) right media frontal gyrus (orbitofrontal cortex; OFC). Differential hemodynamic response curves during the application of noxious thermal stimuli 10 to 15 minutes following VNS (turquoise) and sham treatments (pink) were generated with a group × time, linear mixed-effects analysis showed that (b) subjects in the sham group had greater activity in the bilateral postcentral gyrus (SI; $p = .0006$). Treatment with nVNS significantly decreased the response of the postcentral gyrus during and after the application of noxious thermal stimuli (5 seconds each), up to 12 seconds after cessation of the painful stimulus. Subjects in the sham group had greater activity in the bilateral SII (d, e) (mean and SE shown) (right $p = .0009$, left $p = .0009$)]. Subjects in the sham group had greater activity in the left posterior insula (g) (mean and SE shown; $p = .0004$), during and after the application of noxious thermal stimuli (5 seconds each). This result demonstrates blunting of the usual temporal dynamic response of the insula (as seen in the sham group) that is most evident during and up to 10 seconds after cessation of the painful stimulus. The sham group showed significantly greater activity in the medial thalamus and anterior cingulate (area 24) (i, j) (mean and SE shown; mediodorsal thalamus $p = .0004$, area 24 $p = .0004$), during and after the application of noxious thermal stimuli (5 seconds each). Subjects in the nVNS group had significantly decreased activity in right middle frontal gyrus (l), overlapping with the medial and lateral OFC (mean and SE shown; $p = .0002$) followed by an increase in OFC response (greater than sham) that was most evident at the 10 to 15 second mark.

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demonstrated greater activity in the medulla/brainstem, compared with other groups (Fig 4C and Fig 4D).

## 4. Discussion

The effects of VNS on the central and peripheral neural circuits involved in pain and autonomic physiology are not well elucidated. In this study nVNS treatment (when compared to

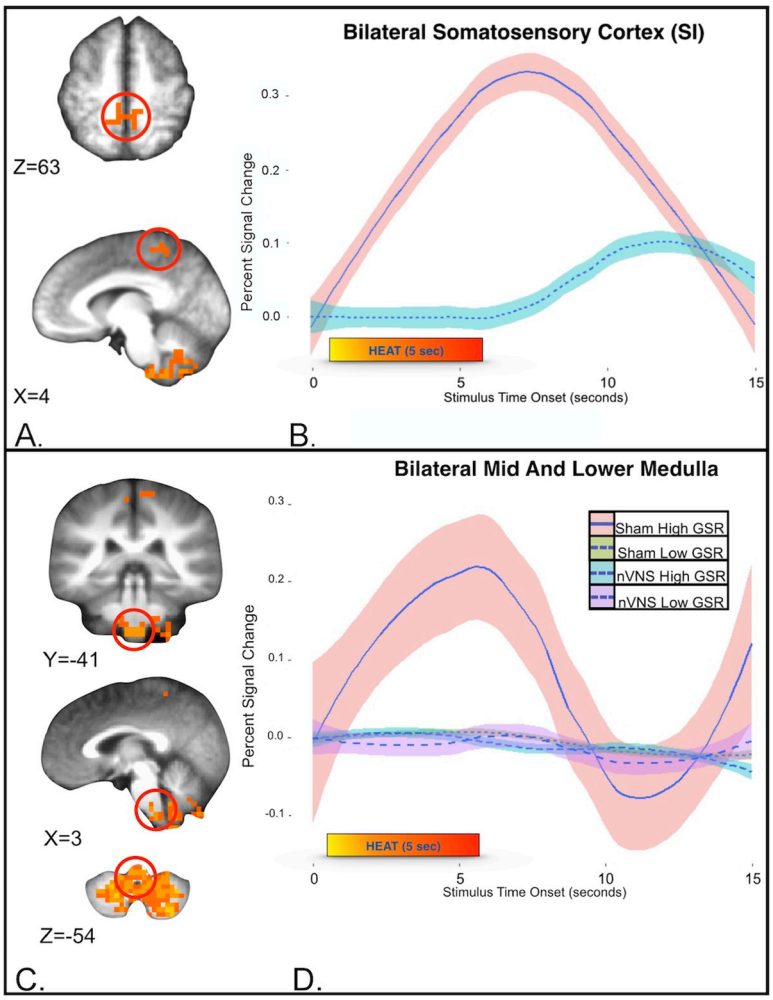


**Fig 4. Neural and autonomic measures taken during the application of thermal stimuli (mean GSR, measured from the peak after the application of the thermal stimulus for 15 seconds).** Group (nVNS vs sham) × linear time x GSR linear mixed-effects analysis. (A) Compared with subjects in the nVNS group, subjects who underwent sham treatment showed significantly greater activity in the bilateral somatosensory cortex. (B) Differential hemodynamics of pain following nVNS (turquoise) and sham (pink) treatment. (SI; mean and SE show; $p = .0002$). (C) Cerebellum/ medullary brain stem measures taken during the application of thermal stimuli show (D). To assist in visual representation of this region of interest, the sham and nVNS groups were separated into high and low mean GSRs (using a group median of 16 microsiemens; the high group included 5 subjects who received sham treatment and 7 subjects who received nVNS treatment). (D) Only the high-GSR sham group (pink shade with blue line) demonstrated greater activity in the medulla/brain stem with the application of noxious thermal stimuli. At this medullary level (i.e. the level of the olive from the lower pons, spanning to the lower medulla) multiple afferent fibers enter the brainstem, including the vagus, glossopharyngeal, hypoglossal, and accessory nerves, that synapse on multiple brainstem nuclei [i.e. the nucleus tractus solitarius (NTS), nucleus ambiguous (NAmb), and dorsal motor nucleus of the vagus nerve (DMNX)]. Other brainstem nuclei important for nociception [i.e. the rostral ventrolateral medulla (RVLM), rostral ventromedial medulla (RVM), and nucleus reticularis (Rt)] are also found at this level.

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sham) resulted in reduced responses in highly relevant pain-processing nodes. There was a significant alteration of autonomic tone, as determined by a decrease in sympathetic activity (measured with GSR) and attenuated activity in brainstem nuclei known to contribute to pain-mediated autonomic responses. These results provide preliminary evidence of significant nVNS modulation of central and peripheral autonomic neural circuits relevant to pain perception.

**Table 4. Cluster results of group × time × GSR LME analysis.**

| Voxels | x | y | z | Within | BA | *t test* | *p* value |
|---|---|---|---|---|---|---|---|
| 414 | 3 | -49 | -54 | Bilateral Medulla Cerebellum | | 5.398 | <0.0001 |
| 25 | 4 | -44 | 63 | Bilateral Primary Somatosensory Cortex (SI) | 3b | 4.200 | 0.0002 |
| 15 | -26 | -92 | -13 | Left Lingual Gyrus | 18 | 4.158 | 0.0003 |

GSR = galvanic skin response; LME = linear mixed effects.

BA = Broadmann's Area

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## Post-Non-invasive vagus nerve stimulation with noxious thermal stimuli; neural effects on bilateral somatosensory cortex 1 (SI) and somatosensory sortex 2(SII) (i.e., Lateral Pain Pathway)

Compared with subjects in the sham group, group × time LME analysis showed that subjects in the nVNS group had decreased neural activation of SI and SII, the medial dorsal thalamus, ACC, IC, and OFC—all brain regions associated with the processing of painful stimuli. Meta-analysis of human data from fMRI, EEG, magnetoencephalography (MEG), and positron emission tomography (PET) studies has shown that the commonest regions found to be active during an acute pain experience [19] are the SI and SII, thalamus, ACC, IC, and PFC, (comparable to areas that show decreased activity with nVNS in this study). Analysis of the group x time interaction showed a decrease in responses of the bilateral SI and SII somatosensory cortex, suggesting that nVNS mediates this signaling during the application of a thermal stimulus. These nVNS-mediated response changes in the SI somatosensory cortex strip match bilateral somatotopy to the lower leg, consistent with the placement of the Peltier heat probe. It is generally believed that somatosensory stimuli are processed primarily or preferentially by the hemisphere that is contralateral to the point of stimulation. However, evidence from clinical studies in patients with brain lesions, and from brain-imaging studies of noxious painful stimuli have called this theory into question [54]. Well-established brain regions that show bilateral activation upon the application of painful stimuli include the ACC, PFC, SII, insula, thalamus, and inferior parietal lobe [55–60]; and, in some instances, SI [55, 61–63]. It is likely that nVNS-mediated bilateral decreases in SI represent modulation of cortical context and or anticipatory neurocircuits. We postulate that the observed effects of nVNS on bilateral pain-processing pathways may represent bilateral nVNS afferent signaling effects; possible afferent to bilateral efferent effects on the thermal (and possibly nociceptive) signaling pathways of the spinal cord; or direct disruption of normal bilateral thermal and nociceptive afferent neural firing patterns that either independently or collectively change the temporal dynamics of pain processing.

## Post-Non-invasive vagus nerve stimulation with thermal stimuli; neural effects on left dorsoposterior insula

In addition to nVNS-mediated bilateral SI and SII responses, our analysis showed a unilateral decrease in the left dorsoposterior insula. The dorsoposterior insula exhibits an anterior-to-posterior somatotopic organization in response to innocuous or noxious/painful stimuli as measured with fMRI [64–68]. Various painful stimuli, including hypertonic saline injection [65], thermal stimuli [66], and laser stimuli [69], have consistently reproduced this anteroposterior somatotopy within the dorsoposterior insula; specifically, rostral targets (head/neck) localizing more anteriorly whereas caudal targets (leg) localizing posteriorly [70].

Dorsoposterior insular stroke results in discrete thermoanesthesia and analgesia that equivalently mapped anteroposterior somatotopy, further supporting the idea that the dorsoposterior IC plays a critical role in the pain experience [71–75]. Neuroanatomical data have demonstrated that the lamina I spino-thalamo-cortical pathway convey both nociceptive and interoceptive information mapped to the viscerosensory cortex in the posterior and mid-insular cortex, which is then represented in the anterior insula [76–78]. Surgically implanted vagal nerve stimulators (FDA-approved for treatment of resistant depression and epilepsy) consistently [79–84] modulate insular cortex activity, thus pointing to the insula as a possible neuromodulatory target for nVNS. Moreover, while insular activity is known to increase during acute VNS [79–81], recent work has shown a resultant decrease in insular activity at 10 to 15 minutes post-nVNS [43]. In our cohort, there was a significant left dorsoposterior insula decrease in activity 10 to 17 minutes post-VNS that further support the temporally dependent dose-response effects of VNS.

As a whole, the observed changes in the response to pain in the SI, SII, and left dorsoposterior insula with nVNS infer possible nVNS-mediated changes in neuronal firing patterns, either through direct brainstem effects, afferent cortical, or afferent cortical-to-efferent brainstem/spinal cord effects on nociceptive signaling.

## Post-Non-invasive vagus nerve stimulation with thermal stimuli; neural effects on bilateral mediodorsal thalamus and anterior cingulate cortex (area 24) (i.e. medial pain pathway)

Beside lateral thalamic nuclei projections (i.e., ventroposterior-lateral and ventroposterior-medial thalamic nuclei) to the SI and SII, known to relate the sensory-discriminative aspects of pain spinal pathways to limbic structures, the medial thalamic nuclei provide inputs to emotion-related brain areas, including the insula, ACC, amygdala, PFC, and other regions important in processing the affective-motivational dimension of the unpleasant pain experience [85]. In our study, the nVNS group showed a decreased response in the bilateral mediodorsal thalamus and dorsal ACC (Brodmann area 24) during the application of thermal stimulation, (with the group x time interaction). Prior clinical work shows that the mediodorsal thalamus is important in antinociceptive regulation [86], the processing of emotions [87], affective pain processing (pain unpleasantness) [66, 88, 89] [90–92],[87], thought to occur through mediodorsal thalamic connections with dorsal ACC (area 24). In an illustrative case study, a patient with a somatosensory cortex stroke that spared the dorsal ACC (area 24) and thalamus (including mediodorsal thalamus) reported usual contralateral limb analgesia to painful stimuli, but the patient continued to report an "unpleasant" feeling with the application of painful stimulus, suggesting *in vivo* separation of the affective and sensory discriminative pain pathways [89]. We observed mediodorsal thalamus and dorsal anterior cingulate deactivation in the nVNS group, which likely indicates a key mechanism of the effect of nVNS on the medial affective pain pathway, in agreement with previous studies [86, 93, 94]. Based on this remarkable (but preliminary) finding in a future study we will measure nVNS effects on affective pain (i.e. pain unpleasantness).

## Post-Non-invasive vagus nerve stimulation with thermal stimuli; neural effects on right orbitofrontal cortex

In addition to the medial dorsal thalamic connections to ACC, there are known medial thalamic projections to the PFC, ventromedial-prefrontal, and orbitofrontal (OFC) cortices [95, 96]. The group × time analysis in the current study showed decreases in the right OFC response, suggesting that nVNS mediates this signaling during nociceptive stimulation (Fig 3K

and Fig 3L). Prior clinical work has also demonstrated involvement of the prefrontal and frontal cortical regions in reflecting the emotional, cognitive, and interoceptive components of pain conditions, negative emotions, response conflicts, decision-making, and appraisal of unfavorable personal outcomes [97, 98]. Multiple pain-imaging studies have found that the frontal cortical regions are critical for controlling functional interactions among key brain loci that produce changes in the perceptual correlates of pain, independent of changes in nociceptive inputs [66, 99, 100]. Manipulating the cognitive aspects of pain, such as reappraisal, control, and coping, produce neural changes in the brain thought to be important in top-down processing. The lateral OFC expresses a contextual modulation of response that is widely implicated in emotional regulation and decision-making behaviors [101, 102], and it has been postulated that the valuation of pain is context-sensitive, as classified by the OFC [103]. Activity in the ventromedial cortex and the OFC has repeatedly been shown to be modulated by acute [80, 104–107] and chronic VNS [80, 104, 108]. In this cohort, we showed initial decrease in OFC activation during nociceptive thermal stimulation followed by an increase in OFC response (greater than sham) that was most evident at the post-thermal stimulation 10 to 15 second mark. This interesting finding suggests a decrease in the OFC affective appraisal of pain (0–6 seconds) followed by a subsequent late hemodynamic response increase that may reflect a resultant increase in pain-coping behavior. The observed nVNS-mediated decrease in the response of the OFC during the application of maximal noxious thermal stimulation is consistent with the results of prior VNS-treatment imaging studies, and suggests that the effect of nVNS on the OFC likely plays a role in the processing of painful and aversive stimuli.

## Non-invasive vagus nerve stimulation; combined neural effects and physiological measures (GSR)

The group × time × GSR analysis highlighted differential interactions among nVNS, GSR, and the temporal dynamic of pain responses in the cerebellum, medulla/brainstem nuclei, bilateral SI, and a right occipital gyrus cluster. In addition to cortical nodes, the mid and lower medullary brainstem have been shown to be important sites that demonstrate an interaction between sympathetic output and pain, with decreases in sympathetic output (as measured with cardiac vagal tone) shown to correlate with brain stem nuclei including: 1) RVLM, 2) Rt, 3) NAmb, 4) DMNX and 5) the RVM, (all found superior to the obex at the level of the olive spanning to lower medulla) [109]. In this study, medulla/brainstem clusters from sham and nVNS groups were separated into high and low mean GSRs. Only the sham treatment group showed a high GSR, demonstrated by greater activity in the medulla/brainstem, compared with other groups (sham low, nVNS high, nVNS low). At the level of the medulla, where this interaction is found (i.e., superior to the obex at the level of the olive from the lower pons, spanning to the lower medulla) multiple afferent fibers enter the brainstem, including the vagus nerve, and the glossopharyngeal, hypoglossal, and accessory nerves, as well as multiple nuclei and tracts (i.e., DMNX, NTS, NAmb, RVM, RVLM, and the Rt). In particular, the Rt is proposed to be primarily a pronociceptive center that integrates multiple excitatory and inhibitory functions important in nociceptive processing [110]. The premotor nuclei (i.e., NAmB and DMNX) are critical in autonomic response patterns evoked by physiological and sensory stimuli [111] that culminate in efferent parasympathetic outflow and play a crucial role in parasympathetic reflexes, accepting input from the NTS that is the principal nucleus for incoming afferent signals from the vagus nerve [112]. The RVM is intricately involved in areas of endogenous pain modulation in the brain, conveying descending pain modulatory influences from the PAG to neurons located in the dorsal horn of the spinal cord. The ON and OFF cells of the RVM increase or decrease activity during the application of painful stimuli, respectively [113], with

notable effects on descending pain-inhibitory circuits [114]. In sum, decreased activity in the medulla found in this study can be seen in reduced autonomic tone (reduced GSR in the lower GSR sham group), or through vagal nerve stimulation (in both nVNS groups, regardless of GSR (high or low)) suggesting that the default regulation of GSR can be decoupled through nVNS. We postulate that, even with increased GSR output (high GSR in the nVNS group), nVNS inhibits the response of the central nervous system to pain (in part) by blunting the response in key nuclei in the medulla that relay autonomic responses. Support for the relationship between the nVNS neural response and physiological response stems from altered autonomic sympathetic output (i.e., time to peak GSR and decrease in GSR slope). Future study is planned to examine this interaction in disease states such as Posttraumatic Stress Disorder or Major Depressive Disorder where dysfunctional emotional regulation and dysregulated autonomic output coincide.

