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Two-Week Cervical Vagus Nerve Stimulation in Chronic Pancreatitis Patients Induces Functional Connectivity Changes of Limbic Structures

Janusiya Anajan Muthulingam, PhD[1,2]; Tine Maria Hansen, PhD[1,2]; Søren Schou Olesen, PhD[2,3]; Asbjørn Mohr Drewes, DMSc[2,3]; Jens Brøndum Frøkjær, MD, PhD[1,2]

ABSTRACT

Objectives: Noninvasive vagus nerve stimulation (nVNS) has not only shown antinociceptive effects, but also demonstrated anti-inflammatory and antidepressant effects. These effects could be beneficial in chronic pancreatitis (CP) patients suffering from chronic abdominal pain, even though the underlying central mechanisms remain unclear. The aim was to investigate the effect of cervical nVNS in patients with painful CP on brain functional connectivity and cerebral metabolites.

Materials and Methods: In a randomized double-blind, sham-controlled crossover trial, we used resting-state functional magnetic resonance imaging to investigate functional connectivity changes of limbic structures (seed-based analysis) after two weeks cervical nVNS treatment (GammaCore) as compared with two weeks sham treatment. Similarly, magnetic resonance spectroscopy was performed in the anterior cingulate cortex (ACC) with assessment of glutamate/creatine (Glu/cre) and N-acetylaspartate/creatine (NAA/cre).

Results: Sixteen CP patients (mean age 56.6 $\pm$ 9.4 years) completed the trial. nVNS induced reduced functional connectivity compared to sham treatment between 1) bilateral thalamus and bilateral superior frontal gyrus, 2) ACC and putamen, and 3) posterior cingulate cortex and right thalamus (all $p < 0.05$). No changes were observed in Glu/cre ($p = 0.96$) and NAA/cre ($p = 0.43$) levels between the nVNS and sham treatments.

Conclusion: In our population of CP patients, cervical nVNS compared with sham treatment induced reduced functional connectivity of limbic structures, as also observed in other patient groups. The findings are relevant, since we have previously demonstrated an effect on pain scores in CP patients for both nVNS and sham treatment. Our results elucidate the effects in the central nervous system following nVNS treatment of CP patients, pointing at potential beneficial effects in this patient group.

Keywords: Chronic pancreatitis, functional magnetic resonance imaging, magnetic resonance spectroscopy, neuromodulation, vagus nerve stimulation

Conflict of Interest: The authors reported no conflict of interest.

INTRODUCTION

The vagus nerve provides an afferent and efferent network of innervation for the viscera playing a critical role as an interface between the central nervous system and gastrointestinal tract (1). Among others, the vagus nerves can be activated with exogenous electrical stimulation with noninvasive vagus nerve stimulation (nVNS) (2). nVNS has provided evidence of therapeutic effects in the treatment of migraine, and is approved to treat episodic cluster and migraine headaches (2). In addition to an antinociceptive effect, nVNS also mediates anti-inflammatory effects (3), improves memory and cognition (4), and induces antidepressant effects (5). Although the mechanisms are not all clear, nVNS is exceedingly being explored as a treatment option for several disorders including chronic pain conditions such as chronic pelvic pain (6) and fibromyalgia (7).

Address correspondence to: Jens Brøndum Frøkjær, MD, PhD, Mech-Sense, Department of Radiology, Aalborg University Hospital, P.O. Box 365, DK-9100 Aalborg, Denmark. Email: jebf@rn.dk

[1] Mech-Sense, Department of Radiology, Aalborg University Hospital, Aalborg, Denmark;
[2] Department of Clinical Medicine, Aalborg University, Aalborg, Denmark; and
[3] Centre for Pancreatic Diseases, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark

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Functional magnetic resonance imaging (fMRI) studies have shown that nVNS affects brain regions important in pain processing such as the thalamus, anterior cingulate cortex (ACC), insula and prefrontal cortex, indicating a supraspinal influence on pain perception (2). Particularly, nVNS reduced the physiological response (decreased activity in thalamus and cingulate cortex) to noxious stimuli and impacted pain related brain regions (2,8). In addition, cerebral metabolic changes reflecting neurotransmitter signaling have also been observed in neuropathic pain after neuromodulation with transcranial direct current stimulation, showing increases in both glutamate/creatine (Glu/cre) and N-acetylaspartate/creatine (NAA/cre) in the ACC (9).

