Neuromodulation: Technology at the Neural Interface

Received: February 10, 2025 Revised: April 6, 2025 Accepted: May 5, 2025

https://doi.org/10.1016/j.neurom.2025.05.003

Abdominal Vagus Nerve Stimulation Increases Firing in the Rat Locus Coeruleus

Tomoko Hyakumura, PhD[1,2]; Sophie C. Payne, PhD[1,2]; Jerico V. Matarazzo, BSc (Hons)[1]; Wendy K. Adams, PhD[1]; James B. Fallon, PhD[1,2,3]

ABSTRACT

Background: Cervical vagus nerve stimulation (VNS) is a clinically available treatment for refractory epilepsy and depression. Animal studies show that electrical activation of the noradrenergic brain region, locus coeruleus (LC), is essential for the therapeutic effects of cervical VNS for the treatment of these conditions. Cervical VNS often causes side effects such as coughing, headache, and apnea-hypopnea. Such side effects can be mitigated by reducing stimulation intensity; however, evidence suggests this reduces efficacy of treatment. Abdominal VNS, targeting the vagus nerve below the nerve branches that cause these side effects, is an alternative strategy to deliver VNS.

Objective: This study aimed to assess whether abdominal VNS increases spike activity in the LC without causing any off-target effects.

Materials and Methods: Cervical and abdominal vagi of anesthetized male Sprague-Dawley rats were implanted with cuff electrode arrays, and a tungsten electrode was used to record neural activity in the LC. Changes in the firing rate of LC neurons and changes to the heart and breathing rate were recorded during cervical and abdominal VNS.

Results: Cervical VNS significantly reduced heart and/or breathing rate (two-way repeated measures analysis of variance, $p < 0.05$; $n = 6$) when stimulation was 0.82 ± 0.09 mA or higher. This stimulation level was termed the "off-target effect threshold." Abdominal VNS did not produce any changes in heart and breathing rate at any stimulus level tested. Cervical and abdominal VNS, delivered at 2 mA (maximum tested) significantly increased spike activity predominantly in the anteromedial LC, compared with prestimulation baseline (paired t-test, $p < 0.001$, $n = 6$). However, when "safe levels," that is, below the off-target effect threshold, of VNS were applied, only abdominal VNS increased spike activity in the LC.

Conclusion: Abdominal VNS activated the LC without causing changes to vitals and could be used as an alternative approach to providing VNS therapy for brain disorders such as drug-resistant epilepsy and depression.

Keywords: Noradrenergic nucleus, neural recordings, neuromodulation, peripheral nerve stimulation, subdiaphragmatic vagus nerve

INTRODUCTION

The vagus nerve is the major component of the parasympathetic nervous system, comprising both afferent and efferent nerve fibers that connect most of the visceral organs and the brain. The vagus nerve regulates a variety of bodily functions including blood pressure, respiration, and feeding.[1] Electrical stimulation of the cervical vagus nerve is clinically available as a treatment for drug-resistant epilepsy, drug-resistant depression, and ischemic stroke rehabilitation.[2,3] Although cervical vagus nerve stimulation (VNS) is a Food and Drug Administration (FDA)-approved therapy of drug-resistant epilepsy, stimulation is often associated with coughing, sore throat, headache, hoarse voice, and worsening of apnea-hypopnea. The main strategy for reducing these side effects is to reduce stimulation intensity, which compromises the efficacy of the therapy.[4,5] Stimulation parameters are often initially set to low levels (0.25–0.5 mA) after implant surgery, with current gradually increased over time on the basis of patient tolerance to side effects

Address correspondence to: Tomoko Hyakumura, PhD, The Bionics Institute, Fitzroy, Victoria, Australia. Email: thyakumura@bionicsinstitute.org

[1] Bionics Institute, East Melbourne, Victoria, Australia;
[2] Medical Bionics Department, University of Melbourne, Parkville, Victoria, Australia; and
[3] Department of Otolaryngology, University of Melbourne, Parkville, Victoria, Australia

For more information on author guidelines, an explanation of our peer review process, and conflict of interest informed consent policies, please see the journal's Guide for Authors.

Source(s) of financial support: This research was supported by the Bionics Institute Incubator Fund. The Bionics Institute acknowledge the support they receive from the Victorian Government through its Operational Infrastructural Support Program.

and evolution of seizures.[5] Studies report that increasing current improves efficacy (≥50% of seizure reduction) in 20% of patients who were nonresponsive.[6] A meta-analysis of four randomized double-blind trials found that high VNS intensities were 1.73 times (95% CI 1.13–2.64) more efficacious than low stimulation protocols in reducing seizures.[5] Furthermore, there are likely age-dependent differences in activation threshold of vagal fibers.[7] Activation of the locus coeruleus (LC), the central noradrenergic nucleus in the brain stem, is deemed essential for the therapeutic effects of cervical VNS (cVNS) for the treatment of epilepsy[8,9] and depression.[10] The LC is the main source of norepinephrine in the brain and plays important roles in the regulation of attention, arousal, stress responses, and emotional memory.[11] cVNS-induced activation of the LC is associated with increased norepinephrine levels in the cortex and the hippocampus.[12,13] Furthermore, cVNS activates the LC in a dose-dependent manner.[14]

Applying electrical stimulation at the abdominal or subdiaphragmatic vagus nerve is a unique strategy to avoid off-target effects of cVNS,[15] and the safety and efficacy of abdominal VNS are now being investigated as a therapy for patients with Crohn's disease (NCT05469607). Promisingly, unilateral afferent abdominal VNS causes an increase in c-Fos positive cells in the LC in rabbits.[16] However, there have been no direct measurements of abdominal VNS effects on neuronal firing in the LC.

As such, this study aimed to assess whether LC neuronal firing increases during abdominal VNS. Specifically, we assessed changes in LC firing during cervical and abdominal VNS delivered at electrical stimulation over a range of current levels, including those below the level that causes disruptions to vitals (heart rate or breathing). In this study, under nonrecovery anesthesia, we implanted two-pair cuff electrode arrays[15] and measured stimulation-induced changes in heart rate and breathing patterns during cervical and abdominal VNS. Changes in LC spike activity were assessed during bursts of cervical and abdominal VNS delivered with matched current levels.