## Non-invasive vagus nerve stimulation; autonomic measures and pain report

Time to peak GSR (i.e., time from GSR measured immediately prior to each 5 second noxious thermal stimuli to peak post-noxious thermal stimuli) in the nVNS group was more rapid than in the sham group, indicating changes in the temporal dynamics of pain processing and subsequent sympathetic output. The temporal dynamic of GSR during the application of a thermal stimulus is an important component of autonomic responsivity [21–23], and the subsequent emotional regulation of aversive stimuli [115, 116]. Loggia and colleagues demonstrated the existence of a dose-response relationship between the magnitude of a thermal stimulus and the time to peak GSR [24]. Specifically, their study showed that the greater the impact of a stressor (increased thermal temperature), the greater the rise in GSR, thus resulting in a longer time to peak response. In addition to the longer time to peak observed in the sham group, significant differences between the nVNS and sham groups in the slope of the GSR rise from baseline (prior to each noxious thermal stimuli to peak after noxious stimuli) for the latter thermal stimuli (T3-T5) were observed. In particular, the slope of the GSR response decreased across the length of the task in the nVNS group, whereas the slope of the response in the sham group increased. Taken together the longer time to peak and increase in GSR slope in the sham group compared to the nVNS group further suggest nVNS alters sympathetic output, possibly to due to the brainstem and cortical effects described.

We did not detect a statistically significant difference in subjective reports of pain for each thermal stimulus with a near maximal noxious thermal stimulus (S1 Fig). However there was a significant difference in the change in response between subjects who underwent nVNS vs sham stimulation across thermal stimuli (T2-T4) in reports of pain, as measured by the NPRS. The group that underwent sham stimulation showed a progressive increase in NPRS (across thermal stimuli T2-4), whereas the nVNS group demonstrated a significant decrease in NPRS (across thermal stimuli T2-4). To further characterize the effects of nVNS, additional work is needed that carefully measures affective pain, such as unpleasantness and catastrophizing, associated with the application of noxious thermal stimuli.

**Non-invasive vagus nerve stimulation potential temporal dependent effects on brain & pain.** Henry and colleagues [79] first argued (2002), that neural effects which occur during VNS are very different from those that occur after VNS, while others continue to confirm this phenomenon [43, 44, 117]. In this study, we showed that subjects in the nVNS group had nVNS-mediated activity decreases in the dorsoposterior insula, low medullary brainstem, medial thalamic, and ACC compared with subjects in the sham group (occurring 10 to 17 minutes after nVNS treatment). Similar to our post-nVNS effects observed on the low medullary

brainstem, Frangos and colleagues also show post-cervical transcutaneous VNS effects in this time frame, (13–15 minutes after stimulation), in posterior insula, lower medullary brainstem and medial thalamic/ACC deactivation at rest [44], that provide a convergence of preliminary evidence supporting a temporal nVNS dose-response curve [44]. In line with the aforementioned post-VNS neural effects, emerging clinical literature also demonstrate post-VNS antinociceptive effects [40, 118] while pronociceptive effects during VNS have also been reported [45, 46]. Both prior literature and this study suggest that the temporally dependent neural effects (i.e. during vs post-stimulation) of VNS may be critical to clinically relevant pro- or anti-nociceptive effects of VNS treatment, and therefore should be taken into account in future clinical study designs. Moreover, future studies are planned to determine the temporal dose response curve on affective pain processing that may also be of clinical import to guide efficacious use of VNS for clinical comorbid pain and psychiatric syndromes.

## Limitations

Our work has some important limitations. The study was carried out in healthy control subjects. Because, as a pilot study, we involved only 15 subjects per group, the small sample size may not adequately represent a larger population. Therefore, our results as described here should be considered preliminary. However, the positive findings observed in this small cohort of healthy control subjects were robust and significant, warranting further investigation of the effects of cervical transcutaneous nVNS on the brain in a larger cohort of healthy control subjects, and in subjects who may experience a greater magnitude of affective pain subtypes, that may include Posttraumatic Stress Disorder or Major Depressive Disorder. Our study found significant neural alterations in the temporal dynamics of noxious thermal-stimuli processing known to be important in affective pain processing, and group differences in changes in the subjective pain report across the thermal stimuli (T2-T4). But we did not detect a statistically significant difference in subjective reports of pain for each thermal stimulus with a near maximal noxious thermal stimulus (S1 Fig). We chose a near maximal noxious thermal stimulus to ensure clear autonomic responses (GSR). Our own work [48] as well as that of other studies has described maximal noxious stimuli that result in maximal reports of pain and, therefore, blunting of group differences in mean reports of pain (i.e. a ceiling effect on pain report) [119–121] [122]. This phenomenon also could have occurred in this study. Future studies that measure affective pain (such as pain unpleasantness and catastrophizing) using maximal and submaximal noxious thermal stimuli are now needed to further characterize the antinociceptive effects of nVNS, as measured by reports of pain. While we correct for motion artifact at the brainstem level with the (Group x time x GSR) interaction, this area can be artifact-prone due to motion and decreased spatial resolution. Although others have shown a similar pain and autonomic tone interaction at the same medullary brainstem level (Sclocco and colleagues [109]) future study is planned in larger cohorts and with high spatial resolution multiband imaging to confirm this interaction at this brainstem level.

## Conclusion

We examined the neural effects of nVNS during a noxious thermal stimulus challenge, in the context of autonomic responses. We demonstrated 3 major findings; first, nVNS activity not only reduces peak responses to thermal stimuli in the SI, SII, medial thalamus, dorsal anterior cingulate (area 24), dorsoposterior insula, and OFC, which are important nodes in sensory discriminative pain, affective emotional pain, and interoception pathways, but also changes temporal dynamic responses within these nodes. Second, nVNS alters autonomic responses to noxious thermal stimuli, as measured by GSR, and therefore affects critical autonomic pain

networks. Third, even with a higher GSR response being provoked by the application of noxious thermal stimulus, nVNS decreased the central nervous system response by blunting the usual reactions in key nuclei in the medulla that relay autonomic responses. These significant findings may improve effectual nVNS that, if tuned with careful dose-response curves in mind, could translate into efficacious targeted effects on pain and autonomic neural circuits.

## Supporting information

**S1 Fig. nVNS versus sham numerical pain rating with maximal noxious thermal stimuli.** After either nVNS or sham stimulation, 5 successive noxious thermal stimuli were applied (up to 49.8˚C) for 5 seconds each (T1-T5). Mean pain, as reported by subjects using the numerical pain rating scale (NPRS) after each noxious thermal stimulus did not differ between the sham and nVNS groups. Both groups had lower NPRS scores at T5 compared with T1 (NPRS decreased by -0.678 ± 0.209; $t$ = -3.241; $p$ = .002). In contrast to findings for the nVNS group, subjects who underwent sham stimulation had a positive slope in NPRS scores across thermal stimuli (i.e. the change in NPRS score with successive noxious thermal stimuli T1-T5) for T2 to T4 that was significantly different (slope in the sham group, 0.150 ± 0.122; vs the slope in the nVNS group, -0.233 ± 0.122; $p$ = .0301) and also approached significance from T1 to T4 (sham group, 0.010 ± 0.847; vs nVNS group, -0.203 ± 0.847; $p$ = .0785). Red circles = nVNS group. Blue circles = sham group. $^{*}p < .05$; $^{\delta}p < .08$.
(DOCX)

**S1 Table. Between-group comparisons for the time to peak and absolute mean GSR.** The nVNS group showed significant decreases in the time to peak GSR for T1 and T2. There was no difference in GSR absolute mean change between groups for all time points examined.
(DOCX)

**S2 Table. Within-group comparisons for the time to peak GSR and absolute mean GSR for the sham stimulation group.** In the sham group, the time to peak GSR increased from T1 to T4 and T5. The mean GSR measured after the application of noxious thermal stimuli consistently increased from T2 to T5 and from T1 to T4.
(DOCX)

**S3 Table. Within-group comparisons for the time to peak GSR and absolute mean GSR for the nVNS group.** In the nVNS group, the time to peak GSR did not change between each successively applied noxious thermal stimulus. The mean GSR measured after each noxious thermal stimulus did not increase from T4 to T5, or from T1 to T2.
(DOCX)

**S1 File. Inclusion and exclusion criteria. Heat tolerance and threshold measurements. Correlations between autonomic tone and pain reports.** This file contains information on inclusion exclusion criteria, a description of heat tolerance and threshold measurements and finally pertinent correlations between autonomic tone and pain reports.
(DOCX)

**S1 Data Set. Demographic and pain data sets.** This data set contains demographic information and pain rating data sets.
(XLSX)

**S2 Data Set. fMRI and gsr measures during thermal stimulus.** This data set contains in MRI scanner GSR measures in sham and nVNS groups during five thermal stimuli.
(XLSX)

**S3 Data Set. fMRI cluster data sets for all subjects GroupXTime.** This data set contains cluster results for group × time analysis of noxious thermal stimuli.
(CSV)

**S4 Data Set. fMRI cluster data sets for all subjects GroupXTimeXGSR.** This data set contains cluster results for group × time x GSR analysis of noxious thermal stimuli.
(XLSX)

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# Clinical Effectiveness of Percutaneous Auricular Vagus Nerve Stimulation in Chronic Back Pain Patients – A Single-Centre Retrospective Analysis

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# Clinical Effectiveness of Percutaneous Auricular Vagus Nerve Stimulation in Chronic Back Pain Patients - A Single-Centre Retrospective Analysis

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## Abstract

**Objectives:** Chronic back pain is one of the biggest causes of disability today. The objective of this study was to evaluate the safety and effectiveness of percutaneous auricular Vagus Nerve Stimulation (pVNS) for chronic back pain patients in routine clinical practice.

**Methods:** Data were retrospectively sourced from a clinical database. Mean reduction in average and maximum pain intensity at three weeks as compared to baseline using Numeric Rating Scale (NRS) pain intensity was assessed. A patient responder was defined as having at least 50% improvement in average NRS pain intensity, assessed at 1-, 3- and 6-weeks, as well as 3 months. In addition, analgesic intake, subjective well-being and number and type of Adverse Events (AEs) were reported.

**Results:** A total of 148 patients underwent pVNS stimulation and met all inclusion criteria. Average NRS pain intensity significantly decreased from 6.36 ± 2.18 at baseline to 3.25 ± 1.83 ($p<0.001$) at three weeks of treatment. One week into treatment, the responder rate was 32.4%, while reaching a maximum of 58.8% at six weeks of treatment. 60% of patients taking opioid analgesics at baseline were able to decrease or stop their opioid usage. Reported AEs were mild and pVNS was well-tolerated.

**Discussion:** Our results suggest that pVNS may be a safe and effective adjunct treatment for difficult to treat chronic back pain patients. Given the retrospective nature of this study, further research is warranted to confirm these findings.

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## Introduction

Chronic pain conditions are by far the biggest cause of disability today [1]. Estimates suggest that every second person in the EU will suffer from back pain at some point in their life. 15% of these patients will be on sick leave for one month or longer because of their condition [2,3]. Besides the personal dimension, this generates costs to the European Union of up to 441 billion Euros each year [4]. For the US, the economic burden is in the range of €468 to €530 billion per year, including both the cost of healthcare and loss of productivity [5].

The current standard of care following international guidelines suggests as first-line therapy the use of acetaminophen and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) [6,7], and under specific conditions short courses of skeletal muscle relaxants or opioid analgesics, in conjunction with non-pharmacological strategies such as multidisciplinary rehabilitation, cognitive-behavioral therapy, or acupuncture. Given the fact that those therapies may often provide only mild symptomatic improvements [6], big efforts are currently put in towards finding adjunct, non-pharmacologic treatment options.

With the recent advances in bioelectronics, growing evidence suggests that neurostimulation of the vagus nerve may be used to modulate nociception and pain perception [8,9]. Vagus nerve stimulation using implantable neurostimulation devices is used for the treatment of refractory epilepsy and major depression [10,11]. Disadvantages of implantable systems are frequent Adverse Events (AEs) due to surgical procedure and stimulation of efferent vagus nerve fibers (e.g., hoarseness,

sore throat, shortness of breath and coughing). Non-invasive or minimally-invasive stimulation techniques can mitigate these disadvantages [12]. Percutaneous auricular Vagus Nerve Stimulation (pVNS) allows for a minimal-invasive electrical stimulation of the auricular branch of the vagus nerve [13]. Several small studies and randomized controlled trials have demonstrated safety and efficacy of pVNS in producing antinociceptive effects for various pain conditions [9]. These conditions include postoperative acute pain, chronic low back pain, or cervical syndrome. Sator-Katzenschlager et al. [14], conducted a randomized control trial in patients with chronic low back pain and found over 70% reduction in pain intensity in those patients receiving auricular electrical stimulation over six weeks as compared to sham. Pain reduction came along with reduced intake of opioid rescue medication (over 95% reduction in intake of tramadol), as well as improved quality of sleep, well-being, and physical activity. All positive effects sustained up to a 12 weeks' follow-up. Similarly, a high trial success rate was observed in patients with chronic cervical pain during a six-week therapy [15]. So far, there are no studies investigating the clinical safety and effectiveness of pVNS in a larger cohort of patients in clinical routine.

This retrospective study aims at evaluating the safety and effectiveness of pVNS in chronic back pain patients that had previously failed first-line therapy, in a real-world clinical setting.

## Materials and Methods

### Study design and procedures

This was a monocentric, retrospective data analysis study. Data for this study were drawn from medical records of all attending patients who were trialed and/or treated for pain with pVNS at the outpatient clinic for special pain therapy at the Medical University of Vienna, Department of Surgery (Vienna, Austria), from February 2002 to June 2010. Combined, a total of 349 patients underwent treatment with pVNS. The study was approved by the ethics committee at the Medical University of Vienna (1789/2020). All authors had full access to the study data.

### Inclusion/Exclusion criteria

Patient data were included in an Intention-To-Treat (ITT) analysis if the patient met the following criteria: (1) Adult patients 18 years of age or older with a history of back pain, meeting the diagnostic criteria listed in ICD-10 M54 (2019); (2) have not had an adequate response to first-line pharmacological therapy with acetaminophen, NSAIDs, and/or opioid analgesics; (3) plausible pain diary documentation, i.e., Numeric Rating Scale (NRS) scorings higher or equal than 0 and smaller or equal 10, maximum > average > minimum NRS scorings; and (4) received at least one pVNS therapy, which included a minimum of two documented visits (baseline and one consecutive therapy visit, with a maximum of 21 days in between the two visits).

Patient data were included in a Per Protocol (PP) analysis if the patient additionally had: (1) At least four documented consecutive visits (baseline and three consecutive therapy visits); and (2) the interval between two visits was between three and eight days.

### Stimulation procedure

pVNS was performed using P-STIM (Biegler Medizinelektronik GmbH, Mauerbach, Austria). P-STIM is a single-use miniaturized (Figure 1), battery-powered, percutaneous electrical stimulator with a pre-programmed amplitude (3.8 V), stimulation frequency (1 Hz), pulse width (1 ms), and duty cycle (3 h ON/3 h OFF). The procedure has been described previously in [14,15]. Needles were positioned in the cymba and cavity of concha as well as the crura of antihelix, i.e., regions partly or solely innervated by the auricular vagus nerve [16,17]. Positions were chosen close to local blood vessels, running in parallel or close to targeted nerve fibers [9,18-19]. Each patient received pVNS continuously over a period of four days a week. At each therapy visit, a new device was applied.

### Data collection and outcome measures

Standardized data, collected by clinical personnel under supervision of the first author, at baseline and/or at each scheduled therapy visit, were retrieved retrospectively from medical records at the Medical University of Vienna. Baseline data refer to the data collected at the time of patient consent prior to pVNS treatment. These included: patient sex, age at start of the therapy, medical history, presenting pain symptoms, and pain severity on a NRS 11-point scale (from 0 = no pain to 10 = worst imaginable pain; [20]). From the last visit, additional variables were extracted related to number and type of AEs, demand for additional medication (i.e., increased, decreased or unchanged), and change in subjective well-being on a 6-point scale (from 0 = very good to 5 = very bad).

The primary endpoint of the study was the mean reduction in average and maximum NRS pain intensity at three weeks as compared to baseline in the PP analysis. Secondary endpoints were: (1) Percentage of patients achieving different thresholds of pain relief in maximum and average NRS pain intensity compared to baseline [21], i.e., ≥ 30% (moderate), ≥ 50% (substantial), and ≥ 80% (which we defined as extensive improvement), at one week, three weeks, six weeks, and three months; (2) percentage of patients decreasing or not requiring additional analgesic medication as a result of the treatment; (3) change in subjective well-being; and (4) number and type of AEs.

### Statistical analysis

Analyses were conducted in both PP group (i.e., patients completing primary endpoint assessment) and ITT group (i.e., patients who were administered the treatment at least once).

For the PP analysis, if a patient visited the outpatient clinic more than once per week, the data from the last visit of that week was taken. All other data from that week were omitted. For the ITT analysis, only the baseline and the last visit of a patient were considered. If a patient visited the outpatient clinic irregularly with a break of more than 21 days between two consecutive visits, only the data up to this point were considered. All the data after the break were omitted. For the ITT analysis, missing data for time points after the last visit of a patient were imputed using last observation carried forward. A decrease of ≥ 50% in average NRS pain intensity was considered significant. Patients reaching this improvement were called responders. Responder analysis was performed for two time points, i.e., after one week and after three months of treatment.

NRS pain intensity is presented as mean ± standard deviation, unless otherwise stated. Comparison between baseline and therapy visits was performed using $\chi^2$-tests and paired t-tests. To compare responders and non-responders, a Welch t-test and $\chi^2$-test were performed. Threshold for significance of statistical comparisons was set to $p<0.05$. Bonferroni correction was used for multiple comparisons. AEs and medication usage were reported descriptively for all patients. Statistical analysis was done using Python 3.7.4 with NumPy 1.18.1 and SciPy 1.4.1.