Severe chronic abnormal pain is one of the hallmarks of chronic pancreatitis (CP) and is difficult to treat (10,11). Today there is strong evidence that peripheral and central sensitization as well as impaired descending pain modulation contribute to the development and chronification of pain in CP (12). In addition to pain, CP patients are also experiencing psychosocial symptoms, including depression, anxiety, and reduced quality of life (13,14). Therefore, there is a need for development and evaluation of effective treatments targeting altered brain functioning in CP, where nVNS could have a potential effect. Patients with painful CP are known to exhibit structural (15) and functional reorganization of the central nervous system (16) as well as cerebral metabolic disruption (17). Specifically, increased functional connectivity has been demonstrated in the default mode and salience networks (16), and in addition also increased Glu/cre and reduced NAA/cre levels (17). Thus, in attempt to modulate and normalize the functional connectivity and the cerebral metabolites in CP patients, nVNS may be used to potentially reduce functional connectivity of limbic structures (including thalamus, ACC, and posterior cingulate cortex [PCC]) and normalize the cerebral metabolite levels.

Altogether, there could be a potential effect of VNS in CP patients. Thus, in the present randomized sham-controlled crossover clinical study in CP patients with chronic abdominal pain, we used both resting-state fMRI and magnetic resonance spectroscopy to characterize the cerebral changes induced by two-week cervical nVNS in comparison to sham treatment. We previously reported that both nVNS and sham treatment induced improvement in subjectively assessed pain scores, but with no significant better pain improvement when comparing nVNS to sham treatment (18). However, to explore the underlying central mechanisms objectively, a first step will be to explore if that nVNS as compared to sham treatment can induce expected functional changes also in CP patients, despite that these patients are known to have pronounced structural, functional, and metabolic brain changes (15–17).

We hypothesized that 1) cervical nVNS treatment induces decreased functional connectivity of the limbic structures (thalamus, ACC, and PCC) as compared with sham treatment and 2) nVNS treatment normalizes the cerebral metabolites in ACC by decreasing Glu/cre and increasing NAA/cre as compared with sham treatment. Hence, the primary aim was to determine the functional connectivity changes of limbic structures after two-week cervical nVNS treatment as compared with sham treatment. The secondary aim was to detect cerebral metabolite changes after two-week nVNS treatment as compared with sham.

MATERIAL AND METHODS

Study Overview

This study was a part of an investigator initiated randomized, double-blind, sham-controlled, crossover study conducted at Departments of Radiology and Gastroenterology, Aalborg University Hospital, Denmark. The study was approved by the North Denmark Region Committee on Health Research Ethics (N-20170023) and the Danish Medical Agency (2017023686). Also, the study was monitored by the Good Clinical Practice Unit, Aalborg, Denmark and conducted according to the Declaration of Helsinki. The clinical trial was registered at ClinicalTrials.gov (NCT03357029). All patients provided written informed consent. The protocol for this clinical trial and results from the primary clinical endpoints including pain scores has been published elsewhere (18,19).

Study Subjects

A medical doctor screened the patients for the inclusions/exclusion criteria. The patients were included if they had a CP diagnosis based on the Mayo Clinic diagnostic criteria (20) and suffered from chronic abdominal pain more than three days per week for longer than three months. The pain should be considered refractory to treatment with usual analgesics. Furthermore, patients should be above 18 years old. Patients were excluded, if they had ongoing alcohol or illegal drug dependencies, any clinically significant abnormalities that in the opinion of the investigator could increase the risk associated with trial participation, cardiovascular diseases, low blood pressure (<100/60 mmHg), elevated intracranial pressure, females who were pregnant or lactating, contraindications for MRI, previous surgery on the vagus nerve, and known neuropathy.

Randomization and Treatment

Eligible patients were randomly assigned to receive a two-week nVNS and sham treatment in a randomized order with a washout period of two weeks between treatments. The two-week washout period was chosen based on previous studies of neuromodulation and has shown to be sufficient to reset the effects of neuromodulation (21,22). An automatic web-based randomization program was used to generate the randomization list. Patients and those administrating medical devices and assessing the outcomes were blinded to the group assignment.

