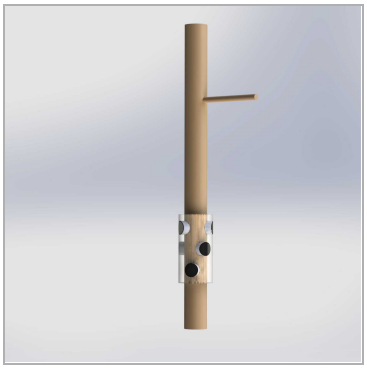

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Spatially selective stimulation of the pig vagus nerve to modulate target effect versus side effect

Forked from Vagus Nerve Stimulation Evoked Electroneurography and Electromyography Recordings in Swine

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ABSTRACT

This protocol was used to collect data now published in the Journal of Neural Engineering, **Spatially selective stimulation of the pig vagus nerve to modulate target effect versus side effect** using the multi-contact ImThera stimulating device. **https://doi.org/10.1088/1741-2552/acb3fd**

Dataset has been published to pennsieve.io and can be found at: **https://doi.org/10.26275/efbj-8evl**

GUIDELINES

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75784

Keywords: electrophysiology, electromyography, swine, vns, vagus nerve stimulation, vagus

MATERIALS

Stimulation Electrode six-contact ImThera cuff electrode (LivaNova, London, UK)

Recording Electrodes (made in-house, Longitudinal Intrafascicular Electrodes) components:
75μm outer diameter PFA-coated platinum wire (AM-Systems, Sequim, WA)
suture needle (Item No. 12050–03, Fine Science Tools, Foster City, CA)
insulated copper extension wire with touchproof connector (441 connector with wire, Plastics1, Roanoke, VA)

Stimulation and Recording Devices:
Tucker Davis Technologies system (Alachua, FL; W8, IZ2MH, RZ5D, RZ6, PZ5, and S-Box units)

Data Analysis:
Python

Animal preparation and initial administration of anaesthesia

1

Note

Consult your institution's IACUC before attempting any of the following.

What we did (approved by the University of Wisconsin-Madison IACUC):

Weigh pig, and perform an IM injection of a mixture of telazol (6 mg/kg) and xylazine (2 mg/kg). Intubate and ventilate the pig and anesthetize using isoflurane gas (0.5-3% in room air) and IV Fentanyl (12-30 mcg/kg/hr) via Landmark Veterinary Anesthesia Machine VSA-2100 (Louisville, KY) with Midmark Matrx Model 3000 ventilator (Dayton, OH) and SurgiVet SOMNI 3 vaporizer (Plymouth, MN).

2 Position the pig in supine position and record heart rate via EKG 5-lead EKG setup, pulse rate and SpO_2 via infrared (IR) probe placed on either the tongue or lip (AD Instruments PowerLab 8/35).

Invasive blood pressure recording

3 Place and invasive blood pressure catheter (Millar Inc., Houston, TX, Model #SPR-350S) into either the right or left femoral artery.

Note

Various approaches can be used to insert an invasive blood pressure catheter. Some surgeons may feel comfortable using a "blind stick," where a needle and catheter are inserted through the skin. The needle is then withdrawn and the Millar catheter is inserted through the lumen. Others may feel more comfortable making an incision in the inguinal space, locating the femoral artery and being able to insert the Millar cather visually.

Cervical vagus nerve preparation

4 Ensuring the pig is in supine position with neck extended, and make a single incision through the skin and superficial fat layers between the mandible and sternal notch using a cautery, approximately 9-12 cm in length. Use blunt dissection to expose the right carotid sheath and isolate the vagus nerve from the carotid artery.

5 Use blunt dissection to locate the superior and recurrent laryngeal branches of the vagus nerve.

5.1 The superior branch inserts into the vagus trunk near the nodose ganglion and projects towards the thyroid cartilage. The nodose ganglion should be near the carotid bifurcation of the carotid artery, and will look like an enlargement (diameter) along the length of the nerve. Place a loose suture tie around the branch to locate the branch easily later.

5.2 The recurrent branch inserts into the vagus trunk within the thorax, and thus cannot be identified within the cervical surgical window for the left or right side vagus trunk. The branch must be located along the length of the trachea projecting towards the thyroid cartilage. Place a loose suture tie around the branch to locate the branch easily later.