MATERIALS AND METHODS

VNS Electrode Array

A custom-designed, two-bipolar paired platinum electrode cuff array (exposed surface area: 0.44 mm^2) was used, which had a distance between adjacent electrode pairs (center to center) of 4.7 mm.[15,17]

Animals and Anesthesia

Male Sprague-Dawley rats (eight–11 weeks old, Animal Resource Center, Western Australia) were kept on a 12-hour light/dark cycle and allowed ad libitum access to standard chow and water before the experiment. Animals ($n = 6$) were anesthetized using isoflurane (2%–3% isoflurane in 1 L/min oxygen) and given an analgesic (carprofen 5 mg/kg, subcutaneous) and local anesthetic at the surgical incision sites (bupivacaine 1 mg/kg). Before recording brain activity in the LC, animals were given an intravenous infusion of medetomidine (0.06 mg/kg/h), and the isoflurane dose was reduced (0.5%–0.75% in 1 L/min oxygen). Isoflurane was adjusted to allow the breathing rate to be maintained at 36 to 48 breaths/minute; animals were kept warm and hydrated with intravenous infusion of Hartmann's solution (1 mL/h) for the duration of the procedure. At the conclusion of the nonrecovery experiment, the rats were euthanized (Lethabarb 300 mg/kg, intraperitoneal).

Surgical Procedures

Vagus Nerve Implantation

The left cervical vagus nerve (in the neck) and anterior abdominal vagus nerve were both implanted. As previously described,[18] the abdominal cavity was opened and the subdiaphragmatic anterior abdominal vagus nerve identified and implanted. Next, the left cervical vagus nerve was exposed and identified,[19,20] and the VNS electrode array was implanted around the nerve. All cavities and skin were sutured closed.

Femoral Artery and Vein Cannulation

The right femoral artery was cannulated (PE tubing, 0.61 × 0.28 mm, Microtube Extrusions, North Rocks, NSW, Australia) for blood pressure monitoring, and the left femoral vein was cannulated (PVC tubing, 0.96 × 0.58 mm, Microtube Extrusions, North Rocks, NSW, Australia) for intravenous delivery of Hartmann's solution.

Stereotaxic Targeting

The head of the rat was secured to a stereotaxic frame with ear bars and an incisor bar. An incision was performed along the midline, and the skull was exposed to identify bregma and lambda. The skull was leveled on the basis of the stereotaxic coordinates of these landmarks. To target the LC, a burr hole was created (3.7 mm posterior, 1.1 mm lateral from lambda) and a tungsten microelectrode (1 MΩ, World Precision Instruments, Inc) was inserted at a posterior angle of 15° from vertical. The electrode was inserted (−5.5 to −6.5 mm from dura) using a micropositioner (Kopf), with placement adjusted in 0.2-mm increments to identify the LC or mesencephalic trigeminal nucleus (Me5) directly adjacent to the LC. DiI (Sigma-Aldrich) was used to label the electrode for easy identification of electrode tracks (Fig. 1c).

Electrode Impedance Testing and Electrophysiological Recordings

Common-Ground Electrode Impedance

Biphasic current pulses (100 μs per phase with 100-μA current) were passed between the electrode of interest and all other implanted electrodes in the same array (common ground impedance). The peak voltage at the end of the first phase (Vtotal) of the current pulse was used to calculate total impedance (Ztotal) using Ohm's law (Z = voltage/current), and to assess electrode shorting or breakage.[22]

Evoked Compound Action Potentials

Evoked compound action potentials (ECAPs) were recorded to ensure stimulation levels delivered to the cervical or abdominal vagus nerve were above the threshold required to electrically activate the nerve.[17,22] ECAPs were recorded from the most proximal pair of electrodes in each array (abdominal/cervical) relative to the brain after stimulation of the distal pair of electrodes. Electrical stimulation ranged from 0 to 2 mA in 0.1-mA steps at frequency 30 Hz using 100-μs pulse width (PW) and 50-μs interphase gap (IPG) using a custom stimulator.[23] Recordings were amplified (ISO-80, World Precision Instruments), sampled (200 kHz; USB-6255 National Instruments), and digitally filtered (300–3000 Hz; Igor Pro 9, Wavemetrics). The ECAP threshold was defined as the minimum stimulus intensity producing a visible response of ≥0.1μV within a poststimulus latency window of 4 to 10 milliseconds, which is specifically in the range to expect a C-fiber response.[24] ECAP analysis during cervical and abdominal VNS was restricted to

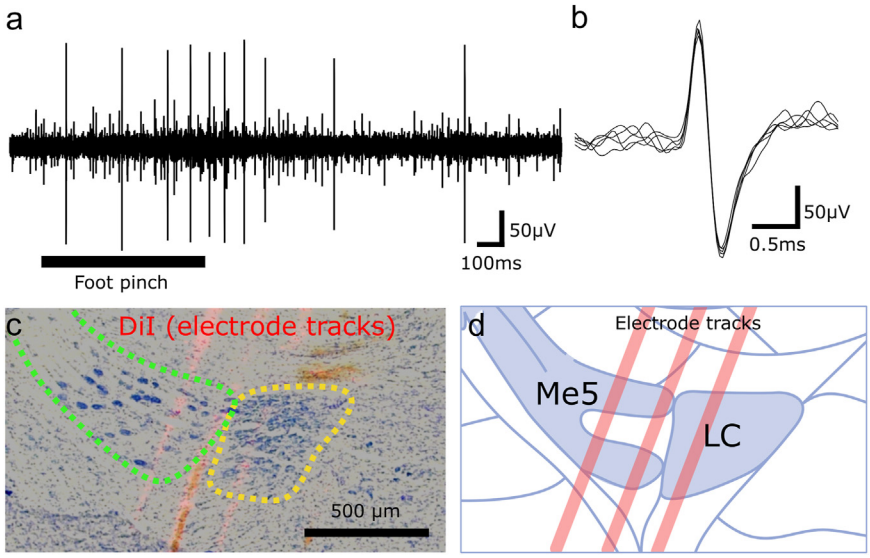

Figure 1. Experimental methods used to confirm electrode positioning in the LC. a. The dark line on the bottom left indicates contralateral foot pinch (~0.5 seconds). The firing rate increased initially and decreased in response to foot pinching, typical of the LC neuron response. b. The spike shape with a notch on the ascending limb, also indicative of LC neuron firing. c. An example image of a sagittal section of the brain stained with cresyl violet (blue) labeling the LC (yellow dotted line) with dense staining and the Me5 (green dotted line) with dark nuclear staining, and a fluorescence image of electrode tracks labelled with DiI (red) overlaid on top. Scale bar = 500 mm. d. A drawing modified from[21] matching the location of panel c, indicating the estimated border of the LC and the Me5 and the electrode tracks in relation to these regions. [Color figure can be viewed at www.neuromodulationjournal.org]

latency window of a C-fiber response because the rat abdominal vagus nerve comprises 97% to 99% C-fibers.[25]

Extracellular Recordings in LC and Me5

ECAPs were measured simultaneously while recording extracellular LC and Me5 neural activity. Extracellular recordings were obtained using a tungsten electrode placed in the LC or Me5, as previously described. Neural signals were amplified from a reference (23-gauge needle) inserted to the skin of the head, digitized (at sampling rate 30 ks/s), and visualized (Cerebus System, Blackrock Microsystems). Electrode placement in the LC or Me5 was identified by a characteristic response to a contralateral hindpaw pinch (Fig. 1) or manipulation of the jaw, respectively.[14,26] LC/Me5 placement was confirmed by an increase in firing rate in response to cVNS[14] (30 Hz, 100 μs PW, 50 μs IPG, 0–2 mA). Once LC/Me5 sites were identified, cervical or abdominal VNS (30 Hz, 100 μs PW, 50 μs IPG, 0–2 mA) was applied for 0.5 seconds every 1.5 seconds.