An FDA approved medical device (GammaCore-S, ElectroCore LLC, Basking Ridge, NJ, USA) was used as active nVNS treatment. The active device produces electrical stimulation at 25 Hz (5 kHz sine wave burst lasting for 1 msec) with a 24 V peak voltage and 60 mA peak output current (19). The units of stimulation (0–40) translate to the voltage in a linear relation. The sham treatment was delivered with a sham device (ElectroCore LLC), which was identical in appearance, weight, visual and audible feedback, but did only deliver vibration instead of electrical stimuli. Patients were trained on correct positioning, parallel with the carotid artery. Patients were instructed to adjust the stimulation intensity with step-by-step increase until a feeling of mild pain and unpleasantness, and then the intensity was decreased one step for a comfortable and sufficient sensation. All patients were instructed to self-administer the treatment with stimulation for 2 min bilaterally on the neck three times per day (morning, afternoon, and evening, aiming at eight hours between stimulations taking daily routines into consideration) for two weeks. For detailed information, see study protocol (19).

Study Visits

During the study period, patients participated in four identical MRI sessions, before and after both treatment periods. All patients fulfilled a daily pain diary to assess the clinical abdominal pain scores, based on a 0–10 visual analogue scale, in which patients rated the average and maximal daily pain intensity (23). These findings are reported in our primary publication (18), and are briefly summarized in the discussion. Patients were instructed not to change their daily pain medication and they were only allowed to take extra pain medication in the case of pain exacerbation. Furthermore, the electronic medical records were reviewed to obtain demographic and clinical characteristics.

MRI Data Acquisition

MRI data were acquired on a 3T General Electric scanner (GE Signa HDxt, General Electric, Milwaukee, WI, USA) with a standard eight-channel head coil. Resting-state fMRI was acquired for 6:32 min as 192 volumes of gradient echo planar images (repetition time [TR]/echo time [TE]: 2000/30 msec, flip angle: 90°, field of view: 24 cm, matrix: 64×64, and voxel size: $2.5 \times 2.5 \times 3.8$ mm). A T1-weighted structural scan was acquired for coregistration of the fMRI data (TR/TE: 9.0/3.6 msec, flip angle: 14°, field of view: 25 cm, matrix: 320×320, and voxel size: $0.8 \times 0.8 \times 1.0$ mm). Patients were instructed to remain awake and with closed eyes. Finally, magnetic resonance spectroscopy was obtained using single voxel PRESS (Point RESolved Spectroscopy) (TR/TE: 2000/30 msec). A voxel of interest measuring $20 \times 20 \times 20$ mm was placed on a sagittal T2-weigted image in the midline of the pregenual ACC with the inferior border along the anterior–posterior commissure line.

Table 1. Demographic and Clinical Characteristics of Chronic Pancreatitis Patients.

	Chronic pancreatitis ($n = 16$)
Age (years)	56.6 ± 9.4
Male, *n* (%)	14 (87.5)
Body mass index (kg/m^2)	21.9 ± 3.6
Etiology of chronic pancreatitis, *n* (%)	
Alcohol	11 (42)
Nicotine	10 (38)
Nutritional	0 (0)
Hereditary factors	2 (8)
Efferent duct factors	3 (12)
Immunological factors	0 (0)
Miscellaneous and rare metabolic factors	0 (0)
Duration of chronic pancreatitis (years)	9.7 ± 8.4
Diabetes, *n* (%)	9 (56.3)
Analgesics, *n* (%)	
Paracetamol	11 (36)
NSAID	1 (3)
Opioids	13 (42)
Adjuvant analgesics, *n* (%)	
TCA	1 (3)
SNRI	0 (0)
Gabapentoids	5 (16)

Values are means ± SD. Percentages may not total 100 due to rounding.
NSAID, nonsteroidal anti-inflammatory drug; nVNS, noninvasive vagus nerve stimulation; SNRI, serotonin-norepinephrine reuptake inhibitor; TCA, tricyclic antidepressant.

Resting-State fMRI Data Analysis

The resting-state fMRI data were preprocessed and analyzed using CONN toolbox version 18.a (https://www.nitrc.org/projects/conn) (24). Data were slice time corrected, head motion corrected, coregistered the anatomical image to the mean functional image, segmented, spatially normalized into the standard space of the Montreal Neurological Institute (MNI) and then spatially smoothed with $8 \times 8 \times 8$ mm^3 Gaussian kernel. Also, the preprocessing included band-pass filtering at 0.008–0.09 Hz.

Functional connectivity analyses were performed using a seed-to-voxel correlation approach in which the time-course signal in a seed region was correlated with all brain voxels (25). The seeds (ACC, PCC, and bilateral thalamus) were extracted from the CONN network cortical region of interest brain atlas.

SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) was utilized to perform the seed-to-voxel group analyses, allowing to study the differences in ACC, PCC, bilateral thalamus functional connectivity 1) between scans after nVNS treatment and scans after sham treatment, and 2) between the two baselines before nVNS and before sham treatment. Additionally, the differences in ACC, PCC, and bilateral thalamus functional connectivity were studied 1) between scans after nVNS treatment and its corresponding baseline, and 2) between scans after the sham treatment and its corresponding baseline. Paired *t*-tests were performed on each resultant connectivity maps. The initial significance threshold was set to $p \leq 0.001$ and a cluster extent of 100 voxels. Results were presented for cluster-level corrected for multiple comparisons with $p < 0.05$ (family-wise error correction).

Magnetic Resonance Spectroscopy Analysis

For magnetic resonance spectroscopy measurements, LCModel (Version 6.3) was used (26). Both water scaling and eddy-current correction were performed, and metabolites were fitted in the chemical shift range 0.1–4.0 ppm. NAA/cre and Glu/cre were analyzed. Metabolites with Cramér-Rao bounds <30% were included. Additionally, quality measurements (signal-to-noise ratio [SNR] and full width at half maximum [FWHM]) were provided from the analyses in LCModel.

Statistical Analysis

All demographic data and clinical characteristics are given as mean ± standard deviation unless otherwise indicated. In view of the non-Gaussian data distribution, nonparametric analysis was performed. Pairwise comparisons were performed to assess differences in stimulation intensities and cerebral metabolites. All the statistical analyses were performed in SPSS (Version 25.0, IBM Corp., Armon, NY, USA). $p < 0.05$ was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

Demographic and clinical characteristics are provided in Table 1. Furthermore, the average intensity of the stimulations was 31.8 ± 6.9 units for nVNS treatment and 36.0 ± 7.9 units for sham treatment ($p = 0.02$).

Decreased Functional Connectivity After nVNS Treatment as Compared With Sham Treatment

As seen in Table 2 and Figure 1, the seed-to-voxel analyses revealed several areas of decreased functional connectivity after cervical nVNS treatment as compared with sham treatment. Hence, decreased functional connectivity was particularly observed between ACC and left putamen ($p = 0.012$) as well as between PCC and right thalamus ($p = 0.020$). Additionally, reduced functional connectivity was observed between left thalamus and left superior frontal gyrus ($p = 0.035$), and between left thalamus and left inferior occipital gyrus ($p = 0.010$). Similarly, reduced functional connectivity was observed between right thalamus and 1) left superior frontal gyrus ($p = 0.010$), 2) right superior frontal gyrus ($p = 0.017$), and 3) right superior frontal gyrus medial segment ($p = 0.006$) after nVNS treatment.

There were no significant clusters where sham treatment had reduced functional connectivity compared to nVNS treatment (Table 2). Also, no significant differences in functional connectivity of areas relevant for the treatment response or pain were seen when comparing the two baseline scans (data not shown).

Additionally, Table 3 illustrates the functional connectivity changes between nVNS treatment and baseline, and between sham treatment and baseline. Briefly, decreased connectivity between nVNS treatment and baseline was observed between right thalamus and orbital gyrus, and between ACC and precentral gyrus, and increased connectivity was observed between ACC and occipital areas. Decreased connectivity between sham treatment and baseline was observed between right thalamus and inferior temporal gyrus/precentral gyrus and between ACC/PCC and precuneus/frontal areas. Increased connectivity between sham treatment and baseline was observed between left thalamus and cingulate areas.

Metabolites of the ACC After nVNS Treatment as Compared With Sham Treatment

There were no differences between the nVNS and sham treatments in NAA/cre (nVNS: 1.04 ± 0.13, sham: 1.07 ± 0.11, $p = 0.43$) and Glu/cre (nVNS: 1.25 ± 0.15, sham: 1.27 ± 0.20, $p = 0.96$) levels of the ACC. No differences between the nVNS and sham treatments were observed for the quality measurements SNR (nVNS: 13.38 ± 4.77, sham: 12.50 ± 4.69, $p = 0.32$) and FWHM (nVNS: 0.054 ± 0.015, sham: 0.053 ± 0.013, $p = 0.53$). No significant differences in NAA/cre and Glu/cre were seen when comparing the two baseline scans (all $p > 0.05$). Finally, no differences were found in NAA/cre between nVNS and baseline ($p = 0.059$) and between sham and baseline ($p = 0.95$), and in Glu/cre between nVNS and baseline ($p = 0.13$) and between sham and baseline ($p = 0.86$).

