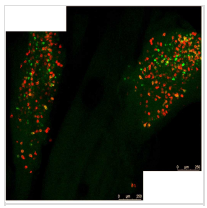

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May 10, 2022

Cholera Toxin Subunit B (CTB) Retrograde tracing from the mouse colon and bladder wall. V.1

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Andrea Harrington

This protocol is used to label the neurons and their central terminals of the sensory neurons innervating the colon and bladder in an experimental adult male or female mouse. The protocol is performed under anesthesia and should incorporate all local requirements for standards of animal experimentation, including methods of anesthesia, surgical environment, and post-operative monitoring and care.

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protocol

Grundy, L et al. Chronic linaclotide treatment reduces colitis-induced neuroplasticity and reverses persistent bladder dysfunction JCI Insight. 2018;3(19):e121841. https://doi.org/10.1172/jci.insight.121841.

retrograde tracing, dorsal root ganglia, colon, bladder, spinal cord, visceral afferent pathways

protocol ,

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Cholera Toxin Subunit-B conjugated to AlexaFluor (CTB-AF) 488 (500ug) ThermoFisher catalogue C22841

Cholera Toxin Subunit-B conjugated to AlexaFluor (CTB-AF) 594 (500ug) ThermoFisher catalogue C34777

5ul Hamilton syringe (701N) Hamilton Company catalogue: HAMC7634-01

30 GAUGE HUB RN Needle length 1 inch point style: 4 (10-12) Hamilton Company catalogue: HAMC7803-07

Preparation for surgery

1 Prepare cages for each mouse – place clean bedding, nesting material in cage and water bottle and food pellets in hopper ready for post-surgery

2 Weigh mouse and record on clinical record sheet and cage label

3 Set up surgical area. Turn on heating pad and cover with appropriate barrier pad. Wipe down surgery area with ethanol and wash dissection tools in ethanol.Place sterile saline tubes into warm water . Place sterile gauze, sterile tip applicators, warmed saline and betadine within easy reach

4 Prepare Baytril and buprenorphine. Place empty Hamilton syringe and CTB tracer dye (0.5% dissolved in 0.1I M Phosphate Buffer) within reach ready for use. Check if isoflurane tank is full and fill if needed.

Anaesthesia

5 Place mouse in induction box, and turn on isoflurane to 5% and oxygen flow rate to 0.5-0.6L/min.

6 When paw pinch does not elicit a pedal response remove mouse from anaesthetic induction box and place nose into nose cone taped to heat pad and re-direct the flow of isoflurane to nose cone and reduce isoflurane percent to 2-3%.

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7 Shave and clean the lower abdomen.

Surgery

8 Perform a midline incision in the skin and then the muscle, then gently move organs to visualise the required injection site.

9 Fill Hamilton syringe with 4ul of CTB-AF

10 Microinject sterile CTB tracer solution at the selected injection sites using a Hamilton Syringe attached to a 30G needle. At each injection site after needle removal, blot area with a cotton tip applicator to soak up any leaked dye. This also minimises leakage.

10.1 CTB-594 Injections into the distal colon wall: 1cm proximal to the pelvic bone, insert tip of the needle into the sub-serosal outer layers of the colon wall. Gradually advance needle tip in the rectal direction using a twirling motion until tip sits just in line with the pelvic bone or until resistance is felt. Making sure the needle is always visible (therefore in the sub-serosal layer and not in the lumen of the colon wall). Gradually withdraw the needle whilst injecting 4ul of dye. Perform single injections (or multiple smaller volume injections) in the midline of the colon, one on the left side of the colon and one on the right side of the colon close to the mesenteric border. Do not squeeze the colon or damage the mesentery.

10.2 CTB-488 Injections into the bladder wall: Hold the bladder taught using blunted forceps (tips protect with plastic tubing). Insert needle into the wall of the bladder starting halfway between the dome and the base of the bladder (urethra opening) moving towards the urethra making sure the needle is always visible. Gradually withdraw the needle whilst injecting 2ul. Repeat for each quadrant of the bladder.

11 After final injection, roll exteriorized organs back into original position

12 Drop warm saline into the abdominal cavity and gently squeeze the sides of the mouse to settle organs back into place

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13 Using sterile 6.0 proline suture material, close the abdominal muscle layer incision using individual stitches.Repeat to close the incision in the superficial skin layer.

14 Wipe are with betadine.

15 Administer antibiotics and analgesics

16 Place mouse in a clean cage. Monitor mouse during post-operative period as local approved procedure.

Tissue harvesting

17 To identify tracer dye distribution in sensory neurons within ganglia and sensory terminals within the spinal cord and medulla, 4-7 days after surgery, fix animals by transcardial perfuse fixation, then remove tissues of interest for further study.

17.1 Tissue collected include dorsal root ganglia (DRG) T9-S1 spinal levels and nodose ganglia and the spinal cord between spinal levels T10-L1 and L5-S3, as well as the brainstem.

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