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Dissection and immunohistochemistry of mouse vagal ganglia

In 1 collection

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We use this protocol and it's working

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Abstract

Mice are euthanized, perfused with fixative and the vagal ganglia extracted. Vagal ganglia are then cyosectioned. Slices are stained for protein expression using immunohistochemistry. Expression of specific proteins and reporter proteins isarevisualized using microscopy.

Materials

MATERIALS

- Triton X-100 **Bio-Rad Laboratories** **Catalog #**1610407
- Bovine Serum Albumin (BSA) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A7906
- DPX **Merck Millipore (EMD Millipore)** **Catalog #**1.00579.0500
- Sucrose
- Tween 20 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P1379
- Paraformaldehyde **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P6148
- Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P3813
- alexa fluor 647 chicken anti-goat **Invitrogen - Thermo Fisher** **Catalog #**A21469

1 6 to 8 weeks old mice (male) were euthanized by CO_2 inhalation

2 Mice were transcardially perfused with phosphate buffered saline (PBS) to remove blood followed by 3.7% formaldehyde (PFA) in PBS

3 Vagal ganglia (including both jugular and nodose ganglia) were collected and fixed with 3.7% PFA for 2 hours on ice

4 Vagal ganglia were transferred to 18% sucrose solution for overnight at 4 °C

5 Tissue were mounted in optimal cutting temperature compound and frozen

6 Tissue were cut at 20 µm thickness and collected onto superfrost plus microscopy slide glass

7 Tissue were washed with PBS three times for 10 min

8 Tissue were blocked with blocking buffer (1% bovine serum albumin/10% normal donkey serum/0.3% triton x-100 in PBS) for 45 min at room temperature

9 Tissue were incubated with primary antibody (1:150, goat anti-VR1, SC-12498, Santa Cruz) diluted in blocking buffer overnight at 4 °C

10 Tissue were washed with 0.2% tween20 in PBS (PBST) for 10 min for three times at room temperature

11 Secondary antibody (1:300, Alexa flour 647 Chicken anti-goat, A21469, Invitrogen) was diluted in the 1% bovine serum albumin/5% normal donkey serum/0.2% PBST and tissue were incubated with it for 1 hour at room temperature

12 Tissue were washed with 0.2% tween20 in PBS (PBST) for 10 min for three times at room temperature

13 Tissue were cover-slipped with DPX for imaging and left to dry in the dark overnight.