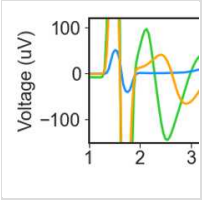

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Electrophysiology from cervical vagus nerve and great auricular nerve in swine

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This protocol was used to collect data in preparation for publication (Characterization of Electrodes to Record Neural Signals in the Periphery). Additionally, the dataset is publicly available on Pennsieve.

In this study, we characterized cuff electrodes, longitudinal intrafascicular electrodes (LIFEs), an intrafascicular electrode used in pre-clinical studies (Yoshida and Stein, 1999, DOI: 10.1109/10.740885; Nicolai et al., 2020, DOI: 10.1088/1741-2552/ab9db8), and microneurography electrodes, a microelectrode used clinically to measure muscle sympathetic nerve activity (MSNA), in their ability to measure neural activity in the peripheral nervous system. The cervical vagus, a major autonomic nerve innervating organs in the thorax and abdomen as well as muscles in the throat, and the great auricular nerve, a sensory nerve innervating the ear and periauricular region, were used as model nerves.

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Vagus nerve, Electrophysiology, Electrode design, great auricular nerve

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Recording electrodes

SIM
Electrophysiology
TDT https://www.tdt.com/component/subject-interface/

Tungsten Microneurography electrode
Recording electrode
FHC **UNA40GCT**

Recording cuff tripole
Recording cuff
Ardiem **Weber cuff 3 mm**

Repeated in text form:
Tungsten microneurography microelectrode 0.8-1.2 MOhm (UNA40GCT, FHC Inc., ME, USA).
Recording cuff platinum and silicone with three cylindrical contacts 1 mm in width, separated by 3 mm from each other, and 2 mm from the edge of the silicone. The three cylindrical contacts themselves split into seven contacts each (1 x 1 mm size) with all seven contacts electrically connected. 3 mm inner diameter (Ardiem Medical Inc., PA, USA).
Longitudinal intrafascicular electrode (LIFE) fabricated in house of platinum iridium wire with 0.004” diameter with insulation and an exposed window length

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of ~2 mm and diameter of 0.002”. The impedance of the LIFEs were 1-10 kOhm at 1 kHz.
The great auricularrecording and stimulation cuffs were identical and fabricated in house of two platinum wires (0.005" diameter), separated by ~1 mm, glued (using a silicone-based glue) into a split silicone tube of 0.75 mm inner diameter with a total length of 3 mm

Consult your institution's Institutional Animal Care and Use Committee (IACUC) before attempting any of the following in a live animal.

Institutional Animal Care and Use Committee Approval

1 Seek approval from your local IACUC.

Anesthesia

2 Deliver intramuscular injection of Telazol (6 mg/kg) and Xylazine (2 mg/kg) to induce sedation.

3 Ventilate the animal and maintain the surgical plane with inhaled Isoflurane (~1-2%) and intravenous Fentanyl (12-30 mcg/kg/hr) administered with lactated Ringer’s solution (LRS) (ICU medical, IM-4389).

Several hours into the experiment, and particularly when peripheral nerves were manipulated, an onset of tremoring and movement was observed in the animals. This movement was observed despite confirmation of the surgical anesthetic plane by means of nose pinch, jaw slackness, unresponsiveness to corneal reflex, and verification of other physiologic parameters such as temperature, blood glucose, and blood pH. The tremoring persisted despite increased dosing of Isoflurane and Fentanyl. Similar intraoperative shivering has been reported under different anesthetic conditions (Dubey, 2004). We found either intramuscular injection of Telazol (4-6 mg/kg) or intravenous Ketamine (10 mg/kg/hr) eliminated the tremoring. The effects of Telazol lasted for ~2 hours while Ketamine effects on tremoring washed in and out in ~15 minutes.

