JAN 23, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5jyl8pop7g2w/v1

Protocol Citation: William Huffman, Nicole A Pelot, Warren Grill 2024. Acute testing of temporal patterns of vagus nerve stimulation on physiological outcomes in mouse. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8pop7g2w/v1

MANUSCRIPT CITATION: Huffman, W.J., Musselman, E.D., Pelot, N.A., Grill, W.M., 2023. Measuring and modeling the effects of vagus nerve stimulation on heart rate and laryngeal muscles. Bioelectron Med 9, 3. https://doi.org/10.1186/s42234-023-00107-4

Acute testing of temporal patterns of vagus nerve stimulation on physiological outcomes in mouse

Forked from Acute testing of temporal patterns of vagus nerve stimulation on physiological outcomes in mouse

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ABSTRACT

This protocol outlines the process for an anesthetized in vivo mouse study to electrically stimulate the cervical vagus nerve while recording EMG of laryngeal muscles and ECG.

Protocol status: Working
We use this protocol and it's working

Created: Jan 13, 2024

Last Modified: Jan 23, 2024

PROTOCOL integer ID: 93476

Keywords: vagus nerve stimulation, in vivo, heart rate, laryngeal EMG, neuromodulation, mouse

Funders Acknowledgement:
NIH SPARC
Grant ID: OT2 OD025340
Wrenn Foundation

MATERIALS

Animals

- 8-10 wk old WT C57BL/6J mice, male and female (RRID:IMSR_JAX:000664)

Software

- MATLAB (Version 2016a used in experiments)
- LabChart (Version v7.3.8 used in experiments)
- Custom MATLAB scripts used for experiment control (Available on request)

Stimulation/Recording Hardware

- PowerLab/16SP (ADInstruments)
- Stimulation Isolater (Model 2200, A-M Systems)
- Pre-amplifier (C-ISO-256, iWorx)
- Biopotential amplifier (ETH-256, iWorx)
- Laptop (for experiment control, ThinkPad used in experiments)
- Bipolar cuff electrode (FNC-200-V-4-A-30 Micro-Leads used in experiments)
- Stainless steel wires (0.0054-in diameter wires used in experiments)
- Platinum Recording Electrodes (C-ISO-PNx, iWorx)
- Alligator Clip Connectors (C-ISO-Ax)

Animal Preparation and Maintenance

- Vet-grade Sevoflurane and Vaporizer (Somni Scientific Sevoflurane Vaporizer used in experiments)
- Induction chamber
- Heated pad
- Pulse oximeter (MouseStat Jr. used in experiments) and hind limb probe
- Hair removal cream (Nair used in experiments)
- No. 11 surgical blade
- Small gauge silk braided suture
- Paper tape
- Forceps (for blunt dissection)
- Saline and bulb pipette (for surgical site irrigation)
- Small surgical scissors

Animal preparation

1h 5m

1

5m

- Gas flow on 1
- Anesthesia induction - place animal in induction chamber and apply anesthesia (5% sevoflurane)
- Place animal on heated pad in supine position and apply anesthesia using nose cone (2-5% sevoflurane)
- Insert rectal thermometer

- Use paper tape to secure upper extremities
- Place pulse oximeter on hind limb and ensure pseudo-real-time read-out (the pulse oximeter should provide a reading at least every second).

2 30m

- Assess anesthesia depth (lack of toe pinch response, heart rate (or HR) between 400 and 500 beats pe minute)
- Perform a midline incision in cervical region overlaying the larynx and trachea (approximately 7.5 to 15 mm)
- Place 4 superficial sutures: upper/lower aspects of incision on left/right side
- Using blunt dissection through connective tissue and the salivary glands at the midline, expose musculature covering larynx
- Blunt dissect on (animal's) right aspect of laryngeal area to expose neurovascular bundle of carotid artery (identified by pulsing), internal jugular vein, and (right) vagus nerve.
- To place the cuff electrode (200 um diameter cuff, Micro-Leads), blunt dissect the vagus nerve away from artery and vein. This should be performed with delicate touch in the longitudinal direction as vasculature can be delicate. Using a single set of forceps and with cuff electrode facing upward, place the cuff underneath the vagus nerve. Use a second pair of forceps to grip cuff electrode wings and open to allow the nerve to slide into the cuff. Make sure nerve is fully and securely settled within nerve. Do not allow air bubbles to enter the cuff as this may affect stimulation.