DISCUSSION

This is the first clinical fMRI trial exploring the effect of cervical nVNS treatment in CP patients with chronic abdominal pain as compared with a sham treatment. We demonstrated that nVNS as compared with sham treatment decreased functional connectivity of ACC, PCC, and bilateral thalamus (limbic structures). No differences were observed in cerebral metabolites between nVNS and sham treatment. Taken together, our findings implicate that a two-week nVNS treatment can modulate functional brain connectivity in CP patients, and in a way that resembles nVNS result from other patient groups and healthy subjects. As reported in our publication of the primary clinical endpoints, we previously found no significant pain improvement when directly comparing nVNS and sham treatment, whereas the reductions in pain scores were similar and significant for both treatments when compared to their individual baseline symptoms (18). Hence, despite a similar effect on pain scores for both nVNS and sham treatment, which opens the possibility for some placebo effect, our explorative functional brain connectivity findings could suggest a genuine effect of nVNS on the brain in our population of patients. Also, our additional comparison of connectivity between sham treatment and baseline could suggest a placebo response involved in our study.

nVNS Induction of Decreased Functional Connectivity

Our results support the hypothesis of decreased functional connectivity of ACC, PCC, and thalamus after nVNS treatment as compared to sham treatment (2,8). This is in line with a previous fMRI study that detected decreased activity in limbic structures (PCC, thalamus) with auricular nVNS as compared with sham stimulation (8). Our findings also resemble observations using invasive vagus nerve stimulation, showing diminished activity in limbic structures such as the cingulate areas (27). Thus, the decreased functional connectivity of the limbic structures in CP patients could indicate that nVNS modulates the brain function.

Table 2. Functional Connectivity Changes Between nVNS and Sham Treatment.

Contrast	Source seed	Regions	Peak *T*-value	Peak MNI coordinates (*x*, *y*, *z*)	Cluster volume (mm^3)	Cluster *p* value
Decreased FC in nVNS treatment compared with sham treatment	Thalamus L	Left superior frontal gyrus/middle frontal gyrus	8.34	−16, 22, 42	225	0.035*
		Left inferior occipital gyrus	5.84	−38, −62, 0	155	0.010†
	Thalamus R	Left superior frontal gyrus	11.19	−18, 24, 40	305	0.010*
		Right superior frontal gyrus/middle frontal gyrus	5.69	18, 22, 42	130	0.017†
		Right superior frontal gyrus medial segment	5.04	6, 46, 26	181	0.006†
	ACC	Left putamen	5.09	−14, 6, 0	136	0.012†
	PCC	Right thalamus	4.85	22,–20, 2	112	0.020†

Only significant clusters are presented.
*Family-wise error corrected *p* values at cluster level.
†Uncorrected *p* values at cluster level.
ACC, anterior cingulate cortex; FC, functional connectivity; L, left; PCC, posterior cingulate cortex; R, right.