Telazol, Ketamine, and Isoflurane have a cardiac blunting effect, by which stimulation induced changes in cardiac function are blunted. As such, Telazol and Ketamine were not used during vagus nerve stimulation and Isoflurane dose was minimized. Instead, a muscle paralytic, Vecuronium (1-1.5 mg/kg/hr), was administered during vagus nerve recordings to abate recording artifact resulting from myogenic electric field spillover due to evoked muscle movement or ongoing tremoring – without cardiac blunting effects. A minimal dose of Isoflurane was still used as it permitted a ‘fast-dial’ (quick wash in and

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out period) to maintain the anesthetic plane.

Cervical vagus nerve preparation

4 With the subject in a supine position, use a midline approach to access the left carotid sheath. The carotid artery is mobilized and carefully retracted to minimize obstruction to blood flow. The cervical vagus nerve is exposed for a length of 9-12 cm.

5 Instrument the vagus nerve with a bipolar stimulation electrode caudal to the superior laryngeal branching. Three replicates of the Longitudinal intrafascicular electrode (LIFE) and microneurography electrodes and a cuff electrode with three recording contacts are instrumented on the nerve caudal to the stimulation electrode. A separation of >4 cm is kept between the stimulation electrode and the closest recording electrode.

6 A reference LIFE electrode and microneurography electrode are inserted in superficial fat. The reference electrode site is selected at a distance approximately equidistant from the stimulation electrode to the recording electrodes to match the representation of the stimulation artifact.

Local tissue reference, compared to on-nerve reference, better preserves conduction speed delays between multiple recording contacts.

7 Evoked compound action potentials (ECAPs) are recorded by delivering 750 biphasic stimulation pulses at 25 Hz and 200 us pulse width with randomized stimulation amplitude between 0 and 10 mA. Time locked recordings are made through the recording electrodes.

Great auricular nerve preparation

8 The great auricular nerve (GAN) is accessed by the following approach: The skin and subcutaneous fat are incised from the medial posterior margin of the ramus. A small notch is palpated at this point and indicates the approximate level of the stylomastoid foramen. Here the facial nerve exits and divided into its various branches.

9 The incision continues dorsal following a line posterior to the temporomandibular articulation and up to the medial base of the ear just inferior to the medial crus of the helix to follow the lateral vein through the base of the ear as a landmark for finding the sensory input for the exterior skin of the auricle. This incision exposes the superficial musculoaponeurotic system (SMAS). The SMAS layer is divided along the posterior margin of the ramus and posterior margin of the temporomandibular articulation process to expose the subfascial level adipose tissue. This underlying adipose tissue along the posterior margin of the temporomandibular articulation is the location where the GAN and branches of the facial nerve are found.

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Running in conjunction with these nerves is the lateral/caudal auricular artery and vein.

10 To determine which of the exposed branches were motor or sensory in origin, electrically stimulate each of the nerve branches to look for a motor response.

11 When the sensory branch of the posterior auricular nerve is identified by no motor response it is instrumented with a stimulation cuff and recording electrodes (two microelectrodes and a two-contact cuff or two LIFE electrodes) and verified by recording sensory evoked responses from the skin at the base of the auricle. Reference electrodes are inserted similarly as described above in the vagus preparation.

12 Sensory-evoked naturally occurring neural activity are recorded by stroking the region of the ear innervated by the GAN with a brush.

13 Transcutaneous electrical nerve stimulation (TENS) electrodes cut to 2 x 2 cm in size are applied to the same region of sensory innervation of the GAN as confirmed by recording of sensory evoked potentials and at the base of the ear through which the main trunk of the GAN courses.

14 Non-invasive ECAPs are recorded by delivering stimulation parameters identical to those reported in the cervical vagus section. Invasive ECAPs were recorded by delivering 250 biphasic stimulation pulses at 25 Hz and 200 us pulse width with randomized stimulation amplitude between 0 and 3-10 mA (determined based on each subject's motor threshold). Time locked recordings are made through the recording electrodes.