Recording Off-Target Effects to VNS

Heart Rate and Blood Pressure

The femoral artery cannula was connected to a calibrated blood pressure transducer (ADInstruments) and the signal amplified (Bridge Amp, ADInstruments) and waveforms recorded (Cerebus System 128 Channel Neural Stimulator, Blackrock Microsystems, MA).

Respiratory Rate

Next, 25-gauge needles were placed in the intercostal muscle to record electromyography (EMG) responses that coincided with the animal's respiration. The signal was amplified using Iso-80 Bioamp (gain: $\times 10^2$; high pass: 5 Hz; low pass: 10 kHz, World Precision Instruments) before being recorded (Cerebus System).

Data Analysis

Off-Target Effect Data

Analysis software (Igor Pro 9) was used to identify the peaks of diastolic blood pressure. The time between two diastolic peaks was used to calculate the estimated heart rate. Igor Pro 9 also was used to visualize and identify the time between the respiration events to calculate the estimated respiratory rate. One-way analysis of variance (ANOVA) with Dunnett's post hoc test was performed to determine change in heart or respiratory rate, and the off-target effect threshold was determined as the minimum amount of current required to induce a significant change to baseline breathing or heart rate. One animal was removed from the heart rate analysis owing to a clogged femoral artery cannula, and another was removed from the respiratory rate analysis owing to a misplaced EMG needle.

Neural Recordings/Spike Processing

Before spike counting, stimulation artifacts were blanked (−1 ms before and +3 ms after each artifact) and neural recording digitally filtered (Igor Pro 9; 30–3000 Hz). The root mean square (RMS) of the total neural recording was calculated, and events that crossed 4× RMS were classified as neural spikes (Fig. 1a,b). The number of spikes in the 0.8 seconds after stimulation onset was counted.

Histologic Processing and Imaging

At the end of the experiment, the rats were deeply anesthetized and intracardial perfusion performed with 0.9% (weight per volume) saline and fixative (10% neutral buffered formalin in 0.1 M phosphate buffer, pH 7.4, 4 °C). The brain was dissected, fixed overnight at 4 °C, and thoroughly washed (3 × 10 minutes in 0.1 M phosphate buffered saline [PBS], pH 7.4). Tissue was cryoprotected (30% sucrose in PBS) and then frozen in Tissue-Tek optimum cutting temperature compound (Sakura Finetek, Torrance, CA) (−20 °C). Sagittal sections of the brain (40 μm) were collected and

stained with cresyl violet (C5042, Sigma, Fig. 1c). Images were taken using a Zeiss M2 Imager microscope (Carl Zeiss Microscopy, Jena, Germany) and Zen Pro Software (Zeiss, Germany and New York, NY). Red fluorescence micrographs of electrode tracks labeled with DiI were captured before cresyl violet staining and overlaid with light micrographs of corresponding cresyl violet-stained sections containing the LC and the adjacent ME5 (Fig. 1c,d).

Statistics and Figure Production

Details of each statistical test are stated in the relevant Results section. Differences among normally distributed data were tested using a one- or two-way repeated measures (RM) ANOVA with post hoc tests as appropriate. The off-target effects threshold for each animal was determined using a one-way ANOVA with Dunnett's test, and the average off-target threshold for all animals was determined by a paired *t*-test. Effective VNS in each recording site was determined by testing differences in LC firing between baseline and maximal or "safe" VNS using *t*-test. Statistically significant differences were accepted as *p*-values of <0.05, and GraphPad Prism 10 (GraphPad Software) was used for all analysis.

For figure production, light microscope images were white color-balanced and adjustments made when necessary in contrast and brightness to best represent that observed under the microscope (Inkscape; free and open-source software licensed under GPL).

RESULTS

ECAPs Confirmed Activation of the Cervical and Abdominal Vagus Nerve Through Electrical Stimulation

The common ground impedance of electrodes in vivo was 6.6 ± 0.2 kΩ (mean ± SEM, $n = 6$ rats). After implantation, ECAPs were successfully recorded from both abdominal and cervical vagus nerve sites (Fig. 2a). Analysis was restricted to the window latency of that expected for a C-fiber response (4–10 milliseconds; 0.5–2 m/s).[24] The conduction velocity of the response evoked during cVNS was 0.68 ± 0.02 m/s, whereas abdominal VNS predominantly produced a response of 0.70 ± 0.02 m/s.

A two-way RM ANOVA was performed to analyze the effect of Location (cervical vs abdominal) and Time (start vs end of experiment) on ECAP thresholds. There was no significant interaction between Time × Location ($F_{(1,5)} = 4.31$, $p = 0.093$) or Location as a main effect ($F_{(1,5)} = 1.156$, $p = 0.33$) (Fig. 2b). However, Time had a significant effect on neural threshold, with start of experiment being higher than end of experiment ($F_{(1,5)} = 9.00$, $p = 0.030$, $n = 6$; Fig. 2b).

cVNS Reduced Heart Rate and Respiratory Rate

Reduction in both heart rate and respiratory rate was observed in all six rats during 5-second cVNS (30 Hz, 100 µs PW or 10 Hz, 200 µs PW, up to 2mA). However, when shorter 0.5-second VNS (30 Hz, 100 µs PW, up to 2 mA) was applied, $n = 3$ animals had a reduction in heart rate only; $n = 2$ had reduction in respiratory rate only, and $n = 1$ had a reduction in both respiratory and heart rate. In the four of six animals that had a significant reduction in heart rate ($p <$ 0.0001, one-way ANOVA Dunnett's post hoc test), the average decrease was −83.30 ± 52.37 beats per minute at 2-mA cVNS (Fig. 3a). The average current level that caused a decrease in heart rate was 0.88 ± 0.17 mA. In contrast, there were no changes to heart rate during abdominal VNS ($p = 0.06$, Fig. 3a) up to 2 mA.