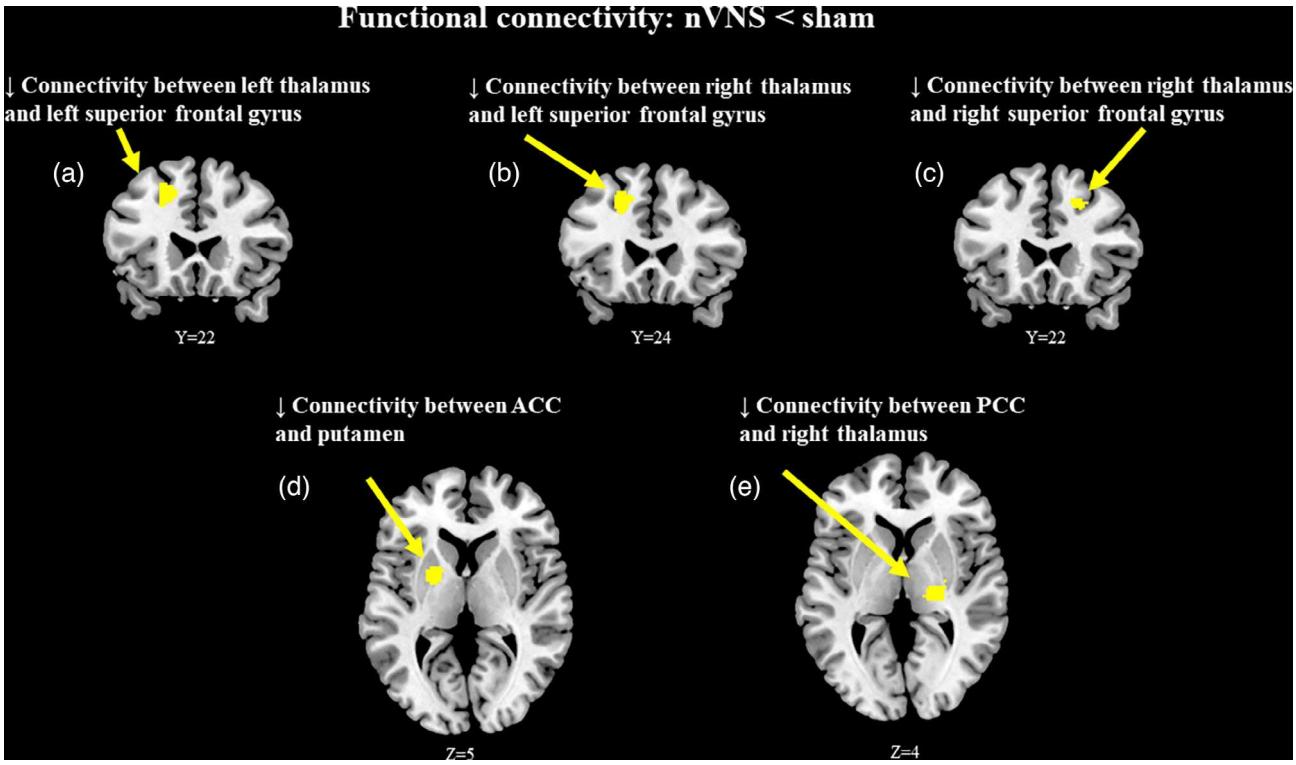

Figure 1. Resting-state functional connectivity was decreased after a two-week cervical nVNS treatment as compared with sham treatment. a. Connectivity between left thalamus (seed) and left superior frontal gyrus. b. Connectivity between right thalamus (seed) and left superior frontal gyrus. c. Connectivity between right thalamus (seed) and right superior frontal gyrus. d. Connectivity between ACC (seed) and putamen. e. Connectivity between PCC (seed) and right thalamus. ACC, anterior cingulate cortex; nVNS, noninvasive vagus nerve stimulation; PCC, posterior cingulate cortex. [Color figure can be viewed at wileyonlinelibrary.com]

ACC is a part of the limbic system and is involved in cognition, decision-making, memory, and emotion (28). Also, ACC may be a key region that is functionally disrupted across different disorders of cognition (29). Some studies have shown altered functional connectivity of ACC in patients with depression (30). Thus, reduced functional connectivity of ACC may be related to cognitive disturbance known to be prevalent in CP patients (14). ACC is involved in pain modulation, and is one of the most commonly reported areas activated by noxious stimuli and may influence affect-related nociceptive connectivity in other regions (9). Consistent with this, we showed that nVNS induced reduced functional connectivity between ACC and putamen, indicating that nVNS possibly reduce connectivity relevant for pain perception. This is also in line with previous studies suggesting that chronic pain induce functional alternations in the ACC, and that an inhibition of ACC activity is known to induce a consistent analgesic effect (28). Taken together, in our study, the nVNS induced functional connectivity reduction of ACC could indicate 1) a potential effect on pain modulation of nVNS, 2) a potential effect on cognitive disruption such as deficient emotional regulation in CP patients, or 3) a combination of both. Since we previously observed similar improvements in pain scores after both two-week nVNS and sham treatment, a pronounced placebo effect could likely be present in both treatments (18). However, since nVNS (as compared to sham) induced relevant reductions in functional connectivity resembling other VNS studies, there seems to be a genuine effect of nVNS on brain function. Indeed longer-lasting treatment may be needed to improve the chronic pain condition in CP patients and prove that nVNS could be better than sham treatment. Also, the effect of placebo/sham is known to involve brain areas such as the rostral ACC, dorsolateral prefrontal cortex (DLPFC), and so on (31–33), making it difficult to distinguish between the brain effects of nVNS and placebo. The effects of nVNS, vibration, and placebo are difficult to isolate, and the potential effects can be individual (responders/nonresponders). A real placebo effect could be present in our study, since our comparison of connectivity between sham treatment and its baseline could suggest a placebo response on the brain involving meaningful cingulate and frontal areas. Since also the typically expected effect of placebo/sham treatment on pain is 30% or more (34), future studies using neuromodulation should consider a sham-controlled study design.