**Table 1:** Patient characteristics at baseline.

| | ITT group (n=148) | PP group (n=59) |
|---|---|---|
| Age (years) | 62.9 ± 15.7 | 64.3 ± 13.9 |
| Number of female/male patients | 96/52 | 39/20 |
| Dorsalgia (ICD-10, M54) (%) | 100 | 100 |
| Radiculopathy (M54.1) | 5.4 | 8.5 |
| Cervicalgia (M54.2) | 18.2 | 16.9 |
| Lumbago with sciatica (M54.4) | 36.5 | 33.9 |
| Low back pain (M54.5) | 23.6 | 22.1 |
| Dorsalgia, unspecified (M54.9) | 16.3 | 18.6 |
| NRS Max ± STD | 7.49 ± 1.94 | 7.42 ± 1.88 |
| NRS Mean ± STD | 6.56 ± 2.15 | 6.40 ± 2.36 |
| NRS Min ± STD | 5.52 ± 2.60 | 5.27 ± 2.88 |

Data are expressed as n, mean ± STD or n (%). ITT: Intention-to-Treat group; PP: Per Protocol group; NRS: Numeric Rating Scale; STD: Standard Deviation

# Results

## Patients and baseline statistics

During the study period, a total of 349 patients were treated with pVNS at our institution. Patients presented to the outpatient clinic with a range of chronic pain conditions, including back pain (51%), abdominal pain (4.3%), pain localized to other parts of lower abdomen (3.2%), shoulder pain (3.7%), postoperative pain (3.7%), migraine (2.3%) and other complex pain patterns (31.8%, either different location or not sufficiently documented). Of the total 349 patients, 171 patients were excluded due to not meeting the diagnostic criteria listed in ICD-10 M54 (dorsalgia). From the remaining 178 chronic back pain patients, 30 had to be excluded due to a missing therapy visit within 21 days after the baseline visit. The remaining 148 patients met all inclusion criteria and constituted the ITT population. Of those patients, 59 (39.9%) met the PP criteria. Patient baseline characteristics and demographics are summarized in Table 1.

Patients for the ITT analysis were 62.9 ± 15.7 years of age, 64.9% female. Among these, 36.5% suffered from lumbago with sciatica, 23.6% from low back pain, 18.2% from cervicalgia, 16.3% from unspecified dorsalgia, and 5.4% from radiculopathy. The minimum, average and maximum NRS pain intensity at baseline was 5.52 ± 2.60, 6.56 ± 2.15, and 7.49 ± 1.94, respectively. Baseline characteristics for the PP population were comparable to those of the ITT population (Table 1).

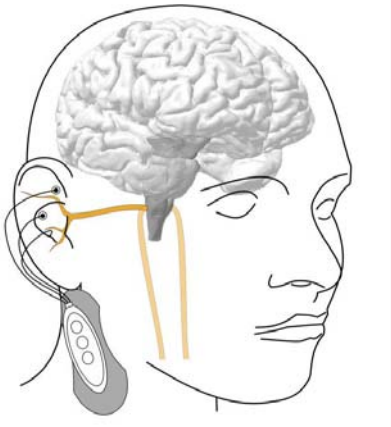

**Figure 1:** P-STIM® device.

## Pain reduction and responder rates

**Per protocol (PP) analysis:** Maximum and average NRS pain intensity decreased significantly over the first three weeks of treatment in the PP analysis (n=59), as shown in Figure 2. Average NRS pain intensity decreased from 6.36 ± 2.18 at baseline to 4.31 ± 1.70 ($p<0.001$) at one week, to 3.68 ± 2.20 ($p<0.001$) at two weeks, and to 3.25 ± 1.83 ($p<0.001$) at three weeks. Similarly, the maximum NRS pain intensity decreased from 7.42 ± 1.88 at baseline to 6.41 ± 1.99 ($p=0.002$) at one week, 5.25 ± 2.58 ($p<0.001$) at two weeks, and 4.88 ± 2.55 ($p<0.001$) after three weeks.

**Intention-to-treat (ITT) analysis:** Pain intensity changes from baseline to the last therapy visit of each patient in the ITT analysis (n=148) were analyzed for four separate time points (one week, three weeks, six weeks, and three months), with regards to the percentage of patients experiencing an average and maximum NRS pain intensity reduction of ≥ 30%, ≥ 50% and ≥ 80%, respectively.

As shown in Table 2 and Figure 3, the percentage of patients achieving more than 30% reduction in average NRS pain intensity increased from 51.4% after one week to 70.3% at three weeks and remained relatively constant at six weeks (72.3%) and three months (75.0%). Similarly, 32.4% of all patients in the ITT population exhibited a ≥ 50% improvement of average NRS pain intensity after one week, 49.3% at three weeks and 58.8% at six weeks. The proportion of patients achieving a ≥ 80% improvement in average NRS pain intensity increase slower from 7.4% at one week to 20.3% at six weeks and 25% at three months. The ratio of patients with complete symptom remission increased from 3.4% at one week to 14.2% at three months. In contrast, ratio of patients not improving over the treatment decreases from 31.1% at one week to 11.5% at three months (Figure 2). A similar behavior could be seen for maximum NRS pain intensities, showing a smaller relative reduction from baseline to the last study visit (Table 2).

In this study, responders were defined as patients showing an average NRS pain intensity reduction of at least 50%. When comparing responders with non-responders for one week and six weeks of treatment, significant differences in the baseline NRS pain intensity values of these groups can be found (Table 3). Responders at six weeks had significantly higher minimum NRS pain intensities (6.10 ± 2.45 *vs.* 4.69 ± 2.57, $p=0.014$), average NRS pain intensities (7.06 ± 2.06 *vs.* 5.85 ± 2.07, $p=0.009$), and maximum NRS pain intensities (7.89 ± 1.89 *vs.* 6.92 ± 1.88, $p=0.003$) compared to non-responders. In contrast, this was not the case when comparing baseline values of responders and non-responders at one week of treatment.

## Medication and adverse events

Patients were subject to various pharmacological therapies, prior to pVNS treatment, including the use of acetaminophen (4.3% of all reported medication), NSAIDs (48.7%), muscle relaxants (5.1%), anticonvulsants (4.3%), opioid analgesics (18.8%), and others (18.8%). In 45.3% of patients we had detailed reporting on concomitant medication. From these patients, 26.9% were able to discontinue their pain medication, 22.4% reduced intake, 40.3% did not change, and 10.4% increased their medication intake. Opioid analgesics were taken by 29.9% of patients at baseline. 60% of those patients were able to decrease or stop their opioid usage during pVNS treatment.

Subjective well-being was available for 36.5% of patients. On average, subjective well-being improved by 1.89 ± 1.66 points.

In general, reported AEs were mild and pVNS treatment was

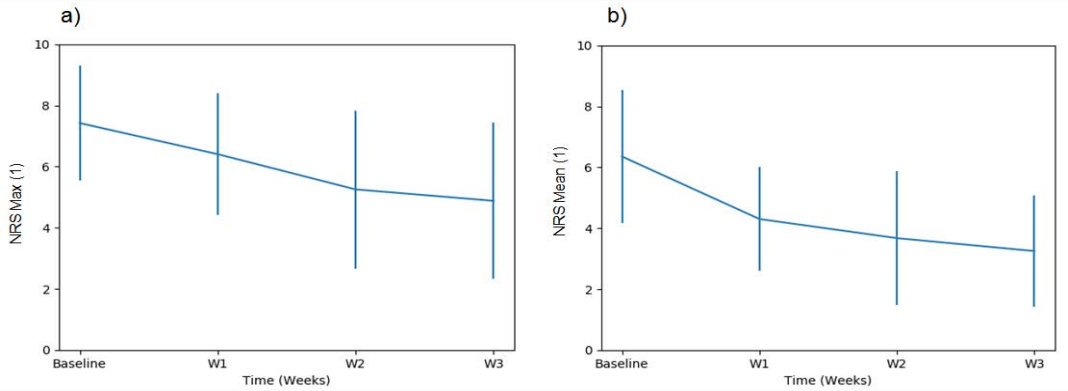


**Figure 2:** Longitudinal (a) maximum and (b) average NRS pain intensity for chronic back pain patients (n=59) over three weeks of pVNS treatment (mean ± standard deviation).

**Table 2:** Percentage of patients reaching a 30%, 50%, and 80% improvement in maximum and average NRS pain intensity at timepoints (one week, three weeks, six weeks, and three months) of pVNS treatment.

| ITT group (n=148) | 1 week | 3 weeks | 6 weeks | 3 months |
|---|---|---|---|---|
| Max NRS 50% reduction (%) | 13.5 | 32.4 | 41.2 | 46.6 |
| Average NRS 50% reduction (%) | 32.4 | 49.3 | 58.8 | 58.8 |
| Max NRS 80% reduction (%) | 3.4 | 12.8 | 14.9 | 17.6 |
| Average NRS 80% reduction (%) | 7.4 | 18.2 | 20.3 | 25 |
| Max NRS 30% reduction (%) | 29.7 | 47.3 | 55.4 | 56.1 |
| Average NRS 30% reduction (%) | 51.3 | 70.3 | 72.3 | 75 |

NRS: Numeric rating scale; ITT: Intention-to-Treat group

**Table 3:** Baseline NRS scores in the responder and non-responder groups (50% reduction in average NRS pain intensity) after 1 week and 6 weeks of pVNS treatment.

| ITT group (n = 148) | NRS baseline responders | | NRS baseline non-responders | |
|---|---|---|---|---|
| | 1 Week (n=48) | 6 Weeks (n=87) | 1 Week (n=100) | 6 Weeks (n=61) |
| NRS Max ± STD | 7.81 ± 1.86 | 7.89 ± 1.89 | 7.33 ± 1.97 (p=1.84) | 6.92 ± 1.88 (p=0.033) |
| NRS Mean ± STD | 7.13 ± 1.90 | 7.06 ± 2.06 | 6.29 ± 2.21 (p=0.25) | 5.85 ± 2.07 (p=0.009) |
| NRS Min ± STD | 6.23 ± 2.29 | 6.10 ± 2.45 | 5.18 ± 2.67 (p=0.19) | 4.69 ± 2.57 (p=0.014) |

NRS: Numeric Rating Scale; STD: Standard Deviation; ITT: Intention-to-Treat group

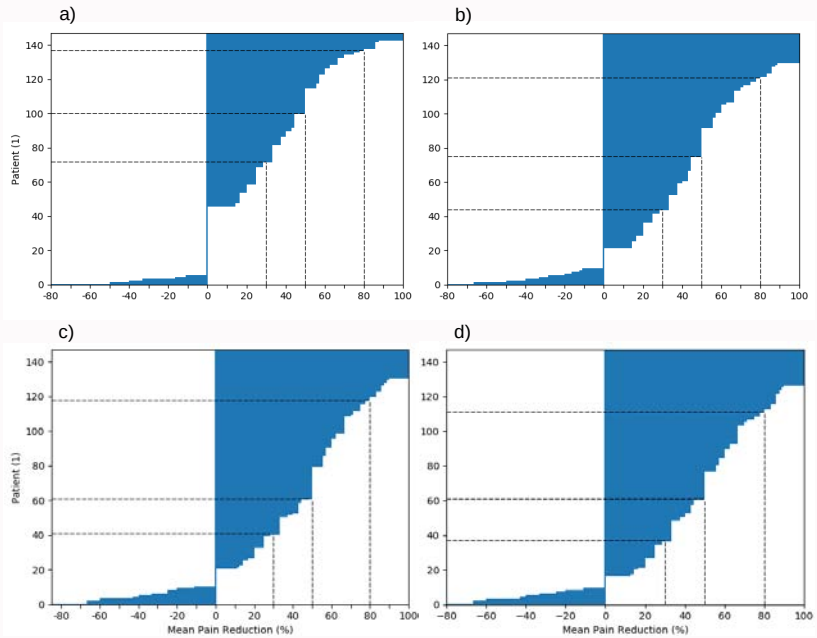


**Figure 3:** Percentage improvement of patients in average NRS pain intensity at the last therapy visit compared to baseline at (a) one week, (b) three weeks, (c) six weeks, and (d) three months of pVNS treatment. Lines indicate patient populations with improvements of ≥ 30%, ≥ 50%, and ≥ 80%.

well-tolerated. Twenty patients experienced an unwanted device disconnection during therapy requiring re-affixation, fifteen patients did not perceive the stimulation at some point of therapy, four patients developed skin irritations due to device application on the neck, and one patient each experienced decreased quality of sleep, dizziness, headache, and pain at stimulation site in the ear, all mild and transient. Twenty patients reported improved motility, three patients reported improved quality of sleep, and one patient reported reduced anxiety. No unexpected side effects were reported. Six patients discontinued therapy, four patients due to insufficient pain reduction, one due to skin irritations on the neck at device application site, and one due to cost of therapy.

### Treatment duration and compliance

From 148 patients, 106 (71.6%) used the device regularly for three weeks, declining to 61 (41.2%) for six weeks, and 28 (18.9%) for three months. Considering the results presented above, with responder rates of roughly 30% at one week, 49% at three weeks, and 59% at six weeks, this indicates moderate compliance with treatment. The duration of therapy for individual patients varied greatly, from 1 day up to 568 days. However, in median each patient had 31 (14-56, 25$^{th}$ to 75$^{th}$ percentile) stimulation days with 8.05 ± 10.01 therapy visits. In addition, the mean interval between two therapy visits was 8.14 ± 3.25 days.

## Discussion

This work constitutes the first study to date evaluating clinical safety and effectiveness of pVNS for patients with difficult to treat chronic back pain in a routine clinical setup. In a total of 148 patients we showed that 32.4% of patients experienced at least 50% improvement in average NRS pain intensity immediately after the first week of treatment, while the responder rate reached a maximum of 58.8% at six weeks of treatment. Additionally, several patients reached full symptom remission, decreased their analgesic usage, and increased their subjective well-being. Thus, pVNS may elicit fast and clinically meaningful responses with a low side-effect profile in this group of chronic back pain patients.

Comparison with other studies on pVNS in chronic pain conditions is difficult, because of inhomogeneous trial designs [9]. A reduction in average NRS pain intensity at six weeks of adjuvant pVNS treatment for chronic cervical pain patients could be shown in [15]. Similarly, a high trial success rate with pVNS was observed in patients with chronic low back pain [14], in comparison to traditional manual auricular acupuncture as sham treatment. The present data extends above findings and shows a clinically significant improvement in a rather inhomogeneous clinical cohort over a comparable timespan of several weeks.

Using the IMMPACT's benchmarks for identifying clinically important changes in pain intensity outcome measures [21], the maximum benefit for patients with a ≥ 30% and ≥ 50% response occurred at three or six weeks of therapy, respectively, and thereafter leveled off, which is in line with published data on Spinal Cord Stimulation (SCS; 22,23), but seems to contradict data on the slow accrual of clinical benefits over time reported in VNS studies in epilepsy, chronic migraine and depression [24-26]. Our data might suggest that participants, who do not achieve minimal or substantial improvement within the first six weeks of treatment, are likely to discontinue the pVNS treatment. In contrast, participants who continued treatment may represent self-identified responders for whom the device is effective, whereas a long-term use of pVNS in treatment responders would be fully justifiable (i.e., beyond 3 months or longer). Hence, a six weeks' timeframe might allow a physician to separate responders from non-responders and to decide on more accurate treatment strategies (i.e., continuing or switching the therapy).

The modulation of nociception and pain perception by pVNS is suggested to be highly dependent on the specific electrical stimulation pattern and localization of stimulation [9,13]. In this study, stimulation amplitude was fixed and mostly produced a tingling (but not painful) sensation at the stimulation region. In particular, pVNS targets Aβ-fibers responsible for cutaneous mechanoreception and touch sensation while avoiding activation of Aδ-fibers, which are involved in affective-emotional pain functions [9]. The frequency of stimulation of 1 Hz was used to interfere positively with the bodies' own cardiac rhythm, facilitating stimulation effects. For instance, the positive influence of the timing between pVNS and the respiratory cycle in pain reduction was demonstrated earlier [27-29].

Furthermore, several patients either substantially reduced or completely abolished analgesic intake, whereas some patients even reported that they stopped or cut down their use of opioid analgesics. Similar results have been described in the pVNS literature for opioid analgesics such as tramadol [14,15], remifentanil [36], morphine hydrochloride [30], naproxen and tramadol and morphine [31,32]. In addition, pVNS reduced anesthetic requirements in response to noxious electrical stimulation, as shown in a clinical trial in [33], and reduced analgesic medication intake after abdominal and accident/trauma surgery, as shown by a case series in Szeles et al. [34] and Qureshi et al. [35].

The lack of AEs typically seen with implantable VNS such as hoarseness, sore throat, shortness of breath, and coughing, might be a factor positively influencing patient's compliance, long-term pain control, and an improvement in function in patients who received pVNS therapy. Similar to our study, many studies have shown that pVNS is a safe therapy in treating chronic pain, with AEs being generally minor and transient [14,15,36-39].

## Study Limitations

As a retrospective investigation of standard clinical practice, this study has several limitations. Since administration in clinical practice is less rigorous than in clinical trials, documentation of outcomes and data from patient follow-up were sometimes inconsistent. Because pain scores were self-reported and assessed in a non-blinded manner, there is a possibility that positive responses regarding the outcome of pVNS treatment were over-reported or under-reported and as such these results should be interpreted with caution. In addition, some patients had a full set of scores for pain, medication, and subjective well-being, whereas others did not. Both factors resulted in an inhomogeneous data set with a declining patient number throughout follow-up, which could hide a sub-cohort of non-responders, potentially biasing the presented outcome. An alternative explanation may be that, if a patient is doing well, they may not feel the need to attend more therapy sessions. In such scenario, there would be a significant potential for under reporting successful clinical outcomes. Whereas randomized controlled trials unquestionably hold many advantages over retrospective studies, the current study serves the purpose of assessing the clinical effectiveness of pVNS treatment in difficult to treat patients seen in general practice, contributing to previous knowledge.

## Conclusion

pVNS treatment led to rapid clinically meaningful pain relieve in patients with chronic back pain that improved with time on treatment. Already after one to six weeks of treatment, substantial reductions in average and maximum pain intensity were observed, along with a decreased need for analgesic medication. Our results suggest that pVNS may be a safe and effective adjunct treatment for difficult to treat chronic back pain patients.