In the three of six animals that had a significant reduction in respiratory rate ($p < 0.05$, one-way ANOVA with Dunnett's post hoc test), the average decrease was −14 ± 5.4 breaths per minute at 2-mA cVNS (Fig. 3b). The average current level that caused a decrease in breathing rate was 0.87 ± 0.07 mA. Again, in contrast to cVNS, there were no changes to respiratory rate during abdominal VNS ($p = 0.6$, Fig. 3b) up to 2 mA.

The off-target effect threshold was defined as the highest current that did not produce either a reduction in heart and/or respiratory rate. Because abdominal VNS did not cause a change in heart or respiratory rate at any current level tested, for the purposes of analysis, the off-target effect threshold during abdominal VNS was set at 2 mA. The average off-target effect threshold was significantly lower during cVNS than abdominal VNS (paired student *t*-test, $p < 0.0001$; Fig. 3c).

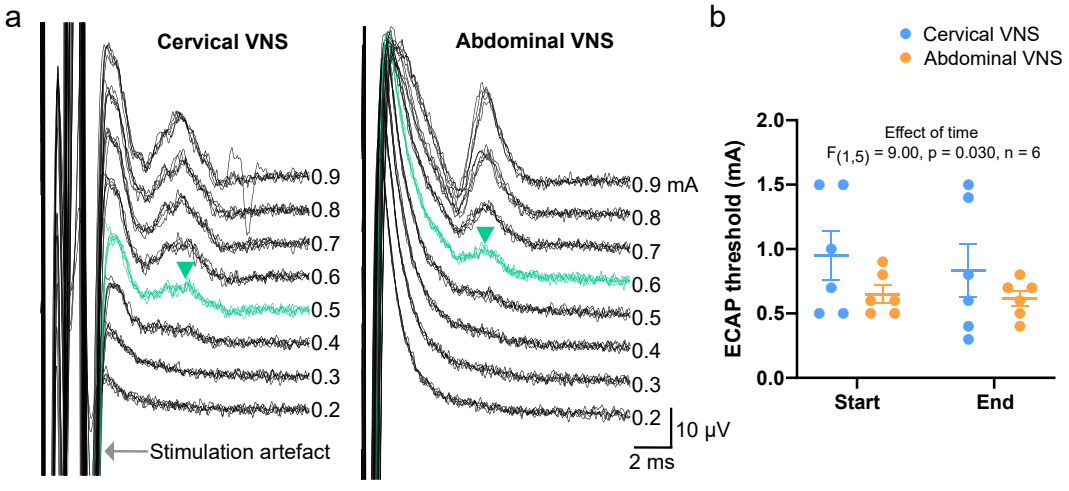

Figure 2. Confirmation of electrode placement by recording ECAPs. a. Representative ECAP waveforms recorded during cervical and abdominal VNS (green denoting threshold responses). b. ECAP threshold of a putative C-fiber response shows there was no main effect of Location, but Time had a significant effect on neural threshold. Data show individual values, mean ± SEM. Significance was accepted as $p < 0.05$; * and ns indicated $p \geq 0.05$. ns, no significance. [Color figure can be viewed at www.neuromodulationjournal.org]

