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AAV injection in the nodose ganglia in mouse

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We use this protocol and it's working

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Abstract

The gut-brain axis links the visceral organs to the medulla oblongata via the vagus nerve. Accessing to the afferent vagal pathway is important to dissect the role of cell populations in the bidirectional communication between brain and body. The jugular-nodose ganglion (JNG) complex contains the cell bodies of many heterogenous neural subpopulations responsible for sensing the physiological and pathological conditions of the thoracic and abdominal organs. However, the study of these ganglia is challenging in small animals due to size and location. Hence, in this protocol we describe a practical surgical approach to the vagal trunk and the JNG complex in mice.

Materials

Equipment

10µl, Neuros Syringe, Model 1701 RN, 33 gauge	NAME
syringe	TYPE
Hamilton	BRAND
65460-06	SKU

Equipment

Sub-Microliter Injection System	NAME
World precision Instruments	BRAND
10uL NANOFIL	SKU
https://www.wpiinc.com/var-3167-sub-microliter-injection-system	LINK

Equipment

NanoFil Application Kits — NAME

World Precision Instruments — BRAND

Beveled (IO-KIT) — SKU

https://www.wpiinc.com/var-3327-nanofil-application-kits — LINK

Equipment

36-gauge Beveled NanoFil needle — NAME

World Precision Instruments, — BRAND

NF36BV-2 — SKU

Equipment

Micromanipulator — NAME

Miller Design — BRAND

P#10 — SKU

Troubleshooting

Before start

Disinfect the surgical work surface with 70% ethanol and prepare in advance sterile instruments (e.g., fine scissors, forceps, and retractor), gauzes, staples, and swabs by autoclaving. For multiple surgeries, clean and re-sterilize instruments with 70% ethanol or a dry bead sterilizer between mice. A surgical mask, clean lab coat, hair bonnet and sterile gloves should be worn. These ganglia are approximately 1mm-wide, and it is located deep in the cervical carotid triangle. Therefore, a surgical microscope will be needed for entirety of the procedure.

Preparation of the surgical setup

1 Turn on the heating pad to 37 °C

2 Position the surgical microscope to be ready when the animal is under anesthesia.

3 Thaw the aliquot of AAV to be used, mix it well, and keep it on ice.

N.B: For intraneural injections (vagus trunk) based on our experience we recommend using the mosaic AAVrg/rh10 to maximize efficiency. This serotype selectively transduces afferent neurons, so none or minimal transduction of efferent vagal pathway should be expected. Titer should be in a range between 1-3×10^{12}vg/ml with an injection volume of 4-6ul. For intraganglionic injections, based on our experience we recommend using AAV9. Titer should be in a range between 1-3×10^{12}vg/ml with an injection volume of 2-3ul.

4 Withdraw the AAV with a 10ul 33G syringe.

5 Remove locking cap and gasket from the 10ul syringe.

6 Connect the NanoFil sub-microliter injection system.

7 Attach the SilFlex tubing to the 10ul syringe and the other end to the Neuros Syringe.

8 Secure the 36G beveled NanoFil needle to the injection holder.

9 De-gas the NanoFil system by slowly pushing the plunger.

Surgery

10 Anesthetize mice using a mixture of ketamine (110mg/kg) and xylazine (8 mg/kg) or isoflurane.

11 Shave the whole anterior cervical area of the mouse with an electric razor or shaving cream.

12 Lay the mouse flat on supine position on a heating pad.

13 Apply ophthalmic ointment of the mouse eyes.

14 Sterilize surgical area skin with 70% alcohol, complex iodine, and 70% alcohol again. Place a surgical gauze in the sterile area. Make a cut/small opening in the gauze at the level of the surgical area.

15 Make sure the mouse is completely anesthetized by applying a strong pinch on the forepaw of the animal with a forceps. Make a small incision in the skin in the middle of the neck with straight thin scissors or a scalpel.

