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A simple approach to identify the influence of left vagal stimulus pulse parameters on vagal and gastric electrical activity in rat

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1 Works for me dx.doi.org/10.17504/protocols.io.2kugcww

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Ward_and_Nowak_2017_BMES2017_LcVNSOverview.pdf

GUIDELINES

All surgical and animal handling procedures are approved by the Institutional Animal Care and Use Committee (IACUC) and adhere to guidelines set forth in the Guide for the Care and Use of Laboratory Animals. All rats are housed in a 12-h light/dark cycle at constant humidity and temperature. Aseptic technique is followed as closely as possible. Analgesia and subcutaneous fluids are provided throughout the procedure as needed and according to IACUC-approved guidelines.

SAFETY WARNINGS

Do not attempt any of these procedures without proper training, experience, and most importantly, authorization from your IACUC to perform these specific surgical procedures.

BEFORE STARTING

Sterile your instruments and ensure a clean, clutter-free operating table.

IACUC Statement

1 All surgical and animal handling procedures are approved by the Institutional Animal Care and Use Committee (IACUC) and adhere to guidelines set forth in the Guide for the Care and Use of Laboratory Animals. All rats are housed in a 12-h light/dark cycle at constant humidity and temperature.

A. Surgical Methods (adapted from DOI:10.1109/TNSRE.2014.2351271)

2 The surgical suite and instruments are sterilized prior to each procedure. Isoflurane gas anesthesia is used for the duration of surgery; it is set to the lowest level that will maintain a stable anesthetic plane (0.5%–3% isoflurane in 2 L/min O)

3 Following induction, the rat is placed in a supine position, the surgical site is shaved and cleaned with alternating scrubs of betadine and 70% ethanol, and an analgesic is provided (butorphanol tartrate; 0.5–2 mg/kg, SC). A small support made by rolling up 3-4 4x4" surgical sponges is placed below the neck for stability and to facilitate easier access to the cervical vagus nerve. Subcutaneous fluids are provided as needed throughout the experiment to prevent dehydration. Heart rate and body temperature is monitored throughout. 5m

4 Once the surgical site is clean, a 1.5–2 cm long midline incision is made from the jaw line to manubrium. A blunt dissection technique is used for the remaining steps of the procedure. 15m

5 With a pair of curved, blunt-tipped scissors, we tunnel through the subcutaneous tissues until the sternohyoid, omohyoid, and sternocleidomastoid muscles are visible. The connective tissue between the left sternocleidomastoid and the sternohyoid/omohyoid is carefully separated until the carotid sheath is visible.

6 Using a small pair of surgical retractors to hold the muscles apart, a 1-1.5 cm segment of the vagus nerve is isolated from the carotid sheath and artery. 10m

7 The incision site is then filled with sterile physiological saline solution warmed to 35 degrees Celsius and covered with moistened surgical sponges before exposing the ventral stomach and ventral gastric branch of the abdominal vagus. 1m

8 To expose the stomach to place electrodes on the antrum, we re-clean the shaved surface of the rat abdomen before making a 1-2 cm long incision approximately 1 cm to the left of and parallel to midline, starting approximately 1 cm below the xiphoid process. The skin and abdominal muscle is carefully cut to expose the ventral surface of the stomach. 15m

9 (For ventral gastric branch stimulation procedures, we follow an additional step to locate, isolate and place a cuff electrode around the ventral gastric branch of the abdominal vagal trunk) 20m

B. Electrode Implantation

10 Once the ventral antrum is exposed, we prepare to implant the wire electrodes into the wall of the ventral antrum in order to record the activity during left cervical VNS. A pair of 22 gauge hypodermic needles are used to guide placement.

11 The hypodermic needles are glued together so that the tips of the needles are spaced approximately 5 mm apart. 5m

12 The hypodermic needle assembly is then used to "sew" through a 3-5 mm long section of the ventral antrum along the direction of motility from the corpus to pylorus such that the ends of the needs are visible to the surgeon, taking care to avoid piercing the mucosa or larger blood vessels. 2m

13 The bipolar electromyogram (EMG) electrode, comprising a pair of 7-strand, braided Pt90/Ir wires (100 micrometer outer diameter; Ft. Wayne Metals, Inc.) sewn into a medical grade silicone tube (1 mm outer diameter; AM Systems, Inc.) with a 5 mm spacing and with 1 cm of wire exposed, is then reverse threaded through the hypodermic needles. 2m

14 Once the electrodes are placed through the hypodermic needles, a small amount of tissue glue is applied to the silicone tube holding the Pt/Ir wires. 5m

15 Once the electrode is secure, the hypodermic needles are carefully removed in the reverse direction of implantation so that the electrodes remain embedded in the muscle wall of the antrum with a spacing of approximately 5 mm along the direction of motility. 1m

16 The incision is then retracted, leaving the EMG (and/or ventral gastric branch cuff, ventral forestomach or ventral antrum patch electrode, depending on the experiment) leads outside of the cavity for connection to the recording amplifier. The site is then promptly covered with sterile surgical sponges moistened with warmed physiological saline solution. 1m

17 After closing the abdominal incision, the cervical vagal stimulating and recordng cuff electrodes are implanted along the left cervical vagus nerve (see Ward et al., 2015 for more information on the design and implantation of these electrodes; DOI:10.1109/TNSRE.2014.2351271). 10m

18 After implanting two bipolar cuff electrodes (made with the same Pt/Ir wire as the EMG electrodes), the conduction distance between the innermost electrodes is measured and recorded in the Autonomous Neural Control graphical user interface (DOI:10.1109/TNSRE.2014.2351271). The retractors are removed and the incision site it closed, leaving the leads of the two cuff electrodes exposed. 2m

19 In some experiments, a pair of cutaneous electrocardiogram pad electrodes are placed in a Lead II configuration to measure the off-target effects of cervical VNS on the heart (e.g., bradycardia). 1m

C. Data Collection

10m

20 The left cervical vagal recording electrode, ventral antrum EMG electrode, and in certain experiments, the ventral forestomach patch, ventral antrum patch, ventral gastric branch cuff electrode, or EKG electrodes, are connected to the inputs of an AM Systems Model 2700 preamplifier. An oscilloscope is used to identify the correct gain settings, but the passbands of each channel are determined by the type of signal being acquired. A 10 Hz high pass filter cutoff is used to remove motion artifact and some other low frequency activity from the vagal recording channels. The EMG electrode channel is given a 0.1 Hz high pass filter cutoff. All other parameters and settings are similar to those described in Ward et al., 2015. The outputs of the AM Systems amplifier are fed into an NI USB-6363 data acquisition system (National Instruments, Inc.), which is connected a Windows 7 laptop running Matlab R2015a and the ANC software.

2m

21 The output of a custom-made Howland Current Pump (HCP; with a 1 uF capacitor in series with the output) is connected to the leads of the stimulating cuff electrode, keeping the ground connection to the electrode site farthest away from the recording cuff. The input of the HCP is connected to the analog output channel of the NI-USB-6353 to allow software-generated stimulus waveforms to be converted to constant-current stimuli for VNS.

30m

22 A plugin to ANC is then used to automatically sweep a set of stimulus pulse parameters in a randomized order, interleaving trials of baseline/post-stimulus response recordings.

1h

23 The same automated parameter sweep may be performed 2-3 times per experiment prior to terminating the experiment via IACUC-approved methods. Data are then fed into an analysis package to generate the stimulus-response surface datasets and plots (see example Figure 1.