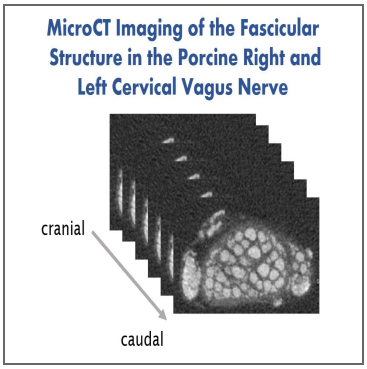

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MicroCT Imaging of the Fascicular Structure in the Porcine Right and Left Cervical Vagus Nerve

Bettina Kronsteiner[1], Max Haberbusch[2], Francesco Moscato[2]

[1]Center of Anatomy and Cell Biology, Medical University of Vienna; [2]Center for Medical Physics and Biomedical Engineering, Medical University of Vienna

SPARC
Tech. support email: **info@neuinfo.org**

Max Haberbusch
Center for Medical Physics and Biomedical Engineering, Medic...

ABSTRACT

This protocol outlines the procedure to dissect, prepare, and perform micro-CT imaging of the fascicular anatomy of the right and left vagus nerve in domestic pigs.

MATERIALS

- Fresh cadavers of pigs (domestic pigs)
- Isotonic sodium chloride solution (NaCl, 0.9%)
- Surgical sutures (5–0 silk suture, Silkam®B-Braun Aesculap, Tutlingen, Germany)
- Polystyrene plate with V-shaped incisions
- PBS (Phosphate-Buffered Saline)
- 4% phosphate-buffered 4% Paraformaldehyde (Roti-Histofix, Roth, Karlsruhe, Germany)
- 1% Lugol's solution
- GIMP open-source software (GIMP 2.10.24, revision 2)
- SCANCO µCT 50 (SCANCO Medical AG, Brutistellen, Switzerland) specimen µCT scanner

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Surgical Dissection and Macroscopic Examination of Nerve Sa...

1 Acquire fresh cadavers of male and/or female domestic pigs.

2 Place cadavers in the dorsal recumbence position.

3 Make a ventral skin incision (15–20 cm) on both sides next to the trachea starting at the level of the mandibles extending to the sternal notch.

4 Dissect tissue and open the carotid sheath to expose the common carotid artery, internal jugular vein, the vagus nerve, and the sympathetic trunk.

5 Begin nerve dissection at the level of the nodose ganglion (NG) and extend caudally to the superior cardiac branch, which originates approximately 2–5 cm cranial to the subclavian artery on both sides.

6 Track branches emerging from and terminating in the vagus nerve (VN) to their origin structures.

7 Identify both recurrent laryngeal nerves running close to the trachea looping back around the subclavian arteries.

8 Open the chest by median sternotomy followed by gentle exposure of the heart.

9 Continue surgical preparation of the VN towards the heart. Dissect the superior cardiac branch from the cardiac branching points at the VN towards the insertion points at the heart, ensuring not to cut any of the cross-connections between the VN and the sympathetic trunk (ST).

10 During dissection, moisten the nerves with isotonic sodium chloride solution to prevent drying.

11 Measure dimensions of the VN using a ruler and capture a photo for documentation.

Vagal Nerve Preparation and Harvesting

12 Fix surgical sutures onto the epineurium of the VN below the nodose ganglion to maintain the anatomical in-situ orientation. Nerves should be harvested 1-2 hours post-mortem.

13 After complete dissection, place and fix nerves onto a polystyrene plate, ensuring the anatomical in-situ orientation is maintained.

14 Wash nerve specimens in phosphate-buffered saline (PBS)

15 Fixate in 4% phosphate-buffered 4% Paraformaldehyde (PFA) at 4°C for 16–18 hours.

16 After fixation, wash the nerve samples again and store in freshly made PBS at 4°C until staining for micro-computed tomography imaging.

Micro-computed Tomography

17 Stain nerves in 14 mL of 1% Lugol's solution for 24 hours.

18 Place nerve specimens in 15 ml conical centrifuge tubes with residual staining solution and the polystyrene plate used for fixation.

19 Perform low-resolution scanning using the SCANCO µCT 50 scanner with 70 kVp (200µA, 0.5 mm Al filter, 550 projections, 100 ms integration time) at an isotropic resolution of 20 µm.

20 Divide the VNs into two nerve specimens for scanning, ensuring proper anatomical orientation for alignment.

21 Perform high-resolution scans at the cardiac branching point with 70 kVp (85 µA, 0.5 mm Al filter, 850 projections, 350 ms integration time) at an isotropic resolution of 8.9 µm over 1 cm of nerve length.

22 Re-stain nerves 24 hours prior to high-resolution scans to maintain full saturation with Lugol's solution.

23 Analyze high-resolution data at the cardiac branching point.