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## Conflict of Interest

We have following conflicts of interest to disclose: J.C. Széles, S. Kampusch, E. Kaniusas are shareholders of SzeleSTIM GmbH. S. Kampusch, E. Kaniusas, V.H. Le are employed at SzeleSTIM GmbH. All other authors declare no competing interests.

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## Author Contributions

JCS, SK, EK, and CN contributed to conception and design of the study. JCS and SK wrote the first draft of the manuscript. JCS, SK, VHL, and DPE performed data collection. JCS, SK, VHL, DPE, EK, and CN performed data analysis and interpretation. JCS, SK, and VHL performed statistical analysis of the data. All authors contributed to manuscript revision, read and approved the submitted version.

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# Evoked Pain Analgesia in Chronic Pelvic Pain Patients using Respiratory-gated Auricular Vagal Afferent Nerve Stimulation

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## Abstract

**Objective—**Previous Vagus Nerve Stimulation (VNS) studies have demonstrated anti-nociceptive effects, and recent non-invasive approaches; termed transcutaneous-VNS, or t-VNS, have utilized stimulation of the auricular branch of the vagus nerve in the ear. The dorsal medullary vagal system operates in tune with respiration, and we propose that supplying vagal afferent stimulation gated to the exhalation phase of respiration can optimize t-VNS.

**Design—**counterbalanced, crossover study.

**Patients—**patients with chronic pelvic pain (CPP) due to endometriosis in a specialty pain clinic.

**Interventions/Outcomes—**We evaluated evoked pain analgesia for Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) compared with Non-Vagal Auricular Stimulation (NVAS). RAVANS and NVAS were evaluated in separate sessions spaced at least one week apart. Outcome measures included deep tissue pain intensity, temporal summation of pain, and anxiety ratings, which were assessed at baseline, during active stimulation, immediately following stimulation, and 15 minutes after stimulus cessation.

**Results—**RAVANS demonstrated a trend for reduced evoked pain intensity and temporal summation of mechanical pain, and significantly reduced anxiety in N=15 CPP patients, compared to NVAS, with moderate to large effect sizes ($eta^2$>0.2).

**Conclusion—**Chronic pain disorders such as CPP are in great need of effective, non-pharmacological options for treatment. RAVANS produced promising anti-nociceptive effects for QST outcomes reflective of the noted hyperalgesia and central sensitization in this patient population. Future studies should evaluate longer-term application of RAVANS to examine its effects on both QST outcomes and clinical pain.

## Introduction

Previous Vagus Nerve Stimulation (VNS) studies have demonstrated anti-nociceptive effects [1], particularly in patients with depression [2]. However, moderate morbidity has been associated with the surgical procedure and maintenance of VNS [3]. Furthermore, it is

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still unclear whether VNS is an analgesic treatment in general or for a specific chronic pain syndrome. In this study, we propose a novel, non-invasive procedure based on the neurobiology of VNS treatment - Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS), which synchronizes stimulation to the respiratory cycle. The auricular branch of the vagus nerve extends to the pinna of the ear and can be electrically depolarized with minimal invasiveness, a procedure referred to as transcutaneous-VNS, or t-VNS [4, 5]. Respiration is known to cyclically modulate activity in both input and output vagal brainstem regions. Hence, the brainstem vagal input-output system operates in tune with respiration and t-VNS can be synchronized with respiratory events to better optimize stimulation, which may improve the analgesic benefits of VNS.

Multiple studies have suggested that VNS can produce anti-nociceptive effects. Studies in a rat model have linked stimulation of vagal afferents with antinociception [6, 7]. Both animal studies [8] and a recent study in humans [9], suggest that *during* active VNS, pro-nociception can occur when stimulus intensity is low, but anti-nociceptive effects predominate when stimulus intensity is high (non-noxious, detectable stimulation, in mA range). Moreover, Kirchner et al. have found in humans that chronic VNS (at mean 0.7 to 1.4mA) raises pain thresholds for both tonic pinch and heat pain, as well mitigating pain wind-up phenomenon for mechanical stimuli [10, 11]. These results demonstrate promising analgesic effects of VNS, although it is unclear whether findings involving implanted vagal stimulators in patients with intractable seizure disorders will generalize to trials of t-VNS in patients with chronic pain.

Classical VNS involves surgery, with the stimulator lead implanted within the carotid sheath, wrapped around the vagus nerve in the left neck [12]. This can induce morbidity stemming either from co-activation of *efferent* vagal fibers (e.g. bradycardia, asystole [13], larynx/pharynx disorders [14], dysphagia [15]), or from infection or hardware failure [15]. Ultimately, as the mechanisms for VNS likely involve afferent, and not efferent vagal fibers [16], isolation of afferent fibers in vagal stimulation would eliminate potential negative effects due to efferent stimulation, while accessing these fibers without surgical intervention would eliminate infection-associated morbidity. In sum, there are many advantages to a minimally invasive and less costly vagal nerve stimulation device, which would serve to benefit a larger number of chronic pain patients.

The analgesic mechanisms of VNS have not been fully elucidated, but are likely mediated by afferent (not efferent) input to supraspinal brain regions [16]. Vagal afference is relayed to the nucleus tractus solitarious (NTS) in the medullary brainstem. Importantly, the NTS also receives somatosensory afference via the auricular branch of the vagus (ABV) nerve from specific portions of the auricle [17]. ABV afference is transmitted to both the NTS [17] and the spinal trigeminal nucleus (SpV) [18], by neurons located in the superior (jugular) ganglion of the vagus nerve. Respiration can modulate NTS activity directly (the lungs are innervated by the vagus nerve) and indirectly. In regard to the latter, inspiration increases venous return to the thorax, which increases arterial pressure, and hence vagal (and glossopharyngeal n.) afference to the NTS via aortic and carotid baroreceptors, respectively [19]. The NTS then inhibits efferent vagal outflow to the heart [20, 21], leading to a transient inspiratory tachycardia with every breath. This feedback loop is termed “respiratory sinus arrhythmia” [22]. Hence, the dorsal medullary vagal system operates in tune with respiration, and we propose that supplying vagal afferent stimulation gated to the exhalation phase of respiration (i.e. when thoracic baroreceptor afference does not enter the NTS), will optimize t-VNS therapy (see Figure 1 for schematic). Furthermore, such intermittent, irregular stimulation (i.e., varying with respiration) will also mitigate classical neuronal adaptation/accomodation, which can occur with continuous stimulation of NTS neurons [23].

While VNS is a general analgesic mechanism at the level the brain, perhaps enhancing the activity of descending inhibitory systems, the vagus nerve has widespread projections throughout the abdominal and pelvic viscera. Thus, a likely target of VNS in initial clinical use could be abdominal and/or pelvic pain. Chronic pelvic pain (CPP) is a syndrome in urgent need of innovative and effective therapies [24]. CPP encompasses a number of common and debilitating syndromes including interstitial cystitis, endometriosis-mediated pain, and cancer pain [25]. Evidence from quantitative sensory testing (QST) studies has indicated that hyperalgesic mechanisms and central sensitization play a role in the chronicity and severity of this pain syndrome [26–28], supporting the use of QST measures as primary outcomes in evaluating potential therapeutic interventions for pelvic pain. In this study, we evaluated the effects of RAVANS on evoked, experimental pain ratings in patients with CPP due to endometriosis, using a counterbalanced crossover design. Patients completed two sessions utilizing QST evaluations before and after either RAVANS or an active control procedure, Non-Vagal Auricular Stimulation (NVAS). This was identical to RAVANS, except for the auricular location of stimulation. We hypothesized that RAVANS would produce greater evoked pain analgesia compared to the NVAS control.

## Methods

Our randomized, crossover, pilot study was conducted at the Pain Management Center in the Department of Anesthesiology at Brigham and Women's Hospital in Boston, MA. All patients completed informed consent procedures according to the protocol approved by the Partners Human Research Committee (PHRC).

## Subjects

In an effort to select a more homogenous pelvic pain condition, patients with CPP due to endometriosis were recruited from the Pain Management Center of Brigham and Women's Hospital. However, we recognize that endometriosis-linked CPP is difficult to classify and characterize as well, and that the etiology of pain is often unclear. For this initial study of RAVANS treatment, we targeted a sample size of approximately 15 patients. Crossover studies in patient groups which use QST as outcome measures often employ sample sizes of 20 or lower (e.g., n=10 in Staahl et al., 2007).

Inclusion criteria consisted of the following: a) female volunteers between 21 and 64 years of age with chronic pelvic pain for more than six months thought to be due to endometriosis by self report (six months of chronic pain is the criteria most often used in CPP research [24]); b) confirmed by determination of a gynecologist or pain physician specializing in pelvic pain (AV); c) average pain intensity of ≥4 on a scale from 0 to 10; and d) at least an $8^{th}$ grade English-reading level. In addition, exclusion criteria consisted of the following: a) any interventional procedure for CPP two weeks prior to the study or during the two-week study period, such as lumbar epidural steroids, nerve root blocks, etc.; b) any etiology for CPP due to a known other local somatic lesion for the pain (e.g. fibroids) documented by the patient's gynecologist, surgery and/or imaging; c) opioid usage, either oral or intrathecal; d) surgical therapy in the previous 12 weeks, the intent to undergo surgery during the study period, or any clinically unstable systemic illness that is judged to interfere with the trial; e) non-ambulatory status; f) history of severe cardiac or nervous system disease; g) cancer or other malignant disease; and i) pregnancy.

We did not evaluate study subjects during a specific phase of menstrual cycle. While the effects of menstrual cycle phase on pain sensitivity have been controversial [29], we chose not to control for this factor as (1) we anticipated that multiple subjects would be menopausal due to either post-hysterectomy, or other endometriosis treatment, (2) our study

outcomes focused on within-session change scores, and (3) multiple subjects would be on oral contraceptives which are known to blunt any potential cycle related variability in pain sensitivity [30, 31].

## Session Protocol

Subjects completed two experimental sessions, spaced at least one week apart, though given the duration of the treatment, we did not expect any carryover effects. The two sessions included either RAVANS (patent pending by Massachusetts General Hospital, not by the authors) or non-vagal auricular stimulation (NVAS), occurring in a counter-balanced order.

Subjects were seated in a reclined position for both sessions. During the RAVANS stimulation session, two 0.20 x 1.5mm modified press-tack electrodes (DBC, Korea and Vinco, China) were inserted in the left ear. Auricular locations were (1) the cymba concha and (2) the slope between the antihelix and cavum concha (Figure 2). These locations were chosen based on previous knowledge of vagus innervation of the human auricle. While variability exists, anatomic dissection in 7 cadavers (14 ears) found that the cymba concha, anti-helix, and cavum concha were innervated by the afferent branch of the vagus nerve in 100%, 75%, and 45% of ears, respectively [32]. During the control, NVAS, procedure, two electrodes were inserted into the ear lobe of the left auricle. Peuker et al. found that the ear lobe was innervated by the great auricular nerve in 100% of ears studied [32]. The stimulus duration, intensity, pulse frequency, and all other stimulation parameters were the same between RAVANS and NVAS. As all aspects of the protocol including transcutaneous electrical stimulation parameters, but not site of stimulation, were matched in the 2 treatment conditions, NVAS should be considered an active control.

Electrical stimulation was provided by a Cefar Acus II (Cefar Medical, Lund, Sweden). Stimuli consisted of rectangular pulses with 450 μS pulse width, delivered at 30Hz. Stimulus duration was 0.5 seconds, and was gated to the exhalation phase of respiration (see below). Current intensity was set to achieve moderate to strong (but not painful) sensation, and pulse frequency/duration was set following pilot testing to achieve a subjectively comfortable stimulus sensation.

Respiratory gating for stimulation required real-time evaluation of the respiratory cycle. A pneumatic belt was placed around the subject's lower thorax. Low-compliance tubing connected this belt to a pressure transducer (PX138-0.3D5V, Omegadyne, Inc., Sunbury, Ohio), thereby producing voltage data that corresponded to changes in respiratory volume [33]. The voltage signal from the transducer was acquired by a laptop-controlled device (National Instruments USB DAQCard 6009, 14bit i/o, with Labview 7.0 data acquisition software). Computer code detected end-inspiration and end-expiration *in real-time* and a TTL signal was output to a miniature high-frequency relay (G6Z-1P-DC5, Omron Electronics Components, Schaumburg, IL). The TTL pulse was output to the relay 0.5 second after end-inspiration (i.e. during expiration), which allowed stimuli to pass to the ear electrodes for 0.5 seconds. Real-time evaluation of respiratory cycle is non-trivial, and an adaptive threshold detection method was employed. Correct expiratory-cycle stimulation was confirmed in real-time by the experimenter via running chart of the respiration signal and stimulus pulse. Post-hoc review of these tracings was also performed and demonstrated accurate expiratory stimulation.

## Physiological Monitoring

In addition to QST and clinical outcomes, we also collected physiological monitoring data, as our RAVANS intervention was mediated by the afferent vagus nerve, and efferent vagal feedback may have also been affected at medullary and higher brain levels. We collected

both respiratory and electrocardiography (ECG) data, at 400Hz. Respiration was monitored with a pneumatic belt as part of the RAVANS procedure.

Respiration and ECG data were used to calculate respiratory rate, heart rate (HR), and heart rate variability (HRV). HRV analysis has been applied to indirectly estimate sympathetic and parasympathetic modulation to the heart [34–37]. While some controversy in interpretation remains, the spectral peak in a low frequency band (LF, 0.01–0.15Hz) is thought to be influenced by *both* parasympathetic and sympathetic activity, while the peak in a high frequency band (HF, 0.15–0.50Hz) is influenced solely by parasympathetic (cardiovagal) activity [36]. The LF/HF ratio has been used to approximate the balance between sympathetic and parasympathetic modulation to the heart. All physiological metrics were evaluated for 5-minute windows at baseline, and at the end of stimulation (window ending at termination of stimulus). ECG data were processed with the WFDB (WaveForm DataBase) Software Package [38] and MATLAB 7.4.0 (The Mathworks, Inc. Natick, MA). Data were automatically annotated with careful manual correction for QRS peak detection in order to form an *R-R* interval time series. Respiration rate and HRV were evaluated using spectral methods over the window of interest. We used a conventional FFT-based analysis using the Yule-Walker algorithm, a parametric spectral estimation method.

Within each window of interest, modulation of physiological metrics (HR, respiratory rate, LF-HRV, HF-HRV, LF/HF) was evaluated with a 2 x 2 ANOVA (PASW Statistics 18, SPSS Inc., Chicago, IL) was performed with factors STIM (RAVANS and NVAS) and TIME (baseline and end-stim) as independent variables. Post-hoc testing was performed with Student's t-tests, significant at alpha = 0.05.

## Quantitative Sensory Testing (QST)

Our primary outcome measures included psychophysical responses to several forms of noxious mechanical stimulation. QST measures serve as markers of sensitization and hyperalgesia, and have been studied as predictors of pain treatment outcomes. Prior research in a variety of patient samples has indicated that QST measures predict responses to opioid medications in both patients [39] and controls [40]. Other treatment studies have revealed that changes in responses to standardized noxious stimuli are associated with changes in clinical pain [41–44].

Since numerous studies have demonstrated that CPP is associated with generalized hyperalgesia at various body sites [26, 45, 46], we elected to study RAVANS' impact on indices of hyperalgesia and central sensitization. Hence, we evaluated repeated mechanical stimuli that produce windup (a phenomenon related to central sensitization) and tonic, deep-tissue mechanical pain.

During the session, subjects were seated comfortably in a reclining chair. Tonic, deep tissue mechanical pain was assessed using an inflatable cuff. Cuff pressure algometry (CPA) is a recently-characterized method that is now included in many quantitative sensory testing studies. In brief, tonic, deep-tissue, mechanical stimulation is applied using a pneumatic tourniquet cuff, which is inflated to and maintained at a particular pressure [47]. One advantage to the application of cuff algometry is that unlike more superficial methods of evaluating mechanical sensitivity, cuff pain responses are unaffected by sensitization or desensitization of the skin, indicating that this procedure primarily assesses sensitivity in muscle and other deep tissues [48, 49]. The present protocol utilized a Hokanson rapid cuff inflator, as in some of our previous cuff studies [50, 51]. A standard blood pressure cuff was wrapped comfortably around the lower leg, over the gastrocnemius muscle. A computer-controlled air compressor maintained the pressure at a level that was individually tailored, for each subject, to produce a pain intensity rating of 40/100. Cuff inflation was maintained

for 2 minutes, and subjects rated pain intensity and unpleasantness at 30-second intervals. Cuff pain intensity and unpleasantness were averaged across the 2-minute cuff stimulation period.

Mechanical probes were used to assess windup. First, as in previous work [52], participants underwent an assessment of mechanical temporal summation using a set of seven custom-made weighted pinprick stimulators developed by the German research Network on Neuropathic Pain [53, 54]. These punctuate mechanical probes have a flat contact area of 0.2 mm in diameter, and exert forces between 8 and 512 mN. Punctate stimuli were delivered to the skin on the dorsum of the middle finger of the right hand. In each session, we determined the lowest force stimulator that produced a sensation of mild to moderate pain (128 or 256 mN for most subjects), and then applied a train of 10 stimuli at the rate of 1 per second. Participants rated the painfulness of the first, fifth, and tenth stimulus. All ratings were on a 0–100 verbal pain intensity scale used in previous studies [55, 56]. We used these ratings to evaluate temporal summation of mechanical pain (i.e., the human analog to “wind-up”), a frequently used index of central pain facilitation. The assessment of temporal summation involves rapidly applying a series of identical noxious stimuli and determining the increase in pain across trials. Animal studies have suggested that temporal summation occurs centrally in second-order neurons in the spinal cord as a consequence of sustained C-fiber afferent input [57].

