Collection of rat vagal tissue samples for TEM imaging

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This protocol describes the methods used to generate samples of rat vagus tissue suitable for TEM imaging.

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Animals

1 Sprague-Dawley **Envigo**

rats (2–4 months old, male and female) were housed in shoe-box cages with bedding material in an Association for Assessment and Accreditation of Laboratory Animal Care-approved colony room, temperature (22–24°C) and humidity (40%–60%) controlled. The room was maintained on a 12:12 hour light–dark schedule. Pelleted chow

2018 Teklad global 18% protein rodent diet **Envigo**

and filtered tap water were provided ad libitum. All husbandry practices conformed to the

National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th edition) and were reviewed and approved by the Purdue University Animal Care and Use Committee. All efforts were made to minimize any suffering as well as the number of animals used.

Perfusion

2 Animals were perfused according to the following procedure. Specifically, they were overdosed with anesthetic (intraperitoneal injection of ketamine/xylazine [275 mg/kg of ketamine/27.5 mg/kg of xylazine]):

☒ Ketamine **Patterson Veterinary Catalog #07-803-6637**

☒ Xylazine **Akorn Animal Health Catalog #NDC: 59399-110-20**

Animals were then exsanguinated with fresh physiological saline, and then transcardially perfused with a fresh solution of EM-grade fixatives (2% paraformaldehyde/1.25% glutaraldehyde) at 4°C:

☒ Paraformaldehyde BAKER J.T. Baker **Fisher Scientific Catalog #02-003-576**

☒ Glutaraldehyde 50% EM Grade Aqueous **Electron Microscopy Sciences Catalog #16320**

in 0.1 M phosphate-buffered saline ([PBS], pH = 7.3) for 20 minutes.

Dissection and Fixation

3 Nerve bundles, trunks, and branches were immediately dissected out, manipulating the vagus as little as possible, removing only the minimum amount of connective tissue or fat require to access and remove the samples (4mm long sections at each sampled level) and moved into the same fixative combination as in step 2 overnight at 4°C on an oscillating platform.

The following morning, the tissue specimens were rinsed (3 × 30 min at 4°C) in PBS, transferred to individual shipping vials under PBS, and shipped overnight to the TEM laboratory for further processing and microscopy - see the protocol below:

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