We found that nVNS reduced functional connectivity between thalamus and bilateral superior frontal gyrus. The superior frontal gyrus is a part of the DLPFC, which is the key node of several brain networks implicated in the cognitive, affective, and sensory processing (35). DLPFC is shown to be activated in experimental pain studies, and shows abnormal increased function in chronic pain (35). Moreover, neuromodulation studies have revealed that stimulation of the DLPFC has a pain relieving effect on some chronic pain conditions and that this effect is mediated by the descending modulatory system, or through effects on the cognitive or affective aspects of the pain experience (35). Hence, our observed reduction in functional connectivity of the bilateral superior frontal gyrus after nVNS could be related to an nVNS induced reduction in abnormal DLPFC functioning.

Compared with sham, nVNS induced reduced functional connectivity between thalamus and superior frontal gyrus bilaterally. Both brain areas are known to be related to pain (36). A reduction in the thalamic signals in pain patients has shown to be related to alterations in thalamic blood flow and neural activity (37). Overall, thalamus plays an important role in the antinociceptive regulation, the

Table 3. Functional connectivity changes between nVNS and corresponding baseline, and between sham treatment and corresponding baseline.

Contrast nVNS treatment	Source seed	Regions	Peak *T*-value	Peak MNI coordinates (*x*, *y*, *z*)	Cluster volume (mm^3)	Cluster *p* value
Decreased FC in nVNS treatment compared with corresponding baseline	Thalamus L	None				
	Thalamus R	Left medial orbital gyrus	6.54	−10, 24, −28	187	0.007†
	ACC	Left precentral gyrus	6.71	−50, −6, 32	114	0.020†
	PCC	None				
Increased FC in nVNS treatment compared with corresponding baseline	Thalamus L	None				
	Thalamus R	None				
	ACC	Right occipital pole	6.44	12, −94, 12	465	0.001*
		Left superior occipital gyrus	6.09	−26, −94, 22	124	0.016†
	PCC	None				

Contrast sham treatment	Source seed	Regions	Peak *T*-value	Peak MNI coordinates (*x*, *y*, *z*)	Cluster volume (mm^3)	Cluster *p* value
Decreased FC in sham treatment compared with corresponding baseline	Thalamus L	None				
	Thalamus R	Right white matter	6.75	44, −12, −20	349	0.009*
		Right inferior temporal gyrus	5.61	54, −32, −20	124	0.027†
		Right precentral gyrus	5.37	12, −16, 76	281	0.023*
	ACC	Left precuneus	6.22	−8, −42, 56	304	0.013*
		Right middle frontal gyrus	6.21	48, 44, 16	305	0.013*
		Left middle frontal gyrus	5.23	−38, 44, 16	106	0.034†
	PCC	Right precuneus	6.49	8, −52, 42	424	0.002*
		Right precuneus	6.46	6, −54, 68	124	0.022†
		Right middle frontal gyrus	4.98	42, 24, 52	144	0.015†
Increased FC in sham treatment compared with corresponding baseline	Thalamus L	Right posterior cingulate gyrus	7.48	2, −48, 2	289	0.020*
		Right anterior cingulate gyrus	4.80	6, 46, 8	106	0.039†
	Thalamus R	None				
	ACC	None				
	PCC	None				

Only significant clusters are presented.
*Family-wise error corrected *p* values at cluster level.
†Uncorrected *p* values at cluster level.
ACC, anterior cingulate cortex; FC, functional connectivity; L, left; PCC, posterior cingulate cortex; R, right.

processing of emotions, and affective pain processing (37). Hence, reduced functional connectivity may likely play an essential role in sensory discriminative pain and affective emotional pain.

Effect of nVNS on Cerebral Metabolites

It is known that measures of metabolites in the ACC have been related to pain (38,39). Particularly, increased pain intensity has been associated with higher levels of Glu and lower levels of NAA (9). Similarly, we have previously demonstrated that high levels of Glu/cre were related to higher pain intensities in CP patients (17). In our present study, we anticipated that nVNS may influence the cerebral metabolites of the ACC by decreasing Glu/cre and increasing NAA/cre. However, no differences were observed in ACC after nVNS treatment as compared with sham treatment, or between the treatments and baselines. This could be explained by the small sample size. Another potential explanation is that chronic pain is related to disturbance in an extensive neural network distributed cortico-subcortically across the hemispheres and not localized in a single cortical area (40). Also, longer-lasting treatment may be needed to induce sustained metabolic changes and show a difference between nVNS and sham treatment.

