

Apr 23, 2020

Cystometry in awake rats

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DOI

dx.doi.org/10.17504/protocols.io.bakjicun

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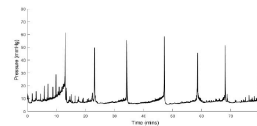
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DOI: <https://dx.doi.org/10.17504/protocols.io.bakjicun>

Protocol Citation: Janet R Keast, Peregrine B Osborne, Nicole Wiedmann 2020. Cystometry in awake rats. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bakjicun>

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Protocol status: Working

We use this protocol and it's working

Created: December 17, 2019

Last Modified: November 27, 2023

Protocol Integer ID: 31083

Keywords: surgery, catheterization, urinary bladder, micturition, cystometry in awake rat, bladder cannulation, cystometry in an experimental adult male, cystometry, bladder, methods of anesthesia, awake rat, surgical environment, anesthesia, female rat, rat, surgery

Abstract

This protocol is used for bladder cannulation and cystometry in an experimental adult male or female rat. The surgery 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

✕ Isoflurane **Zoetis Catalog #10015516**

✕ Lacrilube **Ellar Laboratories**

✕ Loctite super glue liquid professional **Catalog #1365882**

✕ PVC tubing (D: 0.96 × 0.50 mm)

✕ Polyethylene (PE) tubing (D: 0.61 × 0.28 mm)

✕ LabChart 8 **ADInstruments**

✕ PowerLab **ADInstruments**

✕ Syringe Pump **Harvard Apparatus**

✕ 40 PDS® II (polydioxanone) Suture **Ethicon Catalog #D6261**

✕ Disposable BP Transducers **ADInstruments Catalog #MLT0670**

✕ Gentamicin **Ilium Troy Laboratory Catalog #Gentam100**

✕ Silastic tubing (ID 1.6 mm OD 3.2 mm) **Fisher Scientific Catalog #Dow Corning 11-189-15G**

Troubleshooting

Preparation for surgery

- 1 Prepare bladder cannula at least 24 - 48 h prior to surgery. This is comprised of a short length of PE tubing that connects directly with the bladder lumen; this tubing inserts into a long piece of PVC tubing that is exteriorised at the base of the neck.

Note

The length of PVC tubing required is ~25 cm (male rat) and ~20 cm (female rat). This length includes room for the animal to grow and to provide sufficient amount exposed at the neck for daily flushing and experimental cystometry. The length of PE tubing required is ~ 2 cm.

- By carefully holding PE tubing over an open flame, flare the end to form a bell shape. Ensure that the lumen of the tubing does not seal during this process.
 - Cut the flared end to a length of 0.5-1 cm.
 - Insert the non-flared end into the end of the PVC tubing as far east it will go (usually 2-3 mm)
 - Secure by applying superglue to the joint.
 - To make the collar (securing point used for attaching to the neck skin): cut 0.5-1 cm of silastic tubing and glue it to the exteriorised end of the cannula tubing (leaving 5 cm overhang).
- 2 Anesthetise animal (2.5% isoflurane in oxygen, or as required for maintenance)
 - 3 Shave and clean the ventral abdomen and the dorsal neck.
 - 4 Apply eye lubricant and place animal on heated pad.

Surgery

- 5 Perform a ventral midline incision in the skin overlying the region of the bladder. Place gauze dampened with sterile 0.9% saline over the abdominal incision site and turn the animal over.
- 6 Perform a dorsal midline incision in the skin at the base of the neck. This will be the exit site for the bladder cannula.

- 7 Blunt-dissect under the skin from this neck incision site to the ventral abdominal incision side using hemostats. Gently pull the bladder cannula tubing through.
- 8 Perform a ventral midline incision in the muscle. Make a smaller secondary incision in the abdominal muscle on the same side as the cannula tubing being tunnelled. Feed the cannula through the hole to enter the abdominal region.
- 9 Using cotton-tipped applicators dampened with sterile 0.9% saline, gently expose the bladder. Using 4/0 PDS suture, place a very loose purse string knot around the tip of the bladder dome. This should be located between a quarter and a third of the way down the apex of the dome. DO NOT TIGHTEN.
- 10 Pierce the tip of the bladder dome with a 19G needle, carefully absorbing any leaked urine with gauze. Use fine forceps to widen the hole into the bladder.
- 11 Fill the cannula with 200–300 µl sterile saline and insert flared end of the cannula into the bladder, ensuring that the end terminates below the location of the purse string suture. Close the purse string suture and tie off.
- 12 Once secured, infuse 0.2 ml 0.9% sterile saline into the bladder to check for leaks. If there are leaks, re-tie slightly lower than the original suture.
- 13 Close the muscle wall and abdominal skin using approved procedures.
- 14 At the dorsal neck, orientate the collar and cannula tubing so they face caudally. Place a suture through the neck skin, then collar, and then opposite side of the neck skin, then tighten firmly. Repeat this step two more times to secure the collar to the skin.
- 15 Close the end of the exteriorised cannula using a blunt 25G needle that has been sealed at one end with superglue.
- 16 Administer analgesics and monitor animal during postoperative period as per local approved procedures. To maintain patency of the cannula, infuse with gentamicin (0.2 ml, 40 mg/ml in sterile 0.9% saline) for 3 d and then daily with sterile 0.9% saline (0.5 ml).

Habituation

- 17 After post-surgical recovery, habituate the animal to the recording chamber (30 min per day) at least 3 days before cystometry recording



Cystometry recording

- 18 Place the animal into the experiment chamber. Fill up a 25 ml syringe with sterile 0.9% saline. Fill the transducer and connection to the bladder cannula with sterile 0.9% saline.
- 19 Set the baseline pressure (Labchart) by holding a flowing tube of saline at the transducer level prior to inserting it into the cannula line of the rat. Allow this to run for a minute before connecting the line to the animal.
- 20 Slowly infuse sterile 0.9% saline into the bladder at rate 0.1 ml/min or the rate required for your experiment
- 21 After inserting the line into the cannula, record bladder pressure for 30 min while the rat settles. Continue cystometry for approximately another 90 min or the duration required for the experiment.
- 22 Switch off the pump, disconnect the cannula from the pump and return the animal to its home cage for 2 hours.

Tissue harvesting

- 23 To analyse Immediate early gene activation in spinal cord, 2 hours after finishing the cystometry recording, anaesthetise and fix animals by intra-cardiac perfusion, then remove spinal cord for further study. This 2 hour period is required for expression of immediate early genes.