We also evaluated the two measures described above, concurrently, in order to study the modulatory effects of one stimulus on the other. Recent psychophysical pain research has recognized the role of endogenous inhibitory systems in shaping an individual’s perception of pain. In particular, diffuse noxious inhibitory controls (DNIC), refers to one noxious stimulus inhibiting the pain produced by a second noxious stimulus [58]. DNIC depends on opioid-mediated supraspinal mechanisms [59], is a sensitive measure of deficits in pain modulation in fibromyalgia and related disorders [60] and predicts the development of long-term clinical pain [61]. In this study, we assessed the effects of RAVANS and NVAS stimulation on the magnitude of DNIC by assessing changes in the painfulness of punctuate mechanical stimulation during cuff algometry. That is, at the conclusion of the 2-minute cuff stimulus, the sequence of 10 punctate mechanical probe stimuli was repeated while maintaining cuff inflation around the gastrocnemius.

Each set of pain responses (temporal summation, cuff algometry, DNIC) was assessed at baseline, at the midpoint (15 minutes) of a half-hour-long period of RAVANS (or NVAS), immediately post-RAVANS (or post-NVAS), and 15 minutes after the conclusion of RAVANS (or NVAS). A 2 X 3 repeated measures ANOVA was performed on change scores from baseline. The factor with 2 levels was STIM (RAVANS vs. NVAS), and the factor with 3 levels was TIME (change from baseline at the 3 time points: during stimulation, immediately after stimulation, and 15 min following the end of stimulation). Post-hoc testing was performed with Student’s t-tests, significant at alpha = 0.05.

## Exploratory Outcomes

Exploratory, or secondary, outcome measures included clinical pain ratings (on a 0–10 scale), which were obtained at numerous time points during the psychophysical testing session. Sensations evoked by RAVANS and NVAS stimulation were assessed using a psychophysical instrument, the MASS scale, developed for acupuncture and acupuncture-like interventions [62]. The MASS scale can be summarized by the MASS Index, which aggregates the breadth and depth of different sensations evoked by needle penetration and stimulation [62]. In addition, as in prior QST studies (Edwards, Smith et al. 2006; Kuzminskyte, Kupers et al. 2010), current verbal ratings of anxiety (on a 0–100 scale, with

"no anxiety" and "severe anxiety" as the respective anchors) were also obtained during the testing session. Finally, we performed exploratory correlation analyses to evaluate if changes in perceived anxiety were correlated to changes in pain report for both cuff pain ratings and windup scores.

## Results

A total of eighteen (18) women were enrolled in the study. Fifteen (15) women completed the study. Their mean age was 36.3 years old (SD = 10.6, range = 20–58 years), and the mean pelvic pain duration was 12.3 years (SD = 9.2, range = 1–39 years). Subjects completed two experimental sessions, spaced at least one week apart (Mean: approximately 2 weeks, Range: 1 week to 6 weeks). 3 more subjects completed a single session but did not return for the second session. No subjects dropped out due to stimulus discomfort.

All of the subjects tolerated the RAVANS and NVAS procedures. The average electrical current intensity used for stimulation did not differ (p=0.31) between RAVANS (0.43 ± 0.25 mA, $\mu\pm\sigma$), and NVAS (0.34 ± 0.20 mA). Similarly, the intensity of sensations evoked by the stimulation did not differ, as MASS Index (assessed in only 9 of the 15 patients due to a paperwork error) did not differ (p=0.18) across the testing sessions (RAVANS: 3.3 ± 2.3, $\mu\pm\sigma$; NVAS: 2.5 ± 1.4).

Subjects rated the pain intensity and unpleasantness evoked by cuff pressure. One subject's cuff algometry data was dropped due to inadvertent within-session alterations in the cuff pressure. Hence, 14 participants are included in this analysis. Average cuff inflation pressure to reach a 40/100 pain rating did not differ (p=0.34) between RAVANS (133.8 ± 43.0 mmHg, $\mu\pm\sigma$) and NVAS (144.6 ± 45.4 mmHg) visits. In addition, baseline cuff pain intensity and unpleasantness ratings did not differ across study visits (p's > 0.5). A 2 x 3 repeated measures ANOVA with factors STIM and TIME demonstrated that for cuff pain intensity, a significant main effect of STIM was observed [$F(1,13)= 4.7$, $p=0.049$, $eta^2 = 0.27$], with no significant main effect of TIME [$F(2, 12)= 1.8$, $p=0.21$] or interaction [$F(2,12)= 0.5$, $p=0.65$]. Follow-up t-tests (see Figure 3) revealed that cuff pain intensity ratings were reduced from baseline at each time point in both sessions (p's< 0.05), but that the reduction tended to be larger in the RAVANS session for each time period: during the stimulation [$t(13)=1.9$, $p= 0.08$], immediately after the stimulation [$t(13)= 2.0$, $p= 0.07$], and 15 minutes after the end of stimulation [$t(13)= 1.9$, $p= 0.08$]. For cuff pain unpleasantness, neither main effect nor the interaction was significant (p's> .1).

Temporal summation of mechanical pain was calculated by subtracting the pain rating of the first stimulus from the maximum pain rating during the sequence of 10 punctate stimuli. The amount of temporal summation at baseline did not differ significantly (p=0.16) between the RAVANS (30.7 ± 20.8, $\mu\pm\sigma$) and NVAS (20.7 ± 18.9) sessions. As with the cuff algometry data, a 2 (STIM) X 3 (TIME) repeated measures ANOVA was performed on temporal summation change scores from baseline. While no significant main effects of STIM [$F(1,14)= 1.1$, $p=0.33$] or TIME [$F(2, 13)= 0.8$, $p=0.45$] were observed, the interaction was significant [$F(2,13)= 3.6$, $p=0.04$, $eta^2 = 0.20$]. Follow-up t-tests (Figure 4) revealed that the only significant change from baseline was observed during stimulation in the RAVANS session (p=0.05), and there was a similar trend for windup to be reduced immediately post-stimulation in the RAVANS session (p=0.07). At 15 minutes after RAVANS stimulation, and at all 3 time points in the NVAS session, there was no significant change from baseline in windup (p's > 0.1). Comparing change scores in the RAVANS and NVAS sessions, there was a trend for reductions in windup to be greater during stimulation in the RAVANS relative to the NVAS session [$t(15)= 1.8$, $p=0.09$), but no trend for any session differences at the immediate post-stimulation and 15-minute post-stimulation time points (p's>0.4).

DNIC was explored by evaluating temporal summation on the fingers during cuff algometry on the leg, at both RAVANS and NVAS sessions. At baseline, temporal summation of mechanical pain was unchanged during cuff algometry (p's> 0.1 for both RAVANS and NVAS), suggesting an absence of DNIC effects in these patients. A 2 X 3 repeated measures ANOVA on change scores from baseline revealed no significant main effects of TIME or STIM and no interaction (p's> 0.1).

Clinical Pain was also explored by having patients rate (0–100) the intensity of their pelvic pain prior to QST and at each of the study time points. Pain ratings at baseline differed significantly (p=0.02) between the RAVANS (32.8 ± 28.7, $\mu\pm\sigma$) and NVAS (mean= 44.0 ± 27.0) sessions. However, a 2 (STIM) X 3 (TIME) repeated measures ANOVA on change scores from baseline revealed no significant main effects of Time or Session and no interaction (p's> 0.3).

We also specifically assessed anxiety prior to QST at each of the study time points (Figure 5). Anxiety ratings at baseline did not differ significantly (p=0.12) between the RAVANS (17.0 ± 16.9, $\mu\pm\sigma$) and NVAS (10.5 ± 17.2) sessions. A 2x3 ANOVA on change scores revealed a significant main effect of STIM [$F(1,14)= 9.1$, $p< 0.01$, $eta^2 = 0.40$], but no main effect of TIME or interaction (p's> 0.2). Follow-up t-tests revealed that anxiety scores were lower (compared to baseline) at each of the subsequent time points in the RAVANS session (p's< 0.01), but there was no change from baseline at any time points in the NVAS session (p's> 0.3). Direct comparison of change scores at each time point indicated that reductions in anxiety were significantly larger in the RAVANS than the NVAS session at each time point (p's< 0.05).

We examined associations between changes in anxiety and changes in pain responses using correlation coefficients. Because correlations can be strongly affected by outlying values, we evaluated the distributions of change scores. Visual inspection of these distributions did not reveal any obvious outliers, and Grubb's test indicated that no individual values were significant outliers (p's> 0.05). After Bonferroni correction for multiple comparisons, none of the correlations were significant (p's> 0.05), suggesting that treatment-associated changes in anxiety and treatment-associated changes in pain responses were largely independent.

While RAVANS stimulation specifically targeted afferent, and not efferent, vagal stimulation, physiological outflow variables (HR, respiratory rate, and HRV metrics) were evaluated to investigate potential feedback modulation of ANS outflow. These metrics were evaluated at baseline, before QST, and at the very end of RAVANS and NVAS stimulation. Due to excessive noise in the ECG signal (stemming from concurrent stimulation and line noise), the ECG data for some subjects were excluded from HR and HRV analyses. Due to variable cross-interference between electrical stimulation and ECG signal acquisition, we were only able to successfully annotate ECG data for 10 RAVANS and 12 NVAS sessions. A 2x2 ANOVA demonstrated no significant effect of STIM, TIME, or interaction (p's>0.7) on HR. A similar result was also found for HRV indices HF-HRV, LF-HRV, and LF/HF ratio (p's>0.7), with 1 subject's data dropped because their respiratory rate (at both baseline and end-stimulation) was below the HF frequency band cutoff). Respiratory rate was assessed in 11 RAVANS sessions and 14 NVAS sessions. A 2x2 ANOVA demonstrated no significant effect of STIM, TIME, or interaction (p's>0.8) on respiratory rate (RAVANS: baseline = 14.6 ± 1.3 breaths per minute, $\mu\pm\sigma$; end-stim = 14.6 ± 1.2 bpm; NVAS: baseline = 15.1 ± 1.0 bpm; end-stim = 16.1 ± 1.0 bpm).

## Discussion

Our pilot counterbalanced, crossover study found that RAVANS demonstrated a trending reduction of both evoked deep pain intensity and temporal summation of mechanical pain (windup) in patients with chronic pelvic pain due to endometriosis. RAVANS was also found to reduce anxiety levels. These reductions in pain responses and anxiety showed moderate to large effect sizes ($eta^2$>0.2 [63]) and tended to be greater than those produced by control stimulation at auricular sites not innervated by the ABV nerve. Furthermore, analgesic responses were independent of reductions in anxiety, suggesting independent mechanisms. These results are promising and further longitudinal studies are warranted, utilizing QST and clinical outcomes as primary endpoints.

Analgesic effects of the auricular, non-invasive variant of VNS, t-VNS, have been evaluated by several studies in the previous decade. In healthy adults, Johnson et al. found that electrical stimulation of auricular locations, including the cavum concha (a noted site of ABV receptors), increased experimental pain threshold by 30% to 50% in a subset of subjects [64]. While we also did not find modulation of autonomic variables such as HR or HRV, we did find significant evoked pain analgesia in our group of CPP patients. Notable differences between our study and that of Johnson et al. included the group of subjects evaluated (i.e. CPP patients versus healthy adults), and the duration of stimulation, which was only 15 minutes in the study by Johnson et al, and was 30 minutes in our study. Interestingly, several previous longitudinal trials of electrical stimulation on three points on the auricle (one of which was the anti-helix, noted to be innervated by vagal afferents [32]) have demonstrated analgesia for chronic low back pain [65], cervical pain [66], and for acute pain during in-vitro fertilization [67]. Similarly, future studies should also evaluate potential analgesia produced by RAVANS in a longitudinal trial in CPP and other chronic pain populations.

While RAVANS produced more significant analgesia compared to NVAS, some mild analgesia was also noted following this active control stimulation. Auricular vagal stimulation accesses higher brain regions through both the NTS and SpV [17]. As our control stimulation provided input to great auricular nerve receptors localized on the ear lobe, the SpV nucleus would also be processing NVAS stimulation. Thus, the mild evoked pain analgesia imparted by NVAS stimulation, suggests that input to the SpV might also contribute to anti-nociception, though less significant compared to vagal input relayed by both NTS and SpV. In addition to the scientific rationale for being an active control, the lack of differences in stimulation parameters (e.g. current amplitude) or in subject ratings of the stimulation sensations between treatment conditions support the credibility of NVAS as an active control.

Our lack of response in different cardiac autonomic variables (reflecting the safety of RAVANS) may simply reflect the innervation of the auricle, which is innervated by *afferent*, and not efferent, fibers of the vagus nerve [32], the latter of which innervate the chronotropic sinoatrial node of the heart. In fact, this specificity of innervation is one of the advantages of t-VNS stimulation over that of classical VNS stimulation, which affects both afferent and efferent branches of the main vagus nerve, with multiple side-effects resulting from the stimulation of the latter. However, side-effects for t-VNS via afferent-efferent vagal reflexes may also exist and include Arnold's cough reflex (incidence 2.3–4.2%) [18, 68], ear-gag reflex (~1.8%), ear-lacrimation reflex (~2%), and ear-syncope reflex (~0.6%). Thus, feedback loops, similar to the more extensively studied autonomic baroreflex [69], also exist for ABV signaling, but are rare and we did not encounter any side effects consistent with such reflexes in our study. Interestingly, somatosensory afference tuned to the respiratory rhythm has been found in previous studies to modulate autonomic outflow.

For instance, when stimulation was applied to the arm, gated to respiration, heart rate was found to decrease more substantially than for continuous stimulation at the same location [70]. Thus, future studies should continue to evaluate cardiac and other autonomic measures in response to RAVANS, as subtle modulations noted in this study may demonstrate significance with larger sample sizes, and may ultimately relate to clinically-relevant outcomes.

There is a dearth of studies exploring t-VNS mechanisms of action. The afferent vagus nerve, including the ABV, synapses bilaterally on the nucleus tractus solitarius (NTS) in the dorsal medulla of the brainstem. The NTS sends information to efferent (premotor) parasympathetic nuclei, including the dorsal motor nucleus of the vagus (DMNX) and the nucleus ambiguus (NAmb), as well as higher brain regions known to modulate pain, such as the rostral ventromedial medulla, periaqueductal gray, and anterior cingulate cortex [71–74]. Thus the NTS connects with a diffuse system of brain regions modulating pain. This supraspinal network of brain regions has been hypothesized to be the mechanistic substrate of VNS therapeutic effects [16]. In humans, Fallgetter et al. report evoked brainstem potentials following t-VNS [75]. Additionally, fMRI has demonstrated that t-VNS modulates limbic brain regions and induces positive effects on mood [4]. The latter finding is supported by our data, which showed reduced anxiety following RAVANS, and not NVAS. Reduced anxiety was not correlated with reductions in pain outcomes, suggesting an independent mechanism specific to ABV stimulation. More study is needed on the neural mechanisms of t-VNS and on the optimum location for stimulation, as neither of these neuroimaging studies stimulated the cymba or cavum concha, instead focusing on the tragus, which was found by Peuker et al. to be innervated by the ABV in only 45% of ears studied [32].

Future studies will need to more thoroughly optimize various stimulus parameters for longitudinal application of RAVANS. In clinical application, classical VNS uses stimulus parameters that vary depending on patient tolerance. However, typical usage includes a 30–90 second, 20–50hz (0.5mS pulse width) burst of stimulation with current amplitude 1–3mA, which is applied every 5–10 minutes throughout the day [12]. Furthermore, the specific contribution of respiratory gating should be addressed by adding control intervention groups with ABV stimulation only during inspiration, intermittently irrespective of respiratory cycle, and/or continuously throughout the stimulus period. Important design parameters would have to be addressed, including whether stimulation in this control group is continuous at the same frequency (perhaps leading to greater energy input, but also more chance for habituation or sensitization, compared to respiratory-gated stimulation). Another option would be to have pulsed stimuli gated to exhalation (similar to RAVANS), but instead of a fixed delay, these control stimuli could occur after a random delay, i.e. during exhalation or inhalation for the next breath.

Several limitations should be noted. We did not find any reduction of clinical pain by either RAVANS or the control NVAS stimulation. This is not surprising given that chronic pain was assessed after a single treatment. Future studies may need to include longer-duration RAVANS stimulation over the course of multiple treatment sessions. Another issue is the effect size of the analgesia observed. Clinically significant analgesia for clinical pain outcomes is at least a 30% improvement [76]. For evoked pain outcomes (i.e., QST), no consensus has emerged to define the magnitude of clinically meaningful analgesia, and effects vary as a function of numerous factors such as the modality of the noxious stimulus, the location of its application, etc. [77]. However, recent studies of oral opioids have revealed that oxycodone reduces deep-tissue mechanical pain by approximately 15–25% in healthy volunteers [78, 79] and by 40–50% in a study of chronic pain patients [80]. We report RAVANS-associated reductions of evoked pain ratings of approximately 30–50% in

models of deep tissue mechanical pain and mechanical temporal summation. This suggests that RAVANS stimulation may have effects on deep-tissue evoked pain that are comparable in magnitude to those of potent opioids such as oxycodone, though direct comparison studies would be necessary to confirm this. An additional limitation stems from the possibility that CPP patients may have disrupted central pain modulation circuitry [26–28]. While we did not find any significant DNIC effects during RAVANS stimulation, healthy subjects, who would have intact DNIC circuitry, should also be evaluated in future studies, as a comparison group. While we have included our rationale for not controlling for phase of menstrual cycle in our patient cohort, this lack of control should nevertheless be noted as another limitation. Finally, due to technical difficulties we were not able to use the ECG signal in all subjects. Thus, the negative findings of RAVANS effects on autonomic outflow to the heart, while consistent with similar investigations in healthy adults [64], should be confirmed in future studies.