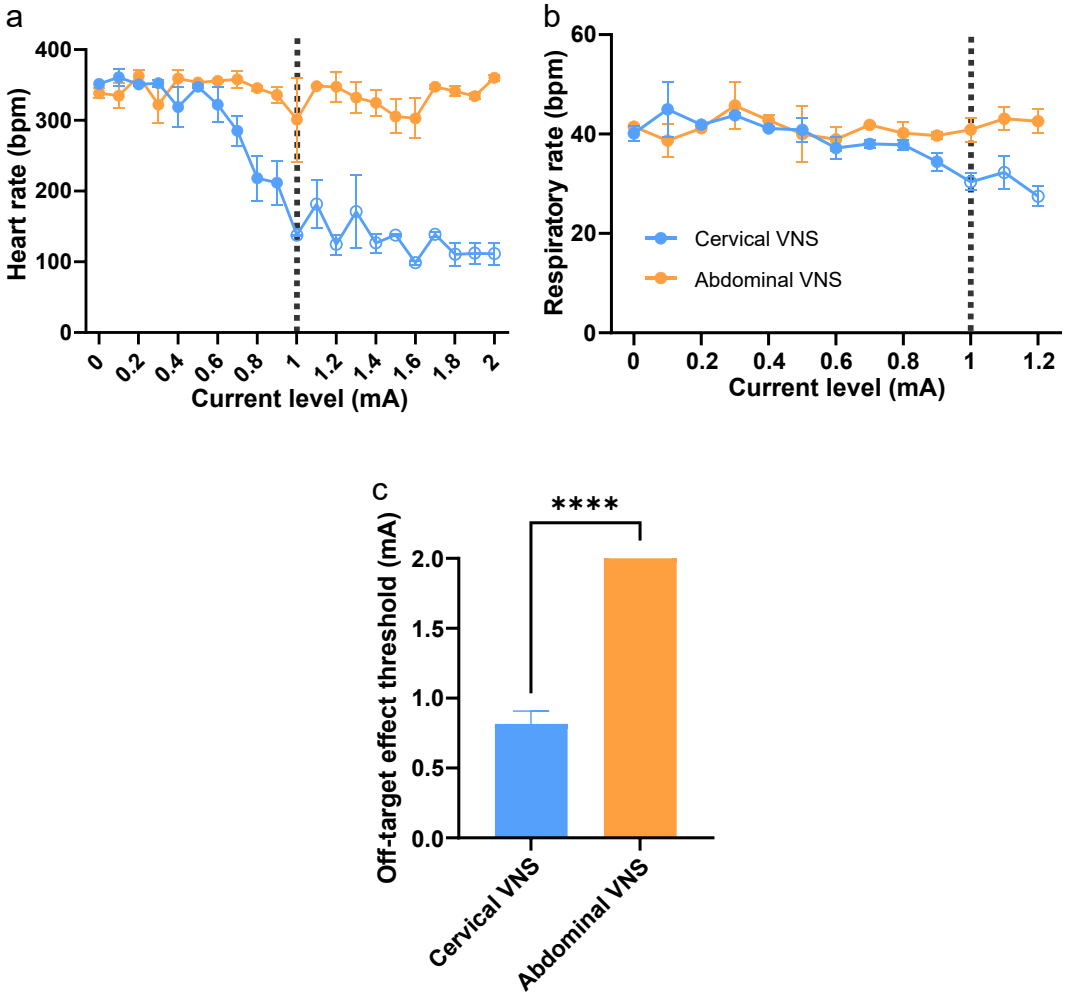

Figure 3. Respiratory and cardiac off-target effects during VNS. a. An example of heart rate reduction in one rat during cVNS showing averages of five repeats per current level. The lowest cVNS current level at which a significant reduction in heart rate (bpm) compared with baseline (0 mA) was observed is marked with a dotted line (one-way ANOVA, Dunnett's test, $p = 0.0024$). b. An example of change in respiratory rate during cVNS, showing averages of five repeats per current level. The lowest current level at which significant reduction in respiratory rate was observed compared with baseline is marked with a dotted line (one-way ANOVA, Dunnett's test, $p = 0.0019$). c. The average off-target effect threshold was determined as 0.82 mA for cVNS, and 2 mA (no off-target effects) for abdominal VNS. The range of safe current level is significantly higher for abdominal VNS than for cVNS (paired *t*-test, $p < 0.0001$). Data in panels a to c show mean ± SEM. In panels a and b, open-circle data points indicate a significant difference from baseline (0 mA) current levels. Significance was accepted as $p < 0.05$; ****indicated $p < 0.0001$. bpm, beats per minute. [Color figure can be viewed at www.neuromodulationjournal.org]

Both cVNS and Abdominal VNS Induced LC Firing, but the Effect Was Greater for Abdominal VNS at Safe Stimulation Current Level

A total of 11 recording sites across all the animals were included in the LC analysis on the basis of the following inclusion criteria: 1) clear ECAPs at the start and end of the experiment; 2) cVNS-induced cardiac and/or respiratory off-target effects; 3) cVNS induced neural spikes in LC (ie, when the number of spikes was plotted against stimulation current level, the trend line shows a positive trend); and 4) increase in LC spike in response to foot pinch and/or histologic confirmation of electrode placement in the LC (Fig. 1).

Statistical analysis was performed using one-way RM ANOVA with Dunnett's post hoc test, to compare baseline (0-mA stimulation) with all other current levels during cervical or abdominal VNS (Fig. 4a). There was a significant stimulation-dependent increase in firing of LC neurons during cVNS at current levels ≥1.2 mA ($p < 0.001$, one-way RM ANOVA with Dunnett's test, $n = 11$) and during abdominal VNS at current levels 1.8 and 2 mA ($p = 0.0084$, one-way RM ANOVA with Dunnett's test, $n = 11$).

At all 11 recording sites, there was a significant increase in firing of LC neurons during cVNS ($p < 0.05$, one-way ANOVA with Dunnett's test, $n = 5$ repeats). During abdominal VNS, there was a significant increase in firing of LC neurons in three recording sites, suggesting there was substantially less innervation of the LC through the abdominal nerve than through the cervical (Fig. 4a presents an example of one recording site).

However, when comparing the effect of VNS at maximum safe stimulation current levels, abdominal VNS had a significant increase in LC neuron firing ($p = 0.0082$), but cVNS did not (range: 0.7–0.9 mA, $p = 0.5122$, one-way RM ANOVA with Dunnett's test, $n = 11$, Fig. 4b).

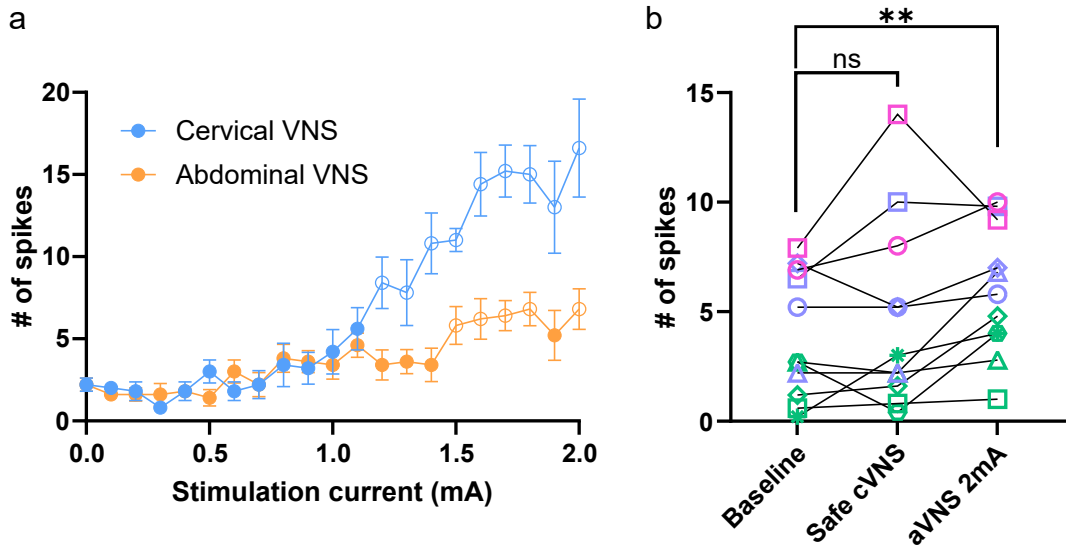

Figure 4. Increases in LC neuron firing during aVNS and cVNS. a. An example of increase in spikes recorded in the LC in a stimulation dependent manner during cervical and abdominal VNS. b. Spike activity increased during 2 mA of abdominal VNS (RM one-way ANOVA, $p = 0.0082$), compared with baseline (0 mA). However, there were no changes in spike activity during cVNS delivered at safe levels below the off-target threshold determined for each animal (0.7–0.9 mA). In panel a, data show mean ± SEM number (#) of spikes recorded in the LC ($n = 5$ repeats per current level at one recording site). Open-circle data points indicate current levels at which spike activity was significantly higher than at baseline (0 mA). In panel b, the data indicate the number of spikes at a recording site ($n = 11$ recording sites). Different colors indicate different animals, and different symbols indicate individual recording sites. Significance was accepted as $p < 0.05$; **indicated $p < 0.001$, and ns indicated $p \geq 0.05$. aVNS, abdominal vagus nerve; ns, no significance. [Color figure can be viewed at www.neuromodulationjournal.org]

Only Abdominal VNS-Induced Neural Activity Was Observed in the Anteromedial LC at Safe Stimulation Levels

Histologic assessment based on the electrode tracks marked with a fluorescent marker (DiI) indicated the activation of VNS-induced response was often observed in the anteromedial region of the LC (Fig. 5). "Effective VNS" for each recording site was determined by testing the difference between baseline and maximal or safe stimulation using a paired *t*-test. The arrangement of cervical and abdominal VNS-induced neural activity in the LC was mapped during maximal (Fig. 5a) and safe (Fig. 5b) stimulation.

At the maximal stimulation level (2 mA), cVNS induced firing of the LC at more recording sites in the LC than did abdominal VNS (Fig. 5a) and induced firing in the Me5.

