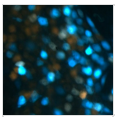

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Use of tracer dyes to label neural projections to lower urinary tract organs

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.w2xfgfn

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ABSTRACT

This protocol is used to visualise sensory and autonomic neurons innervating the bladder body (dome), bladder trigone or proximal urethra 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

NAME	CATALOG #	VENDOR
Neuros syringe	65460-02	Hamilton Company
Fluorogold	39286	Sigma Aldrich
Fast Blue	17740-1	Polysciences
Isoflurane	10015516	Zoetis
Fluorogold	View	Fluorochrome
Lacrilube	View	Ellar Laboratories

Preparation for surgery

1 Prepare tracer dye solutions: Fluorogold or Fast Blue (each 2% w/v in sterile water).

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.

Surgery

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

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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 bladder trigone or 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 dye distribution in ganglia or the injection site, 5-14 days after surgery, fix animals by intra-cardiac perfusion, then remove tissues of interest for further study.

For studies of the lower urinary tract innervation, these tissues would typically include dorsal root ganglia (DRG) from L1, L2, L6 and S1 spinal levels and the pelvic ganglia (synonym, major pelvic ganglia). It is also recommended that the lower urinary tract tissues are also removed for microscopic validation of the tracer injection site.

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