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Vagus Nerve Selective Stimulation and EIT recording

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This electrophysiology protocol describes the electrical stimulation and measurement steps needed to localize branches and image functional activity in the cervical; vagus nerve of pigs.

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Neuromodulation, Electrical Impedance Tomography, Vagus Nerve

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ScouseTom EIT System: <https://doi.org/10.3390/s17020280>

EIT/SS nerve cuffs: https://github.com/EIT-team/Electrode-Designs/tree/master/Enrico_Pigs_SS_EIT_Jan2021

Cuff manufacturing process: <https://doi.org/10.1088/1741-2552/aae868>

Laptop or PC, Matlab software

Animal surgery facility, physiological readout system with End-Tidal CO₂, Blood Pressure, and ECG capabilities.

EMG needles and bipolar stimulation cuff for larynx.

Preparation

- 1 Animal (pig) is anaesthetized (Isoflurane) and cervical vagus is exposed through surgery.
- 2 Clean electrodes of the nerve cuffs by dipping into ethanol and then physiological solution (or saline solution).
- 3 Apply nerve cuffs to exposed cervical left vagus and connect to the ScouseTom system.
- 4 Apply other required electrical interfaces: EMG needles on larynx, bipolar stimulation cuff on recurrent laryngeal branch (approx. 40cm from cervical vagus cuffs).

Selective Stimulation - Pulmonary Branch

- 5 Set initial parameters for selective stimulation of the pulmonary branch: suggested 50µs pulse width, 0.4mA pulse amplitude, 20Hz repetition frequency, 30s on / 30s off stimulation. Set animal ventilation setup to spontaneous breathing.
- 6 Run selective stimulation with above parameters and observe ETCO₂ response to stimulation (breathing slowdown or complete block) on different electrode pairs.
- 7 Repeat stimulation rounds with increased or decreased pulse amplitude until ETCO₂ alteration is localised to one or a few stimulation pairs. Restore mechanical ventilation after completion.

Selective Stimulation - Recurrent Laryngeal Branch

- 8 Set initial parameters for selective stimulation of the recurrent laryngeal branch: suggested 50µs pulse width, 50-100µA pulse amplitude, 20Hz repetition frequency, 5s on / 5s off stimulation. Animal can stay in mechanical ventilation during this phase.
- 9 Run selective stimulation with above parameters and observe EMG needle response to stimulation (activation of larynx shows bursts of activity on EMG recordings) on different electrode pairs.
- 10 Repeat stimulation rounds with increased or decreased pulse amplitude until EMG bursts in response to stimulation are localised to just one or a few stimulation pairs.

Laryngeal EIT


- 11 Connect stimulator output of ScouseTom to bipolar cuff placed on laryngeal branch. Connect ScouseTom in EIT mode to vagus nerve EIT cuff.
- 12 Perform evoked fast neural EIT according to published work with following parameters:
 - EIT: skip-5 pattern, 200 µA, 6kHz, 15s each injection pair
 - Stimulation: 50 µs pulse width, 1.2 mA amplitude, 20 Hz repetition during each injection

Selective Stimulation - Cardiac Branch

- 13 Switch anaesthetic from isoflurane to α-chloralose and wait 30-40 minutes for settling.
- 14 Set initial parameters for selective stimulation of the recurrent laryngeal branch: suggested 1ms pulse width, 1mA pulse amplitude, 20Hz repetition frequency, 30s on / 30s off stimulation. Animal can stay in mechanical ventilation during this phase.
- 15 Run selective stimulation with above parameters and observe heart rate response to stimulation, computed in real time from peaks in blood pressure or ECG on different electrode pairs.
- 16 Repeat stimulation rounds with increased or decreased pulse amplitude until heart rate change in response to stimulation is localised to just one or a few stimulation pairs.

Spontaneous EIT

- 17 Perform spontaneous fast neural EIT with following parameters:
 - No stimulation

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- EIT: skip-5 pattern, 200 μ A, 6kHz, 10 minutes each injection pair
 - Recording ECG, blood pressure, End-Tidal CO₂ on auxiliary channels of the recording system