In conclusion, RAVANS demonstrated a trend for reduced evoked pain intensity and temporal summation of mechanical pain, and significantly reduced anxiety in CPP patients. Chronic pain disorders such as CPP are in great need of effective, non-pharmacological options for treatment. RAVANS produced promising anti-nociceptive effects for QST outcomes reflective of the noted hyperalgesia and central sensitization in this patient population. Future studies should evaluate RAVANS for longitudinal reduction of both QST outcomes and clinical pain.

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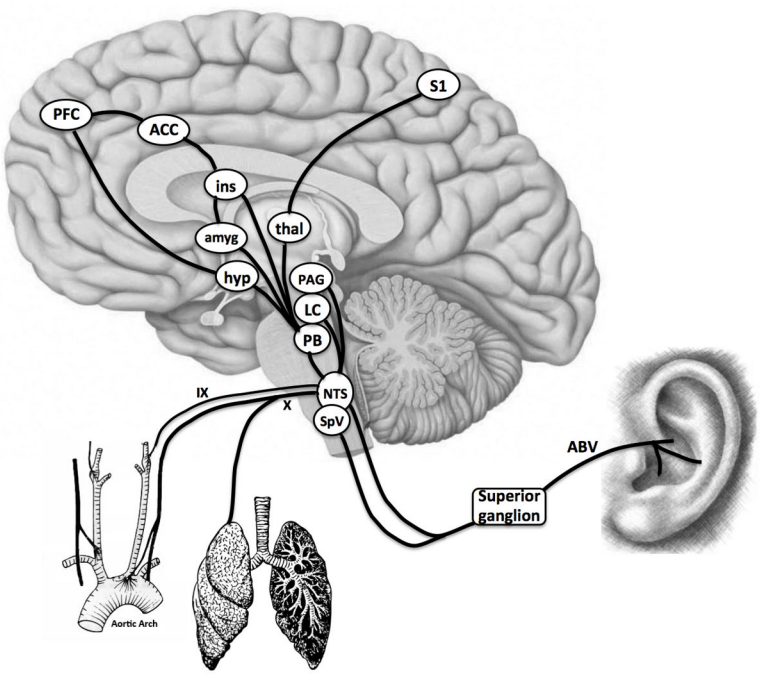


**Figure 1. Schematic of Integrative Innervation of the NTS**

The nucleus tractus solitarius (NTS) in the medulla integrates afferent inputs from the cervical vagus (X, e.g. aortic arch baroreceptors, lungs), glossopharyngeal nerve (IX, e.g. carotid baroreceptors), and auricular branch of the vagus (ABV). NTS input to higher brain regions processing different aspects of pain is thought to underlie the anti-nociceptive effects of vagus nerve stimulation (VNS). N.b. SpV = trigeminal nucleus, PB = parabrachial nucleus, LC = locus ceruleus, PAG = periaqueductal gray, hyp = hypothalamus, amyg = amygdala, thal = thalamus, ins = insula, ACC = anterior cingulate cortex, PFC = prefrontal cortex, S1 = primary somatosensory cortex.

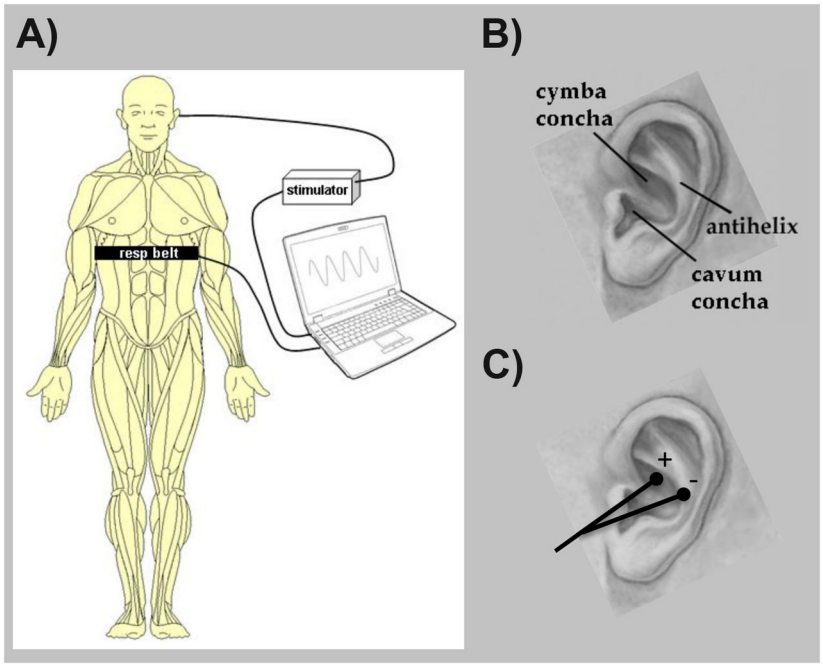


**Figure 2. Schematic of the RAVANS procedure**

(A) Subjects were outfitted with a thoracic belt to measure respiratory excursions. This signal was transduced and fed into a laptop controller, allowing for left t-VNS stimulation to occur only during the expiratory phase of respiration. (B) Auricular anatomy includes important regions including the cymba and cavum conchae, as well as the antihelix. (C) Auricular electrodes were placed within the cymba concha and antihelix, the two regions found to be most consistently innervated by the ABV nerve [32].

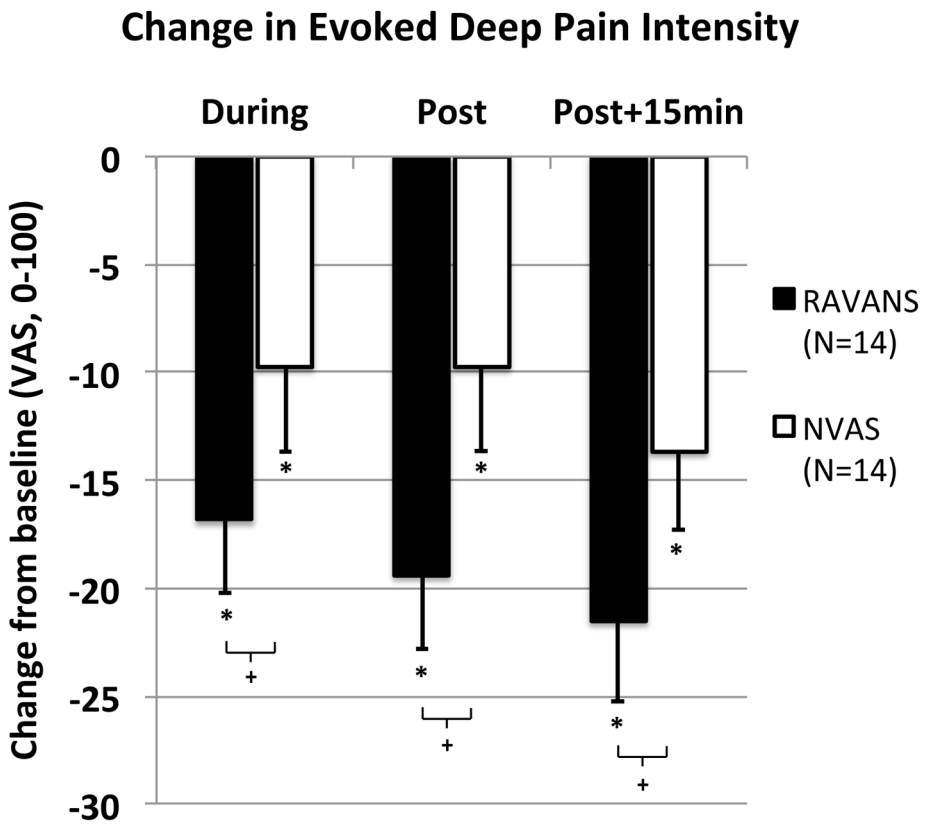


**Figure 3. Response of deep pain intensity to RAVANS vs. NVAS**

Evoked deep pain intensity was reduced ($p<0.05$) during, immediately after, and 15 minutes following cessation of both RAVANS and NVAS, with a trend ($p=0.07–0.08$) for greater pain reduction following RAVANS stimulation. N.b. * = $p<0.05$, + = $0.05 < p < 0.1$; error bars represent SEM.

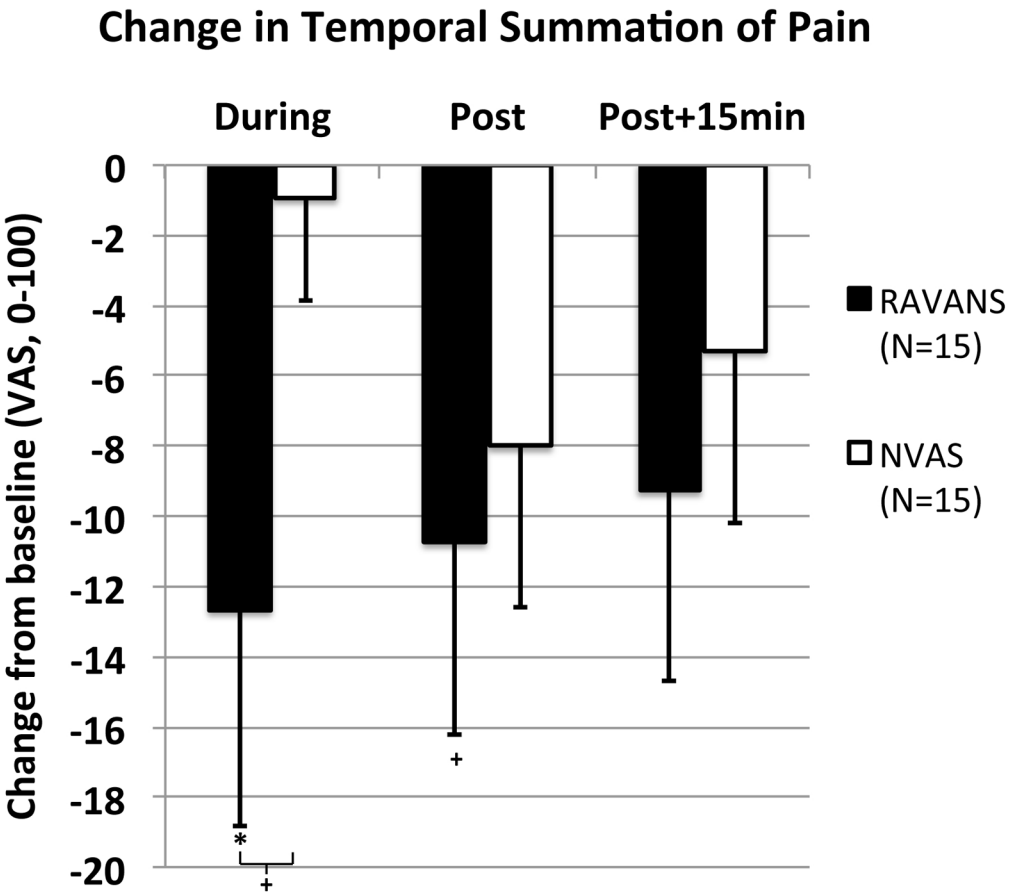


**Figure 4. Response of temporal summation of pain to RAVANS vs. NVAS**

Temporal summation of pain was reduced (p=0.05) during RAVANS stimulation, while a trend (p=0.07) was found for reduction immediately following RAVANS stimulation, and comparing RAVANS and NVAS during stimulation. N.b. * = $p<0.05$, + = $0.05 < p < 0.1$; error bars represent SEM.

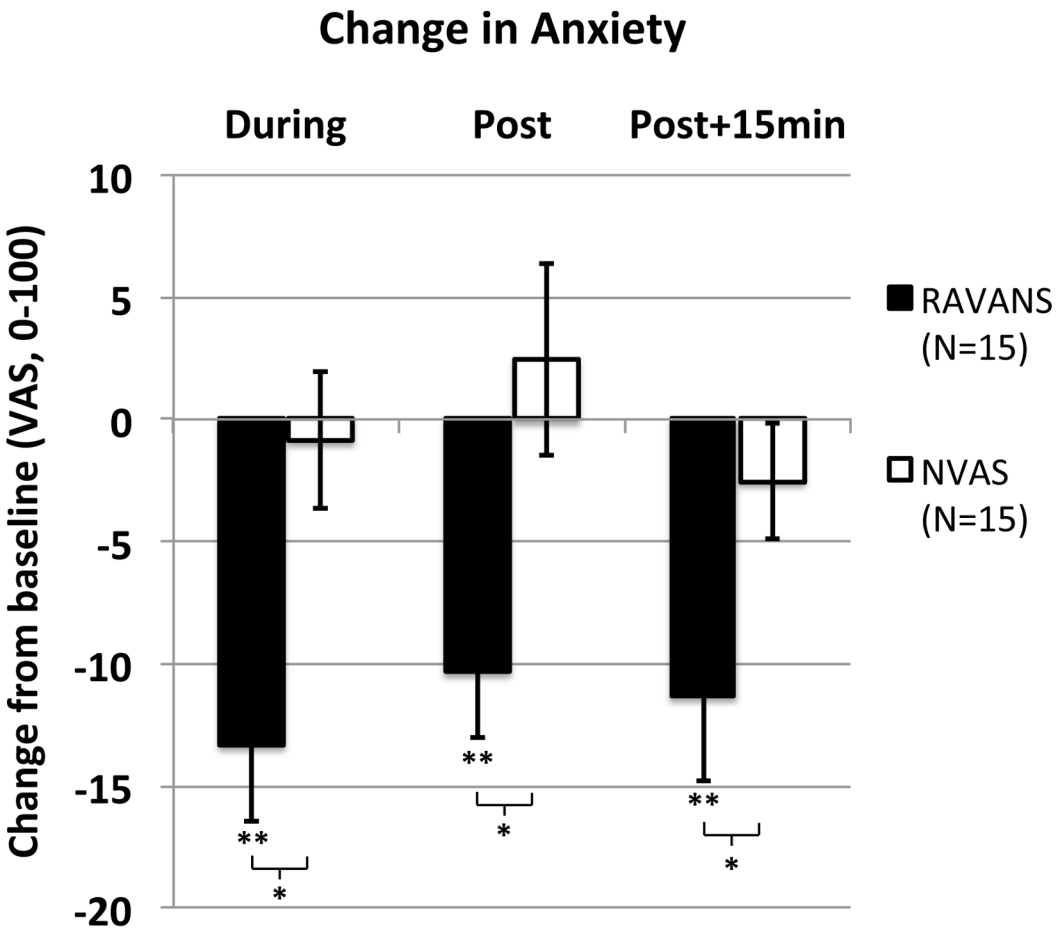


**Figure 5. Response of anxiety ratings to RAVANS vs. NVAS**

Anxiety was reduced (p's < 0.01, compared to baseline) during, immediately after, and 15 minutes after cessation of RAVANS. There was no change from baseline (p's> 0.3) at any time points in the NVAS session. Reductions in anxiety were significantly larger in the RAVANS than the NVAS session at each time point (p's< .05). N.b. * = p<0.05, ** = p<0.01; error bars represent SEM.

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# Different modulation effects of 1 Hz and 20 Hz transcutaneous auricular vagus nerve stimulation on the functional connectivity of the periaqueductal gray in patients with migraine

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## Abstract

**Background:** A growing body of evidence suggests that transcutaneous auricular vagus nerve stimulation (taVNS) may relieve symptoms of migraineurs. Frequency is one of the key stimulation parameters. The aim of this study is to investigate the modulation effect of taVNS frequency on the descending pain modulation system (DPMS) in patients with migraine.

**Methods:** Twenty-four episodic migraineurs without aura (21 females) were recruited for the single-blind, crossover, functional magnetic resonance imaging (fMRI) study. Each participant attended two separate fMRI scan sessions, one for 1 Hz and another for 20 Hz taVNS, in a random order. Seed-based functional connectivity analysis was applied using the ventrolateral periaqueductal gray (PAG) as the region of interest.

**Results:** Compared with the pre-taVNS resting state, continuous 1 Hz taVNS (during) produced a significant increase in functional connectivity between the PAG and the bilateral middle cingulate cortex (MCC), right precuneus, left middle frontal gyrus (MFG), and left cuneus. Compared with 20 Hz taVNS, 1 Hz taVNS produced greater PAG connectivity increases with the MCC, right precuneus/posterior cingulate cortex, left insula, and anterior cingulate cortex (ACC). A significant negative correlation was observed between the number of migraine attacks in the previous 4 weeks and the PAG-MCC functional connectivity in the pre-taVNS resting-state before 1 Hz taVNS.

**Conclusions:** Our findings suggest that taVNS with different frequencies may produce different modulation effects on the descending pain modulation system, demonstrating the important role of stimulation frequency in taVNS treatment.

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**Keywords:** Transcutaneous auricular vagus nerve stimulation, Functional connectivity, Periaqueductal gray, Descending pain modulation network, Frequency

## Background

The vagus nerve consists of a complex system that may regulate pain, mood, and the neuro-endocrine-immune axis [1–7]. Thus, stimulating the vagus nerve to modulate the function of the nerve and related organs has drawn the attention of clinicians and investigators for a long time. Anatomical studies found peripheral branches of the vagus nerve distributed on the ear [8, 9], and according to the bottom-up mechanism of the central nervous system, the propagation of electrical stimuli may follow an afferent path from the peripheral nerves towards the brain stem and central structures [10, 11]. Thus, direct stimulation of the nerve fibers on the ear may produce an effect similar to classic vagus nerve stimulation. This plausibility has led to the development of transcutaneous auricular vagus nerve stimulation (taVNS), a non-invasive, low-cost, and easily implementable alternative to classic vagus nerve stimulation [12–15]. A growing body of evidence suggests that taVNS can induce antinociception, which may affect peripheral and central nociception, inflammatory responses, autonomic activity, and pain-related behavior [1, 16–18].