At stimulation levels below the off-target effect threshold (0.7–0.9 mA, ie, safe current levels below cardiac and/or respiratory off-target effect threshold), only a small fold increase (fold change of 1.77) was observed in one location in the LC (Fig. 5b). Importantly, however, more prominent abdominal VNS-induced firing (fold change of three–20) in the anteromedial region of the LC was observed (Fig. 5b).

DISCUSSION

cVNS is an FDA-approved treatment of drug-resistant epilepsy and depression. However, patients who receive cVNS often report unpleasant side effects. Activation of the LC is central to the effects of cVNS for treatment of drug-resistant epilepsy[8,9] and depression.[10] The key finding of this study was the indication that abdominal VNS increases firing of LC neurons. Moreover, when safe levels of VNS were applied, that is, below the level that caused changes to heart/breathing rate with cVNS, only abdominal VNS increased spike activity in the LC. As such, this study supports the potential use of abdominal VNS as an alternative stimulation strategy for treatment of epilepsy and depression.

The ECAP threshold activation of C-fibers of $n = 2$ rats was >1.5 mA (Fig. 2b) during cVNS, indicating the extent to which the activation threshold of cVNS can vary. Similar clinical heterogeneity in patient responses to cVNS is observed, with 66% of patients experiencing side effects (cough, throat pain, voice alteration, and dyspnea) whereas other patients experience none.[27] A potential explanation for the heterogeneity in evoked responses to stimulation is that the cervical vagus nerve fascicles are highly disorganized, with fascicles splitting and merging approximately every 560 μm in humans.[28] This disorganization of nerve fascicle morphology can cause variability in the electrode-nerve interface and activation thresholds.

Previous studies show that cVNS activates LC neurons in a manner dependent on the stimulation parameters delivered.[14,29] The cVNS-induced activation of the LC, using short bursts of stimulation, reported in this study was similar to that previously described.[14] Although high-stimulation (2 mA) cVNS induced more spike activity in the LC than did abdominal VNS (2 mA), the caveat is that 2-mA cVNS causes significant changes to heart rate and breathing (off-target effects). As such, we conclude that although abdominal VNS activated fewer LC neurons at maximum stimulation levels, it is potentially more efficacious at safe stimulation levels than is cVNS.

Previous cVNS studies in rats defined the lowest current needed to elicit 5% changes in heart rate, which was 0.22 ± 0.20 mA (mean ± SEM), equivalent to 22 ± 20 nC.[30] In this study, a Dunnett's statistical post hoc test was used to determine the off-target threshold defined as the minimum amount of current required to induce a significant change to baseline breathing or heart rate (Fig. 3a,b). The difference in heart rate using this method was 26.20% ± 18.07% (mean ± SD) and produced an off-target threshold of 0.87 ± 0.07 mA, equivalent to 87 ± 7 nC. As such, our method of determining a safe current level produced a higher threshold than that of previous studies[30] and would subsequently have increased the LC drive from safe cVNS.

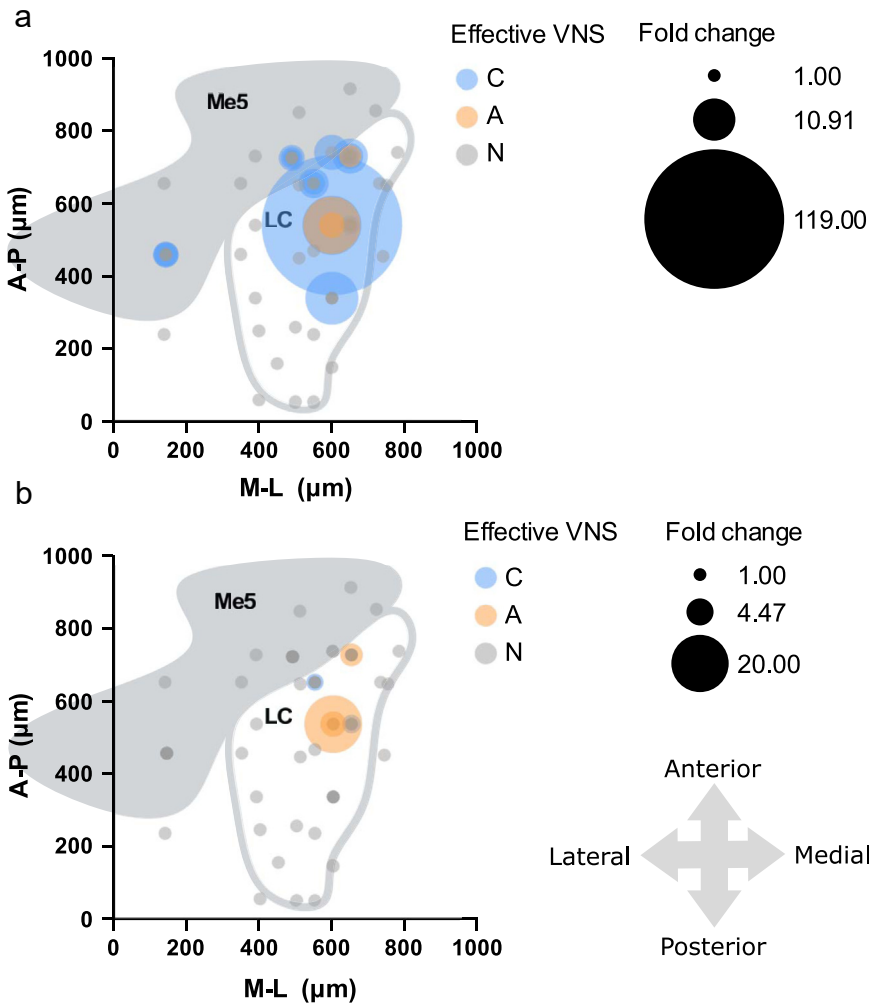

Figure 5. Topographic arrangement of VNS-induced activity in the LC and Me5. a. Location of VNS-induced activation in LC during stimulation levels above the off-target effect threshold (maximal). b. Location of VNS-induced activation in LC during stimulation levels below the off-target effect threshold (safe). At safe stimulation levels (0.7–0.9 mA), most of the VNS-induced firing in the LC occurred because of abdominal VNS. In panels a and b, anatomical maps show the horizontal representations of the LC and Me5.[21] Each gray or colored circle represents a recording site: Gray circles represent no VNS-induced firing; blue circles indicate cVNS-induced firing; orange represents abdominal VNS-induced firing. The size of the circle represents fold change in the level of VNS-induced firing. A-P, anterior-posterior; M-L, medial-lateral. [Color figure can be viewed at www.neuromodulationjournal.org]