3 30m

- Place electrodes for physiological signal recording. Electromyogram of laryngeal muscles will be used quantify activation of laryngeal muscles during stimulation. Electrocardiogram will be used to quantify HR modulation during stimulation.
- Electromyogram (EMG) of laryngeal muscles - Place de-insulated tips of two stainless steel wires under thyroid cartilage on the right aspect. Wires should be placed as to not puncture the esophagus but deep enough to allow sufficient EMG signal collection. Wire tips should not touch as to close the circuit and not allow for signal collection. Signal should be routed through pre-amplifier -> biopotential amplifier -> PowerLab Recording system.
- Electrocardiogram (ECG) - Place platinum recording electrodes on limbs with entry site close to wrist/ankle and needle pointed towards the body. Positive lead placed on right forelimb, negative lead placed on left forelimb, and ground lead placed on left hind-limb. Signal should be routed through pre-amplifier -> biopotential amplifier -> PowerLab Recording system
- Set iWorx biopotential amplifier to band-pass filter both EM and ECG signals between 10 Hz and 1 kHz. Set PowerLab to sample signal at sample frequency of 5 kHz.

Vagus Nerve Stimulation (VNS) 2h

4 2h

- Stimulation is controlled by PowerLab. Voltage-controlled current is applied to a stimulus isolator set to volt - to - 1 milliamp (mA) conversion. Stimulation waveforms are symmetric and biphasic with a 300 microsecond per phase.
- Apply test pulses to assess strength and clarity of EMG signal. Stimulation artifact should be readily observable in signal and scale linearly with stimulation amplitude. EMG signal will have a latency

between 1 and 1.5 milliseconds. If there is no observable EMG signal, wire electrodes may be touching and need replacement. If there is no observable latency between stimulation artifact and EMG signal, the muscles may be directly activated by stimulation and not through efferent fibers within the cervical vagus nerve at the site of cuff electrode placement, requiring replacement of EMG recording electrode.

- Define bradycardia threshold (BCT). Set stimulation frequency to 20 Hz continuous stimulation and stimulation amplitude of 0 mA. Slowly increase stimulation amplitude in increments of 0.02 mA. BCT is defined as the lowest stimulation amplitude required to produce a 10% reduction in HR.
- Define stimulation parameter sets to be tested. This could be anything your heart desires: e.g., ranges of stimulation frequencies and amplitudes, burst patterns, or random patterns. Stimulation trials should include a period of time to record baseline features (10 seconds), a period in which stimulation is applied (30 seconds), and a recovery phase before the next trial begins (30 seconds). Stimulation order should be randomized to preclude bias in analysis between animals. When using MATLAB software to order trials, it is important to note the random number generator must be seeded to avoid re-use of trial order.
- To avoid drift in heart rate responses during experiment, re-assess BCT every 10 trials. Stop automated trials and perform procedure above to assess BCT. This is especially relevant when trial amplitude is normalized by BCT.

Vagotomy (VNX)

30m

5

30m

- Before procedure, re-define BCT and perform a trial using 20 Hz stimulation at BCT.
- Perform transection of the vagus nerve (VNX). Distal to the cuff, use the surgical scissors to gently transect the nerve, taking care not to disturb or damage the surrounding vasculature. The proximal nerve trunk should remain in the cuff with some (less than 1 millimeter) distance between the proximal and distal nerve trunks.
- To assess the affect of VNX on physiological outcomes, perform a second trial after VNX using 20 Hz stimulation at BCT (as defined before VNX). Proper VNX and signal collection will produce no changes in HR during stimulation and no stimulation-evoked EMG signal during the second trial.