Electrophysiology system

15 A Tucker-Davis Technologies (TDT) electrophysiology system is used for stimulation and recording. The front-end and digitization is done on the battery-powered Subject Interface (SIM). Data is collected at 25 kHz.

16 High impedance (microneurography electrode) and low impedance (LIFE and cuff) are recorded into two separate recording cards with an active and passive head stage respectively.

17 Stimulation is delivered in bipolar mode with two floating current sources.

Electrophysiology data analysis

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18 A custom-built and publicly available Python package, PyeCAP (https://github.com/ludwig-lab/pyeCAP), is used for offline analysis of electrophysiology, ECAP, and physiology data.

19 Filtering. Electrophysiology data for ECAP analysis was filtered with a high pass 1st order Butterworth filter with a corner frequency at 100 Hz and a low pass Gaussian filter with a corner frequency at 3 kHz. An additional 60 Hz band stop finite impulse response (FIR) filter constructed with a Hamming window is used on the electrophysiology data for analysis of naturally occurring activity. All filtering is performed on the time series data in both the forward and backward direction to eliminate group delays caused by filtering.

> The low pass gaussian filter negates the possibility of introducing filter 'ringing' artifact that may be introduced by a Butterworth filter, which has an 'overshoot' to an impulse response. The stimulation artifact approximates an impulse and an overshoot following a stimulation artifact – caused by inappropriate filter selection – could be mistaken for an ECAP.

20 Detecting Authentic ECAPs. ECAPs were plotted by averaging (point by point median) the stimulation evoked response across all available consecutive pulses in a particular stimulation train. ECAPs are detected by windowing the trace into time windows according to the distances between recording and stimulation electrode and published conduction speeds by fiber type (Manzano et al., 2008, 10.1590/s0004-282x2008000100033).

21 ECAP authenticity is confirmed by several methods. Firstly, conduction speed delay across recording electrodes are verified to be in the expected nerve fiber conduction speed range (Manzano et al., 2008, 10.1590/s0004-282x2008000100033)

> An artifact (e.g., electromyogram (EMG), motion) could occur at a similar time point as an ECAP but would not show up with a conduction speed delay across spatially separated recording electrodes.

Secondly, a sufficient dose of a muscle paralytic, Vecuronium, is used to abate EMG artifact in the ECAP recordings. Thirdly, transection between the stimulation and recording electrode and subsequent recordings are used to confirm the disappearance of the ECAP signal.

22 Quantifying ECAP strength. Aβ- and B-fiber ECAPs are quantified to investigate their strength. Aβ fibers are used as a measure of fast conducting fibers and B-fibers are used as a measure of slower conducting myelinated fibers.

23 Aβ- and B-fiber ECAP time windows are calculated for each stimulation train based on distance from the recording electrode to the stimulation electrode and published values of

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conduction speed (Manzano et al., 2008, 10.1590/s0004-282x2008000100033). The window are then manually narrowed, without removing the ECAP signal of interest, to be of equal duration across recording channels.

This was done to avoid bias that would arise with increasing distance of the recording electrode from the stimulation electrode and hence increased ECAP time window, which would lead to a skewed ECAP strength measurement.

Root mean square (RMS) are calculated on the narrowed fixed-duration window and used as a measure of ECAP strength.

RMS was selected over alternative methods (e.g., integral or area under the curve) as it indicates the equivalent steady state energy value of an oscillating signal and represents the noise floor of the recording electrode, a useful measure, even when no ECAP signal is present at sub-threshold stimulation currents. Furthermore, the measure of peak-to-peak voltage is more susceptible to noise and amplitude is susceptible both to noise and baseline offsets.

24 Spike detection. Spike detection is done using a thresholding method. Standard deviation (SD) from t=1-6s of the specific recording on the specific channel is calculated and the threshold is set at six times this SD plus the mean, to account for baseline offsets, from t=1-6s of the same recording.

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