Although a role for LC activation is implicated, the precise mechanisms mediating the therapeutic effect during VNS for epilepsy are not known. Early rat studies suggest the therapeutic mechanisms of VNS to suppress pentylenetetrazol (PTZ)-induced seizures require the activation of vagal C fibers.[31] However, in contrast, the destruction of capsaicin-sensitive C-fibers, after multiple subcutaneous injections of noxious capsaicin, did not alter PTZ-induced seizures in awake rats, suggesting capsaicin-sensitive C-fibers are not involved in the therapeutic effects of VNS in seizure reduction.[32] However, this model does not eliminate the proportion of vagal C-fibers that are not sensitive to capsaicin,[33] suggesting vagal capsaicin-resistant C-fibers may still have a role in suppressing seizures. This is important given the abdominal vagus nerve in rats comprises 97% unmyelinated C-fibers, with the remaining 3% of fibers likely myelinated (autonomic motor) B-fibers.[25,34] As such, it is not clear whether abdominal VNS would have a meaningful therapeutic seizure-suppressing effect, and future studies should assess the efficacy of abdominal VNS in a rat model of seizures.

Topographic arrangement of vagal centric nuclei has been observed in regions such as the LC, nucleus tractus solitarius (NTS), and dorsal motor nucleus.[35–37] Here, we report the topographic arrangement of activation of cervical and abdominal VNS within the LC, which was mostly observed to occur in the anteromedial area of the nuclei. A limitation of this study was the use of the anesthetic inhalant isoflurane, which is known to suppress peripheral nerve activity[38] and neurotransmission within the NTS.[39] During our studies, the use of isoflurane was minimized by the addition of medetomidine, an α2-adenoreceptor agonist sedative and analgesic.[40] CVNS-induced increase in the firing rate of LC neurons has been previously reported in animals administered with α2-adenoreceptor agonist.[14] However, because α2-adenoreceptor agonist is known to decrease the firing rate of LC neurons, both the background activity and VNS-induced firing in the LC may be higher in awake animals.[41]

In conclusion, abdominal VNS activated LC neurons without causing changes to heart and breathing rate and shows promise as an alternative approach to the treatment of drug-resistant epilepsy and depression.

Acknowledgements

The authors acknowledge Ms Ella Trang and Lisa Dyball for their histologic expertise, Associate Professor Peta Grigsby for project administration, and Ms Jacinda Nicastri for surgical assistance. They also thank Ms Jenny Zhou for manufacturing the electrode array.

Ethical Statement

All animal procedures were approved by the Animal Research and Ethics Committee of St. Vincent's Hospital (reference number 1_22) and complied with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia, Eighth Edition 2013) and the Prevention of Cruelty to Animals Act (1986).

Data Availability Statement

Data will be made available on request, unreservedly.

Authorship Statements

All the authors made substantial, direct, and intellectual contributions to the study and manuscript, and were involved in reviewing the manuscript. Specifically, Tomoko Hyakumura was involved in all aspects of the study, including experimental design, implantation surgery, and acquisition and analysis of data, and was the primary writer of the manuscript; Sophie C. Payne provided intellectual feedback on experimental design, analysis, supervision, and manuscript writing; Jerico V. Matarazzo provided intellectual and technical support of equipment and software, and was involved in experimental design, data acquisition and analysis; Wendy K. Adams provided intellectual input on experimental design and stereotaxic targeting; and James B. Fallon was involved in the idea conception, data analysis and interpretation, supervision, and manuscript writing.

Conflict of Interest

The authors reported no conflict of interest.

How to Cite This Article

Hyakumura T., Payne S.C., Matarazzo J.V., Adams W.K., Fallon J.B. 2025. Abdominal Vagus Nerve Stimulation Increases Firing in the Rat Locus Coeruleus. Neuromodulation 2025; ■: 1–9.

