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Methods for Visualization of Pig Vagus Nerve "Vagotopy" Using Ultrasound

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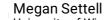
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This protocol describes the surgical, ultrasound, and histology methods for visualizing pig vagus nerve vagotopy.

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Preparing Surgical Pocket for Vagus Nerve Ultrasound

- Surgically Expose the Vagus Nerve
 - 1.1 Place subject in dorsal recumbence position.

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- 1.2 Expose the length of the cervical vagus nerve by creating an incision (15-20 cm long) approximately 3 cm lateral and parallel to midline starting at the level of the mandible.
- 1.3 Divide tissue using blunt dissection to expose the carotid sheath, identifying and removing connective tissue from the carotid artery, vagus nerve and jugular vein.
- 1.4 Particular attention should be paid to clearing the vagus nerve at the most cranial point of interest (superior cervical ganglion, nodose/inferior cervical ganglion) to the caudal region of interest.
- 2 Place wire under the vagus nerve every 2-3 cm, where histology samples will be taken.
 - 2.1 Depending on the diameter and type of wire, artifact will be created in the ultrasound to allow for histological matching. (ex: Silver (Ag) 0.015" bare wire)
- 3 Fill the surgical pocket with room temperature/heated 0.9% Sodium Chloride (saline).
- 4 Collect ultrasound in regions of interest, or along the length of the vagus nerve.

Histology of Vagus Nerve Following Ultrasound

- 5 Pat the vagus nerve dry with surgical gauze, or cotton tipped swabs above each wire.
- 6 Place histology dye as desired to mark the location of each wire along the length of the vagus nerve.
 - **6.1** Recommended Histology Dye: Bradley Products, Inc., Davidson Marking System, Bloomington, MN.

- Once histology dye has been placed in the desired locations, remove the vagus nerve and place in 10% Neutral Buffered Formalin for histological processing.
- 8 For Gomori's trichrome staining, please see our previously published protocol: dx.doi.org/10.17504/protocols.io.9ieh4be