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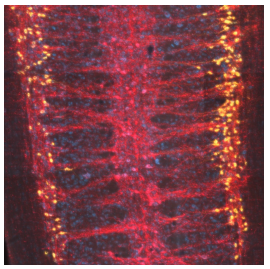
Use of cholera toxin subunit B to label neural projections to major pelvic ganglia



Forked from Use of cholera toxin subunit B to label neural projections to lower urinary tract organs

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is used to visualise preganglionic neurons in the spinal cord innervating pelvic visceral organs (e.g., the lower urinary tract) in an experimental adult male or female rat. 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.

Materials

MATERIALS

- Parafilm
- Isoflurane **Zoetis** **Catalog #**10015516
- Lacrilube **Ellar Laboratories**
- Cholera toxin subunit B **List Labs** **Catalog #**104
- Glass capillaries **Warner instruments** **Catalog #**#GC100F-15
- Sterile Saline (0.9% NaCl)
- Evans Blue Dye **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E2129

Equipment

Picospritzer III Intracellular Microinjection Dispense System	NAME
Injection system	TYPE
Picospritzer	BRAND
052-0500-900	SKU
https://ph.parker.com/us/12051/en/picospritzer-iii-intracellular-microinjection-dispense-systems-picospritzer-micro-dispense-system/052-0500-900	LINK
100 psi, 2 channel	SPECIFICATIONS

Preparation for surgery

1 Prepare cholera toxin subunit B solutions: low salt formulation with 0.05% Evans Blue.

2 Prepare glass pipettes for surgery by prefilling each pulled glass pipette with cholera toxin subunit B

3 Anesthetise animal (2.5% isoflurane in oxygen, or as required for maintenance)

4 Apply eye lubricant and place animal on heated pad.

5 Shave and clean the ventral abdomen.

Surgery

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

The major pelvic ganglia is located on the dorsal lateral lobe of prostate (male) or cervix (female)

7 Using fine angled forceps, gently blunt dissect underneath the pelvic ganglia. Once the ganglia is separated from the underlying tissue, slide a sterile 2mm x 2mm piece of parafilm between it and the tissue.

8 Microinject sterile tracer solution at the selected injection site using a glass pipette attached to a picospritzer. At each injection site, hold the glass pipette in place for ~5 seconds after ejection of the dye, to enable the dye to spread to the underlying tissue. This also minimises leakage. Continue injections until the desired volume is reached.

9 Wash all injection sites with sterile saline.

10 For a bilateral injection, perform steps 6-8 on the alternate side.

11 Close the muscle and skin using approved procedures. Administer analgesics and monitor animal during postoperative period as per local approved procedure.

Tissue harvesting

12 To analyse tracer distribution in spinal cord or the injection site, 3 days after surgery, anesthetise animals as per local ethical requirements, and perform intra-cardiac perfusion with fixative, then dissect tissues of interest for further study.