6 Use blunt dissection to expose as much length of the vagus trunk as possible. Ideally, at least 8 cm from the superior laryngeal branch insertion point (nodose ganglion) to a more caudal location.

Stimulation and Recording Equipment Setup

7 Place the six-contact ImThera cuff electrode (LivaNova, London, UK) on the right VN caudal to the

nodose (~1.1 mm). Use a 7-0 silk suture tied around the device to keep it stationary. Ensure to minimize mechanical forces on the VN from implanting the device.

8 To ensure that no movement had occurred during the procedure, the placement of the cuff on the nerve (longitudinal location and relative rotation) was recorded and measurements to known landmarks (e.g., nodose ganglion) were made before and after the procedure.

9 Five LIFE devices were placed within the VN caudal to the ImThera cuff to record ENG, caudal to the ImThera cuff. These devices were implanted longitudinally (caudal to cranial) such that the entirety of the recording window was parallel to the course of the nerve. The most cranial LIFE served as a reference electrode for the remaining four LIFE devices. The recording electrodes were staggered in the cranial/caudal direction to discern spatiotemporal aspects of the ENG signals. Cranial/caudal staggering ensured signals were confirmed as neural in origin if temporal differences were observed among recording electrodes. Medial/lateral staggering enabled sampling and later analysis of ENG signals from various locations within the nerve. The distance between the stimulating electrode and the recording electrodes was maximized within the surgical window to reduce the contamination of the neural signals with stimulation artifact. This resulted in an average distance of 7.93 ± 1.75 cm from the caudal edge of the cuff to the most cranial LIFE and 0.79 ± 0.38 cm from the most cranial to the most caudal LIFE.

10 EMG needle electrode pairs were placed in the cricothyroid, cricoarytenoid, and pharyngeal constrictor muscles.

11 Stimulation system / Recording system. We used the Tucker Davis Technologies systems (Alachua, FL; W8, IZ2MH, RZ5D, RZ6, PZ5, and SIM) were used to control stimulation and to record EMG and ENG signals.

12 Record some data and check noise levels. Noise should be less than 10 microvolts peak to peak.

Given we performed these studies in a surgical suite without a faraday cage, additional grounding was performed to reduce noise levels. Likewise, some items unnecessary to animal care were removed or unplugged.

Use wires to attach to large metal items in the surgical suite. At the end of the wire should be a banana plug that will go into the ground socket of an outlet. All large metal objects like metal tables and IV poles should be grounded together.

We also unplugged devices like the cautery and water heater pump. Warmed blankets were used to keep the pig warm after recordings began.

Experiments

13 We conducted stimulation trials in the “intact” condition, using a symmetric biphasic rectangular pulse with 0 ms interphase delay, 0.2 ms per phase, cathodic phase first, and 0.4 ms total duration, mimicking common clinical parameters. All stimulating pulses were delivered in a monopolar configuration to avoid potential interactions between simultaneously active cathode/anode electrode pairs along the nerve. We placed an 18-gauge, stainless steel needle (152 mm^2 surface area) within the thoracic wall to serve as the counter electrode. The stimulus was delivered through one of six contacts of the ImThera cuff at 25 Hz with random amplitudes from 50 to 5000 µA. We paused for 60 s between each amplitude to allow for physiological states to return to baseline. The range of randomized stimulation amplitudes was adjusted on a case-by-case basis based on physiological responses (e.g., in some animals, severe bradycardia was evoked during stimulation at high amplitudes). Stimulation was delivered sequentially through electrode contacts 1 through 6. For each contact and stimulation amplitude, a total of 750 pulses (i.e., 30 s of stimulation) per amplitude per stimulation contact were delivered in the intact condition.

14 Subsequently, animals were stimulated under the following conditions to confirm the source of recorded signals: 1) neuromuscular junction (NMJ) block (vecuronium, IV, 0.15 mg/kg bolus, 0.15 mg/kg/hour maintenance rate), 2) transection of the superior laryngeal (SL) branch, 3) transection of the recurrent laryngeal (RL) branch, and 4) transection of the vagus trunk cranial and caudal to the stimulating cuff (double vagotomy). Multiple studies demonstrated that EMG signals can appear as artifacts in neural recordings (Nicolai et al., 2020; Yoo et al., 2013); therefore, we conducted both NMJ block and transections to assess the origin of recorded signals. After stimulating in the NMJ condition, we waited for the paralytic agent to wear off before proceeding with the remaining conditions. In all these additional conditions (NMJ to double vagotomy), we delivered 25 pulses per amplitude (i.e., 1 s of stimulation) due to limitations in our protocol-allotted surgical time.