16 Retract the submandibular glands laterally to expose the cervical musculature.

17 Dissect the sternocleidomastoid muscle to the laterally and the omohyoid muscle medially.

18 Identify the carotid bifurcation and gently dissect the connective tissue surrounding the area.

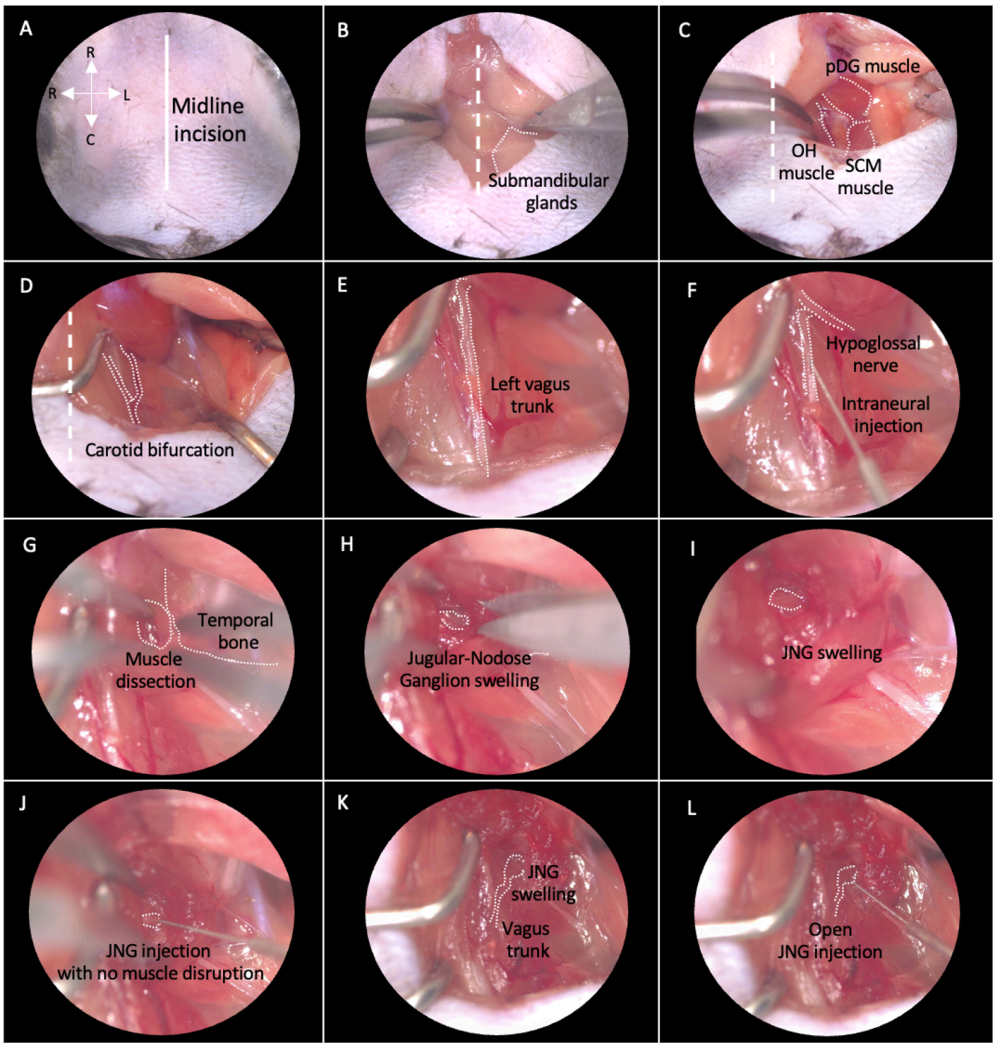
19 Carefully remove the connective tissue surrounding the vagus trunk above the carotid bifurcation. (If intra neural injections are required, proceed with injection.)

N.B: It is crucial that the carotid is treated carefully, otherwise major bleeding could be caused. It is also crucial to avoid excessively disturbing the vagus trunk as this could cause autonomic dysfunction after surgery.

20 Identify the temporal bone underneath the posterior belly of the digastric muscle.

21 Dissect the muscle fibers, and next to the mastoid notch of the temporal bone the JNG swelling will appear.

N.B: Muscle fibers should be gently opened in the same orientation of the fibers to avoid damage of the occipital and auricular artery that irrigates the posterior belly of the digastric muscle.



Stepwise procedure to access the vagus nerve trunk and jugular-nodose ganglion.
A. Make a small incision in the skin in the middle of the neck with straight thin scissors or a scalpel. B. Retract the submandibular glands laterally to expose the cervical musculature. C. Dissect the sternocleidomastoid muscle to the laterally and the omohyoid muscle medially. D. Identify the carotid bifurcation and gently dissect the connective tissue surrounding the area. E. Carefully remove the connective tissue surrounding the vagus trunk above the carotid bifurcation. F. If intra neural injections are required proceed with injection. G. Identify the temporal bone underneath the posterior belly of the digastric muscle. H. Dissect the muscle fibers, and next to the mastoid notch of the temporal bone the JNG swelling will appear. I-J. If the JNG can be fully visualized, proceed with intraganglionic injection. K-L. If needed open completely the posterior belly of the digastric muscle to gain a clear view of the cervical portion of the vagus trunk arriving to the JNG, and then proceed with injection.

22 If the JNG can be fully visualized, proceed with intraganglionic injection. If needed open completely the posterior belly of the digastric muscle to gain a clear view of the cervical portion of the vagus trunk arriving to the JNG, and then proceed with injection.

N.B: Intraganglionic injections can be done with the help of a micropositioner or free-handed, based on operator preference. 10ul syringe should inserted in a pump set up at a infusion rate of 2nl/s, final volume of 500nl.

23 Close the skin with a sterile suture and apply povidone-iodine.

24 Apply antibiotic ointment to the wound. Carprofen (5mg/kg) as analgesic is injected subcutaneously by body weight of the mice (100ul of Carprofen for each 10 g of mouse).

25 After the surgery, place mouse under a heat lamp until fully awake; return the mouse back in the cage when full motility is restored.