While taVNS has demonstrated its potentials, the optimal parameters for taVNS, such as frequency, remain unclear [12]. Accumulating evidence suggests different frequencies may be associated with different physiological and treatment effects. For instance, investigators compared the effect of 2, 10, and 20 Hz stimulation on heart rate in healthy subjects, and they found that both 10 and 20 Hz could decrease heart rate [19]. Furthermore, studies suggest that the optimal taVNS frequency may vary across different disorders. For example, a recent clinical research study on taVNS treatment of drug-resistant epilepsy showed a significant reduction in seizure frequency in patients of the 25 Hz group compared to the 1 Hz group [20]. However, in another clinical study of migraine patients, investigators found that although both 1 Hz and 25 Hz taVNS improved clinical outcomes in patients with chronic migraine, 1 Hz taVNS produced greater improvement [21]. Nevertheless, the underlying mechanism of different taVNS frequencies remains unclear.

Recently, brain imaging has been widely used to investigate the central mechanism of taVNS, and these studies demonstrate that intermittent taVNS can modulate activity of certain brain regions consistent with the vagus nerve central projections [22–28]. For instance, investigators have assessed brainstem fMRI response to 2, 10, 25, and 100 Hz taVNS in healthy individuals, and found that the strongest brainstem response was evoked by 100 Hz stimulation [29]. In recent studies, we also applied the resting-state functional connectivity method to investigate the functional connectivity alteration during "the continuous taVNS" (20 Hz) and found that taVNS can modulate the functional connectivity of the ventral striatum and hypothalamus [30, 31].

Nevertheless, the neural substrates underlying frequency have rarely been investigated in a patient population such as migraine; elucidating how different frequencies can modulate pathways associated with migraine may further facilitate the development of this promising neuromodulation method.

Recently, the role of descending pain modulatory system (DPMS) in pain modulation and the physiopathology of chronic pain has drawn more and more attention [32–34]. Yet, investigating the functional status of the DPMS in humans remains a challenge. In an earlier study [35], we investigated the resting state functional connectivity (rsFC) of the periaqueductal grey (PAG), a key region in the DPMS in healthy subjects and found significant rsFC between the PAG and central regions of the DPMS, such as the anterior cingulate cortex (ACC), rostroventral medulla (RVM) and anterior insula, demonstrating the feasibility of using functional connectivity methods to non-invasively investigate the DPMS in humans.

Following the study, the PAG functional connectivity has been applied to investigate the physiopathology of chronic pain disorders including migraine [35–43], menstrual pain [44–46], postherpetic neuralgia [47], fibromyalgia [48], myofascial pain [49], visceral pain [50], low back pain [36], and neck pain [51]. Further, studies have also shown that effective treatment can significantly modulate the PAG functional connectivity in patients with migraine [41], chronic low back pain [52], and knee osteoarthritis [53]. We also found that continuous electroacupuncture stimulation alters PAG functional connectivity [54]. Taken together, these findings demonstrate the important role of PAG functional connectivity in pain research.

Thus, in this study, we investigate how continuous taVNS at 1 Hz versus 20 Hz (a relatively low frequency versus a moderate frequency) that are widely applied in taVNS studies [12] can modulate the PAG functional connectivity in patients with migraine without aura, using a cross-over design. We hypothesize that taVNS at 1 Hz versus 20 Hz may produce greater PAG functional

connectivity changes due to its greater improvement in patients with migraine [21].

## Methods

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Z2016-079-01). All participants provided written informed consent before starting the study.

### Participants

Twenty-four episodic migraineurs without aura were recruited in the present study from outpatient neurology clinics of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. Similar to our previous studies [55, 56], the diagnosis of migraine was based on the International Classification of Headache Disorders, 2nd Edition (ICHD-II), as diagnosed by a specialist working at the neurology outpatient service.

Patients were eligible for participation if they: (1) were 18 to 45 years of age, (2) self-reported being right-handed, (3) have at least 6 months of migraine duration, (4) have at least one headache attack per month, (5) have not taken any prophylactic headache medications during the past 4 weeks, (6) have not taken any psychoactive or vasoactive drugs during the past 3 months. Patients were excluded if there was a/an: (1) headache induced by other diseases, (2) headache attack within 48 h prior to the experiment or during the experiment, (3) pregnant or lactating, (4) any other chronic pain conditions, (5) severe head deformity or intracranial lesions, (6) score on the Self-Rating Depression Scale [57] or Self-Rating Anxiety Scale [58] > 50, and (7) inability to provide informed consent for oneself.

### Study design

A single-blind, crossover functional magnetic resonance imaging (fMRI) trial design was applied in the present study to investigate the modulation effects of 1 Hz and 20 Hz taVNS in patients with migraine without aura. Specifically, each participant attended two taVNS fMRI scan sessions with identical parameters, one for 1 Hz and another for 20 Hz taVNS in a random order (Fig. 1A). Each session was separated by at least 7 days to avoid sensitization to the stimuli. All scans were applied during an interictal period when the participants were free from headache symptoms.

### Interventions

In the current study, we applied taVNS on the participant's left concha (cymba and cavum, Fig. 1B, Additional file 1: Fig. S1) [59]. The electrical stimulation was performed using the Electronic Acupuncture Treatment

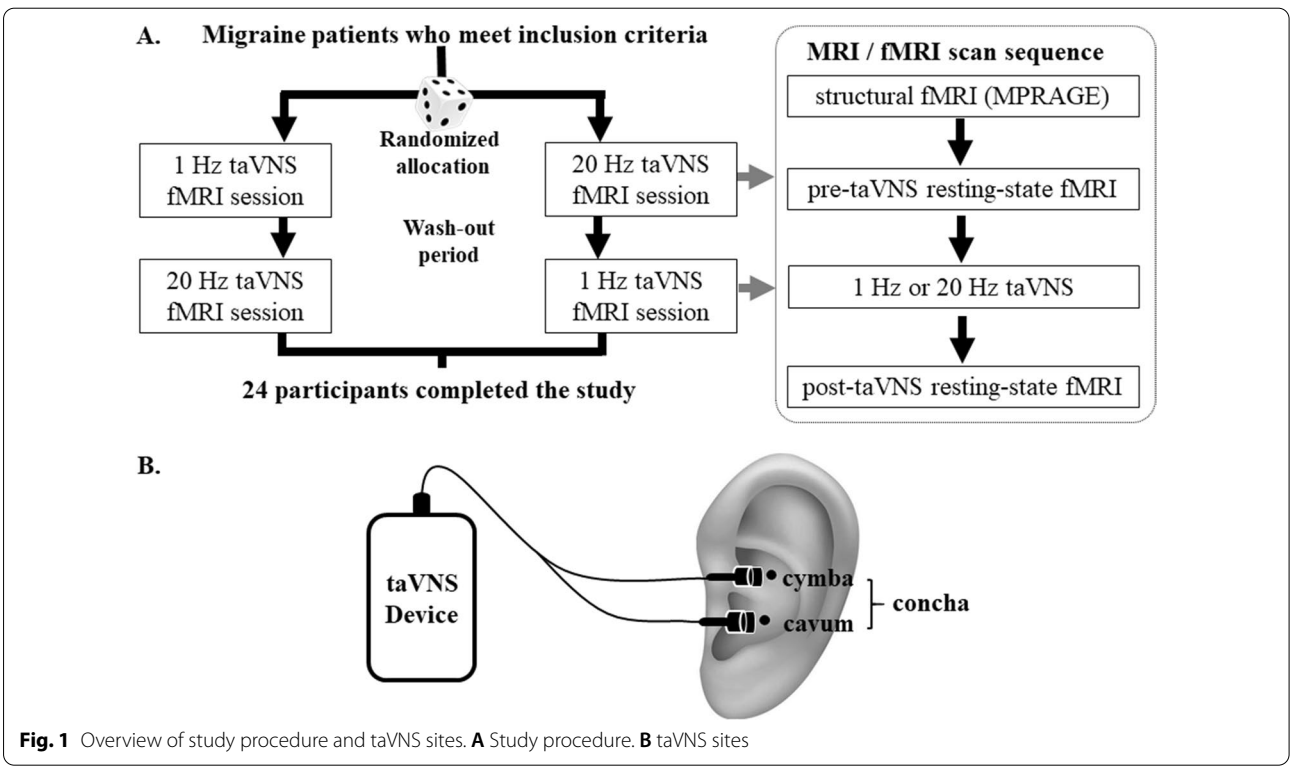


**Fig. 1** Overview of study procedure and taVNS sites. **A** Study procedure. **B** taVNS sites

Instrument (SDZ IIB, Huatuo, Suzhou, China) with the self-made MRI compatible electrode to deliver electric current at 1 Hz or 20 Hz with a continuous wave (width: ~0.2 ms). The 1 Hz/20 Hz taVNS stimulation lasted about 8 min. Similar to our previous studies, stimulation current intensity was adjusted to the strongest nonpainful sensation that participants could tolerate (approximately 4 mA) [31, 55, 60–62].

#### Clinical assessments

Migraine duration, migraine attacks during the past 4 weeks, and average migraine intensity of the past 4 weeks on the 0 ("not at all") to 100 ("extremely") visual analog scale (VAS) were assessed preceding the first MRI scan session. Participants were also asked to complete the Migraine Specific Quality-of Life Questionnaire [63] to measure the impact of migraine on health-related quality of life.

#### MRI data acquisition

All imaging data was acquired at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine using a 3 T MRI System (Siemens MAGNETOM Verio 3.0 T, Erlangen, Germany) with a 24-channel phased-array head coil. Each scan session included a pre-taVNS resting-state fMRI (8 min), the 1 Hz or 20 Hz continuous taVNS (8 min) fMRI, and a post-taVNS resting-state fMRI (8 min).

fMRI scans were acquired with the following parameters: time repetition = 2000 ms, time echo = 30 ms, flip angle = 90°, field of view = 224 mm × 224 mm, matrix size = 64 × 64, slice thickness = 3.5 mm with 0.7 mm inter-slice gap, 31 axial slices paralleled and 240 time points. During the fMRI scans in resting-state and continuous taVNS, participants were asked to stay awake, keep their heads still, eyes closed, and ears plugged and to not think about any particular thing. A T1-weighted structural image was acquired by an isotropic multi-echo magnetization-prepared rapid gradient-echo pulse sequence for anatomic localization of significant signal changes: time repetition = 1900 ms, time echo = 2.27 ms, flip angle = 9°, field of view = 256 mm × 256 mm, data matrix = 256 × 256, and slice thickness = 1.0 mm.

#### Functional connectivity analysis

Data and calculations of functional connectivity were conducted using the CONN toolbox version 18.b (http://www.nitrc.org/projects/conn) [64]. We used the default preprocessing pipeline for seed-to-voxel functional connectivity analysis. The specific steps were as follows: functional realignment and unwarping, slice timing correction, head motion correction, co-registration of the anatomical image to the mean functional image, segmentation of the anatomical gray matter, white matter, and CSF, normalization to Montreal Neurological Institute (MNI) 152 standard template and smoothing with a 6-mm full width at half maximum (FWHM) kernel. A default frequency window of 0.008 to 0.09 Hz was used for band-pass filtering.

To eliminate correlations caused by head motion and artifacts, we identified outlier time points in the motion parameters and global signal intensity using ART (http://www.nitrc.org/projects/artifact_detect). Images whose composite movement exceeded 0.5 mm or whose global mean intensity was greater than three standard deviations from the mean image intensity were treated as outliers. The time series of the head motion matrix of outliers was also entered as first-level covariates.

Similar to our previous studies [35, 36, 41], we selected the right ventrolateral periaqueductal gray (vlPAG) with a 2 mm radius sphere (MNI coordinates x = 4, y = − 26, z = − 14) as the region of interest (ROI). In addition, we also chose seeds with a 2 mm radius in the fourth ventricle (MNI coordinates: x = 4, y = 10, z = 12; x = − 4, y = 10, z = 12) as a control. Seeds were created using the SPM Wake Forest University Pickatlas toolbox (http://fmri.wfubmc.edu/software/pickatlas) [65].

In the first-level analysis, we produced a correlation map for each participant by extracting the blood oxygenation level dependent time course separately from the vlPAG and the control seeds and computing Pearson's correlation coefficients between the time course in the vlPAG/control seeds and every voxel of the whole brain. Correlation coefficients were Fisher transformed into "z" scores to increase normality.

In seed-to-voxel functional connectivity analyses, we first used a pairwise t-test to compare the vlPAG-based functional connectivity between the pre-taVNS resting-state and during continuous taVNS (1 Hz and 20 Hz taVNS, respectively). Next, we compared the difference of vlPAG-based functional connectivity change (during continuous taVNS minus pre-taVNS resting-state) between 1 and 20 Hz taVNS. Finally, we compared the vlPAG-based functional connectivity difference between the pre-taVNS and post-taVNS resting-state between the 1 Hz and 20 Hz taVNS.

For whole brain analysis, a threshold of voxel-wise $p < 0.005$, and $p_{FDR} < 0.05$ at cluster level was applied. Also, given the important role of the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), insula, amygdala, and thalamus in the DPMS [32, 36, 53, 66] and pathophysiology of migraine [41, 67–72], we pre-defined these areas as regions of interest (ROIs), and derived masks of each region from the Automated Anatomical Labeling brain atlas using the Wake Forest University Pickatlas toolbox as ROIs. A threshold of voxel-wise

$p < 0.005$ was used in data analysis. Similar to previous studies [73–75], Monte Carlo simulations using the 3dFWHMx and 3dClustSim (as part of the Analysis of Functional NeuroImages program [http://afni.nimh.nih.gov] released in July 2017) were applied for the p value correction for pre-defined ROIs. For each region, the minimum voxel size required for $p < 0.05$ cluster level p value correction is indicated as the k value in the results presented below.

To explore the association between the initial clinical assessments and the vlPAG-based pre-taVNS resting-state functional connectivity for 1 Hz and 20 Hz respectively, we also selected significantly altered vlPAG-based connectivity clusters (during continuous taVNS minus pre-taVNS resting-state) and extracted the average z-score values of peak MNI of clusters above significance in vlPAG-based pre-taVNS resting-state. Correlation analyses were conducted using the R program in JASP open-source statistical software (Version 0.8.1, http://www.jasp-stats.org), and p values were Bonferroni corrected (see "Results" for details).

**Table 1** Demographic and clinical assessments

| | |
|---|---|
| Demographic | |
| Participant count | 24 |
| Sex (female/male) | 21/ 3 |
| Age (mean ± SE, yrs) | 31.33 ± 1.55 |
| Clinical assessments | |
| Migraine duration (mean ± SE, yrs) | 8.68 ± 1.47 |
| Migraine attacks (mean ± SE) | 1.67 ± 0.25 (ranging from 1 to 5) |
| VAS (mean ± SE) | 38.60 ± 3.30 |
| MSQ (mean ± SE) | 74.83 ± 1.90 |
| SDS (mean ± SE) | 42.14 ± 1.82 |
| SAS (mean ± SE) | 39.69 ± 1.85 |

Migraine attacks assessed attack times during the past 4 weeks. The VAS assessed the average migraine intensity of the 4 weeks preceding the first MRI scan. The MSQ, SDS, SAS were assessed preceding the first MRI scan

*VAS* visual analog scale, *MSQ* Migraine Specific Quality of Life, *SDS* Self-rating Depression Scale, *SAS* Self-rating Anxiety Scale

## Results

### Demographic and clinical assessments

Twenty-four participants completed the study and were included in the data analysis [21 females; age 31.33 ± 1.55 years, mean ± standard error (SE)]. No participant reported administration of acute migraine medication or having an attack 48 h prior to the MRI sessions. Detailed results for demographic and clinical assessments are shown in Table 1. All participants reported acceptable stimulation intensity underneath the electrodes during the continuous taVNS, with no adverse effects reported. The interval period of the two taVNS/fMRI scan sessions was 8.79 ± 0.74 (mean ± SE) days.

### vlPAG-based functional connectivity analysis results

Compared with pre-taVNS resting state, 1 Hz continuous taVNS (during) produced significant functional connectivity increases between the vlPAG and the bilateral middle cingulate cortex (MCC), the right precuneus, the left middle frontal gyrus (MFG), and the left cuneus (Table 2, Fig. 2A). There was no significant finding detected when we applied the same analysis on the 20 Hz taVNS data set.