Histological Analysis

15 The VN was exposed from the nodose ganglion to the recurrent laryngeal bifurcation at the level of the subclavian artery. To match histology to relative in vivo locations, tissue dye was used to mark the ventral and lateral edges of the nerve (Bradley Products, Inc., Davidson Marking System, Bloomington, MN), and sections were removed for fixation and processing.

16 All sections were placed in 10% neutral buffered formalin at 4 °C for 24-48 hours. Samples then were embedded in paraffin wax and 5 µm thick slices, approximately every 40 µm, were collected and mounted on charged slides (Sakura Tissue-Tek VIP).

17 Slices were stained with Gomori’s trichrome and were imaged with a Motic Slide Scanner (Motic North America, Richmond, British Columbia).

Data Analysis

18 The ENG and EMG data were digitally filtered using in-house developed software (pyeCAP is available on PyPI) to increase the signal-to-noise ratio and to remove long-term baseline drift without introducing filter ringing from a large stimulation artifact, which could otherwise be misconstrued as neural signals. Specifically, we subtracted the median filtered signal (kernel size: 201) from the original signal. Next, we applied a Gaussian filter (standard deviation for Gaussian kernel: 0.87) and a finite impulse response filter to reject common-mode noise and its harmonics (60, 120, and 180 Hz) with 1.0 Hz bandwidth. An additional median filter (kernel size: 11) was applied to EMG signals to eliminate intermittent spikes present only in EMG recordings.

19 After filtering, neural signals were averaged across pulse trains and segmented into five time-restricted windows based on known conduction velocities per fiber type (i.e., Aα, Aβ, AϒΥ, Aδ, and B). The onset of stimulation was set as the time zero starting point. The boundaries of the time-restricted windows were calculated by dividing the distance from the stimulating device to the recording electrode by the conduction velocity of each fiber type.

20 For neural signals uncontaminated by stimulation artifact, the root mean square of the measured voltage (VRMS) within each time-restricted window was calculated to determine the magnitude of the eCAP generated by that nerve fiber type.

21 If neural signals contained noticeable eCAPs but were still contaminated by stimulation artifact despite the preprocessing steps, the signals were processed additionally to isolate the eCAP.

21.1 First, to find the onset and offset of the eCAP, the contaminated signals were filtered using a Savitzky-Golay filter to smooth the signal and eliminate false minima (Savitzky & Golay, 1964). Next, a peak-detection algorithm was applied to find the local maximum within the time window for each fiber type. The time points of the closest minima occurring before and after the maximum were classified as onset and offset of the eCAP. The later minimum (offset) could exceed the bounds of the time-restricted window by 25% to allow for the entirety of the signal to be captured. Once the onset and offset of the eCAP were found, these points were superimposed on the filtered signal (without Savitzky-Golay filter). We then calculated the definite integral of the eCAP.

21.2 To account for the stimulation artifact, a simplified model of the stimulation artifact was created by forming a line between the onset and offset of the eCAP. The definite integral of this simplified model was subtracted from the definite integral of the signal to give an area measurement of the eCAP.

22 Dose-response curves (DRCs) were generated for neural, muscle, and physiological responses to randomized stimulation amplitudes. The “intact” condition was used to analyze: 1) nerve fiber responses measured via ENG, 2) muscle response measured via EMG, and 3) changes in HR and BP from baseline measured via EKG and invasive arterial catheter, respectively.
As described above, the magnitude of the eCAPs was calculated either as VRMS or as area-under-the-curve following the peak-detection algorithm. The amplitude of the EMG response was calculated via VRMS; as evoked EMG responses occurred outside of the window contaminated by the stimulus artifact (~5 ms), no stimulus subtraction methods were required to measure the magnitude of the evoked signal.

23 Changes in HR and BP were defined as the maximum change during a stimulation pulse train from the baseline calculated over the interval immediately prior to stimulation (i.e., t-3 to t-1 seconds before stimulation).