Role of Placebo Effect in nVNS Pain Studies

We previously found no significant difference in pain relief comparing nVNS and sham treatment (18). However, we demonstrated that both nVNS and sham treatment significantly induced improvements in pain symptoms when compared to their respective baselines. This could indicate that several possible mechanisms are involved in both treatment arms, including a placebo/sham effect. Although the subjective pain scores were not improved after nVNS as compared with sham treatment, it is based on the findings from this present study likely that nVNS can modulate the cerebral

functional connectivity. We consider that the comparison of connectivity after nVNS and sham treatments probably best reflects the genuine effect of nVNS (canceling out the effect of placebo in both arms), whereas the comparison of connectivity between sham treatment and its baseline probably best reflects the effect of placebo response on the brain. However, this has limitations, since it is very difficult from our seed-to-voxel analysis findings in Table 3, in comparison to Table 2, to derive what is due to a placebo effect and a genuine nVNS effect on the brain, as they a based on statistical maps and not involve the same brain connections. Since sham stimulation provides some vibration that potentially can stimulate the vagal nerve which could potentially induce a minor effect similar to the active treatment, the observed difference between nVNS and sham treatments could potentially underestimate the true effect of nVNS. Hence, we find it likely that nVNS can affect the central sensitization and partially normalize the abnormally increased functional connectivity in CP patients, as demonstrated by Muthulingam et al. (16). In general, clinical studies of neuromodulation should optimally include longer interventions and a sham-controlled study design to address the placebo effects.

Methodological Considerations

There are several limitations of this study. The sham device may not be physiologically inert as it provides some vibration that could stimulate the vagal nerve, which should be taken into consideration when interpreting our results, as discussed above. The many confounders in CP patients with chronic pain, that are typical multimorbid and under treatment with several medications, makes such studies challenging to perform. Also, CP patients are known to be very different and heterogenous in preexisting changes of the sensory system and brain alternations, with makes interpretation and generalizability of our findings even more difficult. Furthermore, the treatments were self-administered by the patients, and thus there was a risk for reduced compliance. Compliance was assessed by reading the remaining stimulation doses on the device display after each treatment period, and to enhance the compliance all patients were required to complete daily records in a diary that was checked at the study visits. Moreover, further studies are needed to evaluate the long-term effect of nVNS treatment on long-lasting chronic pain, and additional studies lasting several months are encouraged. Also, a longer washout period between treatments can be considered. Also, all patients used several types of analgesics, which may influence the effect of nVNS and the observed results. In the resting-state fMRI analysis, we utilized seeds placed with a priori knowledge from the literature, but other areas such as the amygdala and insula could be investigated further, or the thalamus could be investigated in subregions. Also, other analysis approaches could be considered to explore between the placebo and genuine nVNS effects in more details. As the mechanisms of nVNS are relatively unknown, a data-driven analysis could possibly add further information. Finally, psychological, cognitive, and emotional factors could have impact on the brain function (41). Thus, it could be valuable to include psychological evaluations, such as perceived stress, anxiety, depression, coping strategies, and catastrophizing.

CONCLUSIONS

To the best of our knowledge, no previous studies have investigated the effect of cervical nVNS on functional brain connectivity in CP patients with chronic abdominal pain. Despite the limitations, our findings provide important information regarding the potential beneficial brain mediated effects of nVNS treatment. Specifically, our explorative findings implicate that nVNS significantly modulates limbic structures in our population of patients, by diminishing the functional connectivity of the ACC, PCC, and thalamus, even though generalizability to CP in general is very difficult. Altogether, nVNS may potentially have beneficial effects in the central nervous system targeting mechanisms of central sensitization, even though no effect was previously observed on the pain symptom scores (nVNS as compared to sham) in this short-lasting study. However, a potential sham/placebo effect should be considered when designing and interpreting findings in neuromodulation studies. Future research with larger sample sizes, longer duration of treatment, and psychological, cognitive and affective endpoints are warranted.

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Authorship Statement

Jens Brøndum Frøkjær, Janusiya Anajan Muthulingam, Søren Schou Olesen, Tine Maria Hansen, and Asbjørn Mohr Drewes conceived and designed the study and participated in planning of the study. Janusiya Anajan Muthulingam collected the data, analyzed the data, and drafted the initial version of the manuscript. All authors made significant contributions to the development and conceptualization of the protocol. All authors reviewed the draft versions of the manuscript and have read and approved the final manuscript.

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