In addition, we compared the vlPAG-based connectivity difference in 1 Hz vs. 20 Hz taVNS [(during 1 Hz taVNS minus 1 Hz pre-taVNS resting-state) vs. (during 20 Hz taVNS minus 20 Hz pre-taVNS resting-state)], and found that compared to 20 Hz, 1 Hz taVNS produced greater vlPAG-based connectivity increases with the MCC, the right precuneus/posterior cingulate cortex (PCC), the left insula (k = 18), and the anterior cingulate cortex (ACC) (k = 41) (Table 3, Fig. 2C). No significant

**Table 2** Comparisons of the vlPAG functional connectivity change in 1 Hz and 20 Hz taVNS

| Comparisons | Brain Regions | Cluster size (voxel number) | Peak T | MNI coordinates | | |
|---|---|---|---|---|---|---|
| | | | | x | y | z |
| 1 Hz taVNS > pre-resting | MCC | 282 | 6.36 | −4 | −12 | 40 |
| | PCu | 200 | 4.71 | 4 | −50 | 44 |
| | MFG | 232 | 4.56 | −30 | 30 | 42 |
| | Cuneus | 191 | 4.05 | −6 | −76 | 32 |
| pre-resting > 1 Hz taVNS | | | | | | |
| 20 Hz taVNS > pre-resting | No regions survive the threshold | | | | | |
| pre-resting > 20 Hz taVNS | | | | | | |

"Pre-resting" indicated pre-taVNS resting-state. Results were significant at cluster $p_{FDR} < 0.05$, corrected at the whole brain level

*vlPAG* ventrolateral periaqueductal gray, *MCC* middle cingulate cortex, *PCu* precuneus, *MFG* middle frontal gyrus

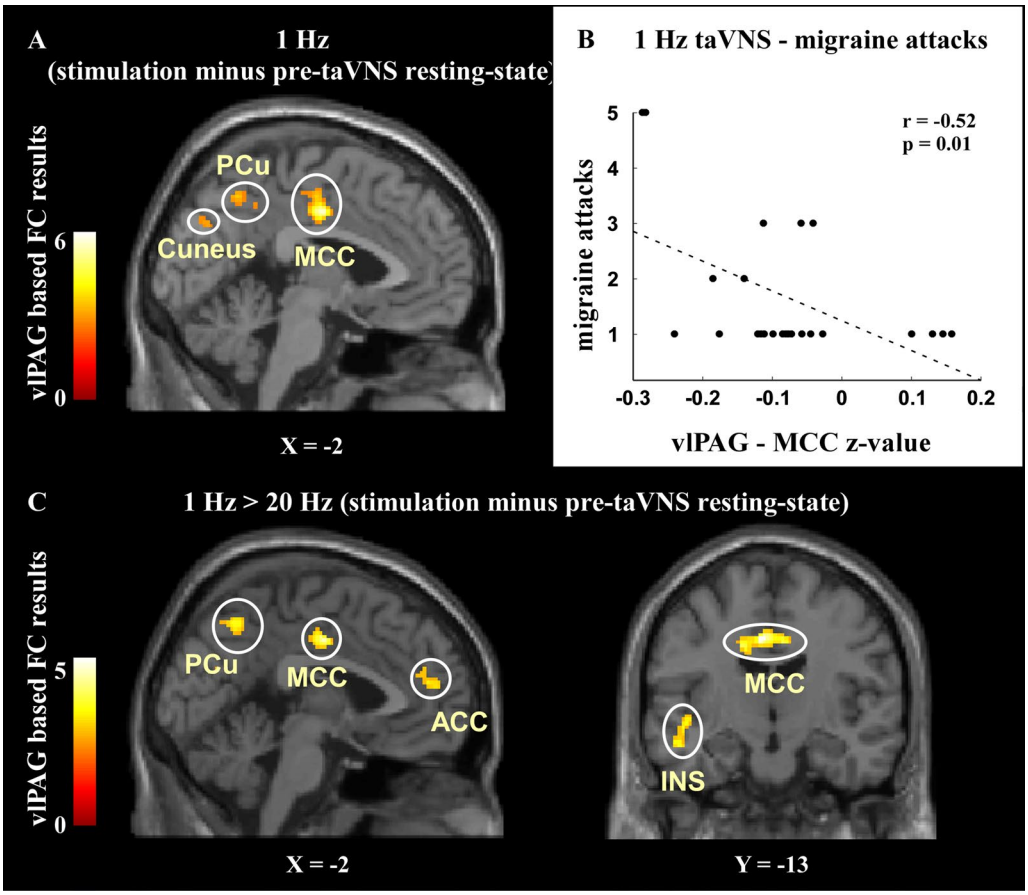


**Fig. 2** vlPAG based connectivity results. **A** Compared to pre-taVNS resting-state, 1 Hz taVNS had significantly greater connectivity with the MCC, precuneus, Cuneus, and left MFG (not present in the figure). **B** Significant negative correlation was observed in migraine attacks and vlPAG-MCC connectivity in pre-taVNS resting-state preceding to the 1 Hz taVNS. Bonferroni correction was applied, and the significance threshold was adjusted to $p < 0.0125$ because four significant clusters were identified. **C** Compared to 20 Hz, 1 Hz taVNS had significant connectivity increases (stimulation minus pre-taVNS resting-state) with the MCC, precuneus, ACC, and left insula. *FC* functional connectivity, *vlPAG* ventrolateral periaqueductal gray, *MCC* middle cingulate cortex, *PCu* precuneus, *MFG* middle frontal gyrus, *ACC* anterior cingulate cortex, *INS* insula

**Table 3** Comparisons of vlPAG functional connectivity change produced by 1 Hz and 20 Hz taVNS

| Comparisons | Brain Regions | Cluster size | Peak T | MNI coordinates | | |
|---|---|---|---|---|---|---|
| | | | | x | y | z |
| 1 Hz > 20 Hz | MCC | 225 | 5.37 | −6 | −12 | 40 |
| | PCu/PCC | 216 | 4.74 | 4 | −48 | 46 |
| | *INS | 148 | 5.35 | −44 | −6 | −6 |
| | *ACC | 45 | 3.50 | −4 | 42 | 18 |
| 20 Hz > 1 Hz | No regions survive the threshold | | | | | |

Change presented in continuous taVNS minus pre-taVNS resting-state. "*" identified results significant at cluster $p < 0.05$ after 3dFWHMx and 3dClustSim correction. Other results were significant at cluster $p_{FDR} < 0.05$ corrected at the whole brain level

*vlPAG* ventrolateral periaqueductal gray, *MCC* middle cingulate cortex, *PCu* precuneus, *PCC* posterior cingulate cortex, *INS* insula, *ACC* anterior cingulate cortex

decrease in vlPAG-based functional connectivity was detected.

With the threshold we set, no significant result has been found in the comparison of vlPAG-based pre- and post-taVNS resting-state functional connectivity differences between the 1 Hz and 20 Hz taVNS.

We found that 1 Hz taVNS increased vlPAG resting state functional connectivity with the MCC, precuneus,

MFG and cuneus compared with the pre-taVNS resting state. To explore the potential clinical meaning of these functional connectivity increases, we performed correlation analyses between the vlPAG resting-state functional connectivity with these regions during the pre-taVNS (1 Hz) and the clinical measures (migraine attacks in the past 4 weeks and VAS). Results showed a significant negative correlation between the number of preceding migraine attacks and the vlPAG-MCC functional connectivity in the pre-taVNS resting-state preceding the 1 Hz taVNS ($r = -0.52$, $p = 0.01$, significant after Bonferroni correction $p < 0.05/4 = 0.0125$ because four significant clusters were identified, please see Table 2 and Fig. 2B for details). No other significant vlPAG-based functional connectivity finding was detected.

We also performed the above analysis using bilateral seeds from the fourth ventricle. No result was found at the threshold we set in functional connectivity analysis.

## Discussion

In the present study, we compared the vlPAG connectivity changes evoked by 1 Hz and 20 Hz taVNS in migraine patients. Results showed that compared to pre-taVNS resting-state, continuous 1 Hz taVNS produced increased connectivity in the MCC, MFG, precuneus and cuneus. Compared to 20 Hz, 1 Hz taVNS produced greater connectivity increases in the MCC, ACC, precuneus and left insula. There is a significant negative association between migraine attacks in the past 4 weeks and the vlPAG-MCC connectivity during resting-state. Our findings suggest that taVNS with different stimulation frequencies may produce different modulation effects on the descending pain modulation system.

As a non-invasive and safe peripheral neuromodulation method, taVNS has been applied in a wide range of disorders such as depression, epilepsy, tinnitus, migraine, as well as cognitive and behavioral disorders [12, 21, 76–78]. Nevertheless, one challenge for the development of taVNS is to elucidate the modulation effect of taVNS with different parameters so that we can optimize its effects for different disorders.

As a key parameter of taNVS, frequency is a continuous measurement. Thus, it is not possible to test/compare the effects of different frequencies in one study. As a start of this line of work, we have chosen 1 Hz as a representative of low frequency. The 20 Hz frequency has been used to treat depression, and previous studies have found that 20 Hz taVNS can significantly modulate the multiple brain networks [27, 60–62], particularly the functional connectivity of the amygdala [62], default mode network [60], hypothalamus [79], and ventral striatum [30], all of which are associated with pathophysiology of migraine [80, 81]. Further, investigators found that 20 Hz taVNS in healthy subjects could decrease heart rate [19], and 20 Hz is also close to the higher frequency used in a previous study in which the authors have compared the treatment effect of 1 Hz and 25 Hz in [21]. Thus, we have chosen 20 Hz to represent a moderate frequency in this study.

We found that continuous 1 Hz taVNS can significantly increase vlPAG-MCC connectivity. In addition, the vlPAG-MCC connectivity during resting-state before 1 Hz taVNS was negatively associated with participants' migraine attacks. Literature suggests that the MCC is involved in the affective, cognitive, attention, and orienting aspects of pain [82–84]. A previous study found that migraine is associated with decreased grey matter at the MCC [85] and increased activation during experimental heat pain (compared to healthy controls). The pain-induced MCC activation is associated with migraine attacks in migraineurs [86]. Interestingly, we found that the vlPAG-MCC connectivity increased during 1 Hz taVNS, but not during 20 Hz taVNS, which may provide a neural mechanistic support to a previous clinical trial [21], in which researchers investigated the therapeutic effects of daily 1 Hz and 25 Hz taVNS on chronic migraineurs over 3 months, and demonstrated that 1 Hz taVNS was more prominent in migraine alleviation.

Furthermore, we observed that continuous 1 Hz taVNS can produce vlPAG-rACC connectivity increases compared to 20 Hz. In addition, we also detected an increase in vlPAG-rACC connectivity (compared to resting-state) at a less conservative threshold ($p = 0.01$, cluster size = 14). The rACC is a key region of the DPMS [35, 36], and contains numerous opioid receptors [87]. Previous studies have suggested that the rACC plays an important role in the pathophysiology of migraine [88, 89]. Findings from the current study are consistent with our prior study, in which migraine patients are associated with reduced connectivity of the PAG-rACC, compared to healthy subjects, and effective acupuncture treatment can normalize the decreased connectivity in PAG-rACC correspondingly [41]. Further, the study demonstrates that a DPMS abnormality might be an underlying pathological mechanism of migraine, and such an abnormality can be normalized by effective treatment.

In addition, we found that 1 Hz taVNS can increase vlPAG connectivity with the precuneus and cuneus. The precuneus is a key region in the default mode network. Studies suggest that the default mode network (DMN) is a pivotal network affected by migraine [90–92]. We found that migraineurs showed decreased functional connectivity between the right frontoparietal network and precuneus compared with healthy controls, and the connectivity significantly increased after effective treatment [93]. In a more recent study, we found abnormal

posterior thalamus (pulvinar nucleus) dynamic network functional connectivity with the precuneus, and the changes were significantly correlated with the headache frequency of migraine [79].

The cuneus is a key region of the visual network. In a recent longitudinal study on grey matter volume of migraineurs, researchers found that migraineurs developed a decreased grey matter volume of visual regions, including the cuneus. The decreased volume was associated with the level of migraine severity, in terms of disease duration, pain intensity, and attack frequency [94]. We found migraine is associated with altered posterior thalamus dynamic network functional connectivity with the visual cortex [79], and the abnormal functional connectivity within the visual, default mode, sensorimotor, and frontal-parietal networks, which could discriminate migraineurs from healthy controls, with 93% sensitivity and 89% specificity [95]. More recently, we found that 4-week taVNS at 1 Hz can decrease the connectivity between the occipital cortex-related thalamus subregion and the postcentral gyrus/precuneus [96]. Taken together, these studies demonstrate the important role of the precuneus and cuneus in the pathology of migraine. Our study further suggests that 1 Hz taVNS may modulate the connectivity between the descending pain modulation system, the default mode network, and the visual network.

Nevertheless, the question whether the effective frequency of taVNS that influences migraine is different from other diseases remains open [19, 20, 97]. Further studies are needed to determine the optimal frequency of taVNS for different diseases. Additionally, as a brain imaging study, the aim of this study was to investigate and compare if 1 Hz and 20 Hz taVNS can modulate the vlPAG functional connectivity in a migraine population rather than assessing the efficacy/clinical effects of 1 Hz and 20 Hz taVNS. In addition, we used seeds in the ventricle as a control ROI, and the lack of significant results further validated our findings.

Potential limitations of this work include a relatively small sample size of migraine participants with low-frequency migraine attacks. Future studies are needed to investigate if the findings can be replicated in migraineurs with high attack frequencies in a larger sample size. Also, there are only three male participants (of 24 in total) included in this study. This ratio is partly consistent with epidemiology studies showing the prevalence rate of female migraineurs is much higher than male migraineurs [98]. Nevertheless, we have applied a crossover design, which should have controlled the potential gender effects in this study. This study is not designed to answer the question of gender differences. A future study is needed to elucidate if male and female migraineurs are associated with same taVNS response.

Furthermore, our MRI scans were applied when participants were migraine-free, so we could not assess the acute effects of taVNS on headache intensity. Also, clinical trials on migraine usually assess the clinical improvement (migraine attack time or pain intensity) in the past month, thus, we could not investigate/compare the clinical improvement produced by single 1 Hz/20 Hz taVNS treatment, as well as the association between functional connectivity changes (evoked by 1 Hz and 20 Hz taVNS) and clinical improvement. Moreover, although still under investigation, some studies suggest that different stimulation frequencies of taVNS may induce different changes in heart rate, which can be considered as a confounding factor of functional connectivity [19, 99]. Nevertheless, the heart rate changes evoked by taVNS are relatively small, and studies also show no significant change on blood pressure values after taVNS [100]. Future study should consider measuring this confounding factor and adjust for it during data analysis.

## Conclusion

In summary, we found continuous 1 Hz taVNS can significantly modulate functional connectivity between the vlPAG and key regions of the DPMS in patients with migraine. Our findings demonstrate the important role of stimulation parameters (particularly the frequency) in taVNS treatment of different disorders.

#### Abbreviations

taVNS: Transcutaneous auricular vagus nerve stimulation; DPMS: Descending pain modulation system; DMN: Default mode network; MRI: Magnetic resonance imaging; fMRI: Functional magnetic resonance imaging; PAG: Periaqueductal gray; vlPAG: Ventrolateral periaqueductal gray; ACC: Anterior cingulate cortex; rACC: Rostral anterior cingulate cortex; MCC: Middle cingulate cortex; PCC: Posterior cingulate cortex; MFG: Middle frontal gyrus; RVM: Rostroventral medulla; mPFC: Medial prefrontal cortex; ROI: Regions of interest; FWHM: Full width at half maximum; VAS: Visual analog scale.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-021-03024-9.

**Additional file 1: Figure S1.** Electrodes and clips used in this study.

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Not applicable.

#### Authors' contributions

JK and BL designed the study. Data collection was performed by YZ, HL, ZY, XL, XH and WC. Data analysis was performed by JC. JC, JK and SH prepared the manuscript draft. All authors read and approved the final manuscript.

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**Availability of data and materials**
Data supporting the findings of this study are available from the corresponding author, upon reasonable request.

## Declarations

**Ethics approval and consent to participate**
Study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Z2016-079-01), and a written informed consent was obtained from all participants in accordance with the Declaration of Helsinki prior to study enrollment.

**Consent for publication**
Not applicable.

**Competing interests**
Jian Kong has a disclosure to report (holding equity in a startup company, MNT, and a patent to develop new neuromodulation devices) but declares no conflict of interest. All other authors declare no competing interests.

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## Publisher's Note

# Acute and long-term VNS effects on pain perception in case of treatment-resistant depression

Borckardt J. Jeffrey, et al. (2005)
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**ABSTRACT**

Vagus Nerve Stimulation (VNS) is approved by the FDA for treatment of both epilepsy and depression. Recent work has shown that VNS acutely affects pain perception in humans, actually increasing pain sensitivity momentarily while the device is firing. It is unclear how this acutely increased sensitivity might change over time with treatment and how it might relate to longer-term therapeutic effects of VNS on pain. We describe a patient with treatment-resistant depression and a history of severe lumbar degenerative disease with resultant chronic low back pain. His depression and pain symptoms both seemed to respond to VNS. He eventually stopped all medications and remained depression and pain free for 35 months with no change in his device settings. Sixty-six months after VNS implantation and 64 months after his initial clinical antidepressant response, under single-blind conditions, we performed quantitative sensory testing with laboratory thermal pain procedures during acute VNS-on and -off conditions. Interestingly, despite a significant and profound anti-nociceptive clinical response for the previous 35 months, he had significant increases in painfulness ratings while the VNS device was actively firing compared with device-off conditions. This case suggests that VNS-induced acute increases in pain sensitivity can coexist with a clinical anti-nociceptive response. If the acutely increased sensitivity sets the stage for the slower chronic anti-pain effects, the increased acute sensitivity does not disappear. Acute and chronic effects of VNS on pain perception merit further research.

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# The effect of transcutaneous vagus nerve stimulation on pain perception - An experimental study

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**ABSTRACT**

**Background**
Recent preclinical work strongly suggests that vagus nerve stimulation efficiently modulates nociception and pain processing in humans. Most recently, a medical device has offered a transcutaneous electrical stimulation of the auricular branch of the vagus nerve (t-VNS) without any surgery.

**Objective**
Our study investigates whether t-VNS may have the potential to alter pain processing using a controlled design. Methods: Different submodalities of the somatosensory system were assessed with quantitative sensory testing (QST) including a tonic heat pain paradigm in 48 healthy volunteers. Each subject participated in two experimental sessions with active t-VNS (stimulation) or sham t-VNS (no stimulation) on different days in a randomized order (crossed-over). One session consisted of two QST measurements on the ipsi- and contralateral hand, each before and during 1 h of a continuous t-VNS on the left ear using rectangular pulses (250 μS, 25 Hz).

**Results**
We found an increase of mechanical and pressure pain threshold and a reduction of mechanical pain sensitivity. Moreover, active t-VNS significantly reduced pain ratings during sustained application of painful heat for 5 min compared to sham condition. No relevant alterations of cardiac or breathing activity or clinical relevant side effects were observed during t-VNS.

**Conclusions**
Our findings of a reduced sensitivity of mechanically evoked pain and an inhibition of temporal summation of noxious tonic heat in healthy volunteers may pave the way for future studies on patients with chronic pain addressing the potential analgesic effects of t-VNS under clinical conditions.