REFERENCES

1. Yuan H, Silberstein SD. Vagus nerve and vagus nerve stimulation, a comprehensive review: part I. *Headache*. 2016;56:71–78. https://doi.org/10.1111/head.12647.
2. Fang Y-T, Lin Y-T, Tseng W-L, et al. Neuroimmunomodulation of vagus nerve stimulation and the therapeutic implications. *Front Aging Neurosci*. 2023;15:1173987.
3. Hulsey DR, Shedd CM, Sarker SF, Kilgard MP, Hays SA. Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. *Exp Neurol*. 2019;320:112975. https://doi.org/10.1016/j.expneurol.2019.112975.
4. Toffa DH, Touma L, El Meskine T, Bouthillier A, Nguyen DK. Learnings from 30 years of reported efficacy and safety of vagus nerve stimulation (VNS) for epilepsy treatment: a critical review. *Seizure*. 2020;83:104–123. https://doi.org/10.1016/j.seizure.2020.09.027.
5. Panebianco M, Rigby A, Weston J, Marson AG. Vagus nerve stimulation for partial seizures. *Cochrane Database Syst Rev*. 2015;4:CD002896. https://doi.org/10.1002/14651858.CD002896.pub:2.
6. Bunch S, DeGiorgio CM, Krahl S, et al. Vagus nerve stimulation for epilepsy: is output current correlated with acute response? *Acta Neurol Scand*. 2007;116:217–220.
7. Koo B, Ham SD, Sood S, Tarver B. Human vagus nerve electrophysiology: a guide to vagus nerve stimulation parameters. *J Clin Neurophysiol*. 2001;18:429–433.
8. Krahl SE, Clark KB, Smith DC, Browning RA. Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia*. 1998;39:709–714. https://doi.org/10.1111/j.1528-1157.1998.tb01155.x.
9. De Taeye L, Vonck K, van Bochove M, et al. The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy. *Neurotherapeutics*. 2014;11:612–622. https://doi.org/10.1007/s13311-014-0272-3.
10. Grimonprez A, Raedt R, Portelli J, et al. The antidepressant-like effect of vagus nerve stimulation is mediated through the locus coeruleus. *J Psychiatr Res*. 2015;68:1–7. https://doi.org/10.1016/j.jpsychires.2015.05.002.
11. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. *Nat Rev Neurosci*. 2009;10:211–223. https://doi.org/10.1038/nrn2573.
12. Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. *Brain Res*. 2006;1119:124–132. https://doi.org/10.1016/j.brainres.2006.08.048.
13. Follesa P, Biggio F, Gorini G, et al. Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Res*. 2007;1179:28–34. https://doi.org/10.1016/j.brainres.2007.08.045.
14. Hulsey DR, Riley JR, Loerwald KW, Rennaker 2nd RL, Kilgard MP, Hays SA. Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. *Exp Neurol*. 2017;289:21–30. https://doi.org/10.1016/j.expneurol.2016.12.005.
15. Payne SC, Furness JB, Burns O, et al. Anti-inflammatory effects of abdominal vagus nerve stimulation on experimental intestinal inflammation. *Front Neurosci*. 2019;13:418. https://doi.org/10.3389/fnins.2019.00418.
16. Gieroba ZJ, Blessing WW. Fos-containing neurons in medulla and pons after unilateral stimulation of the afferent abdominal vagus in conscious rabbits. *Neuroscience*. 1994;59:851–858.
17. Payne SC, Romas E, Hyakumura T, Muntz F, Fallon JB. Abdominal vagus nerve stimulation alleviates collagen-induced arthritis in rats. *Front Neurosci*. 2022;16:1012133. https://doi.org/10.3389/fnins.2022.1012133.
18. Hyakumura T, Fallon JB, Payne SC. Implantation surgery for abdominal vagus nerve stimulation and recording studies in awake rats. *J Vis Exp*. 2024;203:e65896.
19. Savastano LE, Castro AE, Fitt MR, Rath MF, Romeo HE, Muñoz EM. A standardized surgical technique for rat superior cervical ganglionectomy. *J Neurosci Methods*. 2010;192:22–33.
20. Ahmed U, Chang Y-C, Lopez MF, et al. Implant- and anesthesia-related factors affecting cardiopulmonary threshold intensities for vagus nerve stimulation. *J Neural Eng*. 2021;18:046075.
21. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 7th ed. Elsevier; 2013.
22. Fallon J, Payne S. Electrophysiological recording of electrically-evoked compound action potentials. *Protocols.io*. 2020;10. https://doi.org/10.17504/protocols.io.bfwyjpfw.
23. Fallon JB, Senn P, Thompson AC. A highly configurable neurostimulator for chronic pre-clinical stimulation studies. In: *Neural Interfaces Conferences*. Minneapolis, MN. 2018.
24. Castoro MA, Yoo PB, Hincapie JG, et al. Excitation properties of the right cervical vagus nerve in adult dogs. *Exp Neurol*. 2011;227:62–68.
25. Prechtl JC, Powley TL. The fiber composition of the abdominal vagus of the rat. *Anat Embryol (Berl)*. 1990;181:101–115. https://doi.org/10.1007/BF00198950.
26. West CHK, Ritchie JC, Boss-Williams KA, Weiss JM. Antidepressant drugs with differing pharmacological actions decrease activity of locus coeruleus neurons. *Int J Neuropsychopharmacol*. 2009;12:627–641. https://doi.org/10.1017/S1461145708009474.
27. De Ferrari GM, Stolen C, Tuinenburg AE, et al. Long-term vagal stimulation for heart failure: eighteen month results from the NEural Cardiac TherApy foR Heart Failure (NECTAR-HF) trial. *Int J Cardiol*. 2017;244:229–234.
28. Upadhye AR, Kolluru C, Druschel L, et al. Fascicles split or merge every ~560 microns within the human cervical vagus nerve. *J Neural Eng*. 2022;19:054001.
29. Farrand A, Jacquemet V, Verner R, Owens M, Beaumont E. Vagus nerve stimulation parameters evoke differential neuronal responses in the locus coeruleus. *Physiol Rep*. 2023;11:e15633. https://doi.org/10.14814/phy2.15633.
30. Owens MM, Jacquemet V, Napadow V, Lewis N, Beaumont E. Brainstem neuronal responses to transcutaneous auricular and cervical vagus nerve stimulation in rats. *J Physiol*. 2024;602:4027–4052.
31. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. *Epilepsia*. 1990;31(suppl 2):S7–S19. https://doi.org/10.1111/j.1528-1157.1990.tb05852.x.
32. Krahl SE, Senanayake SS, Handforth A. Destruction of peripheral C-fibers does not alter subsequent vagus nerve stimulation-induced seizure suppression in rats. *Epilepsia*. 2001;42:586–589. https://doi.org/10.1046/j.1528-1157.2001.09700.x.
33. Berthoud HR, Patterson LM, Willing AE, Mueller K, Neuhuber WL. Capsaicin-resistant vagal afferent fibers in the rat gastrointestinal tract: anatomical identification

and functional integrity. *Brain Res*. 1997;746:195–206. https://doi.org/10.1016/S0006-8993(96)01222-X.
34. Stakenborg N, Gomez-Pinilla PJ, Verlinden TJM, et al. Comparison between the cervical and abdominal vagus nerves in mice, pigs, and humans. *Neurogastroenterol Motil*. 2020;32:e13889. https://doi.org/10.1111/nmo.13889.
35. Waterhouse BD, Border B, Wahl L, Mihailoff GA. Topographic organization of rat locus coeruleus and dorsal raphe nuclei: distribution of cells projecting to visual system structures. *J Comp Neurol*. 1993;336:345–361.
36. Huang XF, Törk I, Paxinos G. Dorsal motor nucleus of the vagus nerve: a cyto- and chemoarchitectonic study in the human. *J Comp Neurol*. 1993;330:158–182.
37. Dufour A, Tell F, Kessler JP, Baude A. Mixed GABA–glycine synapses delineate a specific topography in the nucleus tractus solitarii of adult rat. *J Physiol*. 2010;588:1097–1115.
38. Silverman HA, Stiegler A, Tsaava T, et al. Standardization of methods to record vagus nerve activity in mice. *Bioelectron Med*. 2018;4:1–13.
39. Peters JH, McDougall SJ, Mendelowitz D, Koop DR, Andresen MC. Isoflurane differentially modulates inhibitory and excitatory synaptic transmission to the solitary tract nucleus. *Anesthesiology*. 2008;108:675–683.
40. Savola MKT, MacIver MB, Doze VA, Kendig JJ, Maze M. The α2-adrenoceptor agonist dexmedetomidine increases the apparent potency of the volatile anesthetic isoflurane in rats in vivo and in hippocampal slice in vitro. *Brain Res*. 1991;548:23–28. https://doi.org/10.1016/0006-8993(91)91101-6.
41. Jorm CM, Stamford JA. Actions of the hypnotic anaesthetic, dexmedetomidine, on noradrenaline release and cell firing in rat locus coeruleus slices. *Br J Anaesth*. 1993;71:447–449. https://doi.org/10.1093/bja/71.3.447.

COMMENT

This study shows that abdominal VNS can potentially be as effective as cVNS but without the off-target effects on heart rate and blood pressure that are common in cVNS. Although cVNS is approved by the FDA for treating refractory epilepsy and depression, it may be worth further investigating the alternative of using abdominal VNS.

Mayuresh V. Kothare, PhD
Bethlehem, PA, USA