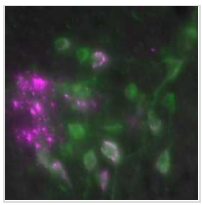

Dec 15, 2021

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

Use of tracer dyes to label neural projections to lower urinary tract organs [keast-001-stage01]

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This protocol is used to visualise sensory and autonomic neurons innervating organs of 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.

DOI

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Visualizing lower urinary tract afferent projections in the lumbosacral spinal cord in rats

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lower urinary tract, retrograde tracing, neural tracing

protocol ,

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Part of collection
Visualizing lower urinary tract afferent projections in the lumbosacral spinal cord in rats

MATERIALS

Neuros syringe **Hamilton Company Catalog #65460-02**

Isoflurane **Zoetis Catalog #10015516**

Lacrilube **Ellar Laboratories**

Cholera toxin subunit B **List Labs Catalog #104**

Preparation for surgery

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

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

3 Apply eye lubricant and place animal on heated pad.

4 Shave and clean the ventral abdomen.

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Surgery

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

6 Microinject sterile tracer solution at the selected injection site using a Hamilton Neuros Syringe attached to a 33G needle. At each injection site, hold the needle in place for ~5 seconds after ejection of the dye, to enable the dye to spread to the underlying tissue. This also minimises leakage.

Injections into the bladder body (dome) are made bilaterally on dorsal and ventral aspects; total volume ~ 5 µl. Single injections are made into the dorsal aspect of the proximal urethra (maximum 0.3 µl per injection).

7 Wash all injection sites with sterile saline.

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

Tissue harvesting

9 To analyse tracer distribution in ganglia or the injection site, 4 days after surgery, fix animals by intra-cardiac perfusion, then remove tissues of interest for further study.

For studies of the central projections of lower urinary tract afferents, the spinal cord and dorsal root ganglia of segments L5 to S2 would be taken. Lower urinary tract organs would also be taken for confirmation of injection site.

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