



AWAKE CYSTOMETRY

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References:

Pandita RK, Fujiwara M, Alm P, Andersson KE. Cystometric evaluation of bladder function in non-anesthetized mice with and without bladder outlet obstruction. *J Urol* 2000;164:1385-1389.

Mingin GC, Heppner TJ, Tykocki NR, Erickson CS, Vizzard MA, Nelson MT. Social stress in mice induces urinary bladder overactivity and increases TRPV1 channel-dependent afferent nerve activity. *Am J Physiol Regul Integr Comp Physiol* 2015;309:R629-638.

Yaksh TL, Durant PA, Brent CR. Micturition in rats: a chronic model for study of bladder function and effect of anesthetics. *Am J Physiol* 1986;251:R1177-R1185.

1. Cystostomy tubing. Most references indicate the use of PE10 tubing in mice and PE50 tubing in rats. We have found that we can use PE50 in mice and that the patency rate of cystostomy tubes is higher if PE50 tubing is used when testing is performed 5-7 days after tube placement. Inflammation of the bladder observed 2 days after cystostomy tube placement in rats appeared to be resolved 6 days after tube placement (Yaksh et al., 1986). However, many investigators perform awake cystometry 72 hours after cystostomy tube placement (Mingin et al., 2015).
2. PE50 is cut into approximately 15 cm lengths. Create flare in one end by dilating the tubing while heating it over a flame. Tubing is flushed and soaked with 70% alcohol for at least 15 minutes prior to use. Immediately prior to use, tubing is flushed and rinsed with 0.9% sterile saline.
3. Weigh mouse.
4. Anesthetize mouse with ketamine (100 mg/kg IP) and xylazine (10 mg/kg IP) or by inhalation of isoflurane (3-5%) in oxygen, administered by mask or induction chamber.
5. Mice are placed on a heating pad or under a lamp to maintain body temperature (37C?).
6. Apply lubricant to eyes of mouse.
7. The caudal ventral abdomen and back of the neck are clipped and prepared for sterile surgery by gentle scrubbing with 4% chlorhexidine. Soap is removed with 0.9% sterile saline.

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8. Incise lower abdomen midline with scissors or scalpel and carefully expose bladder. Use 6-0 silk suture with swaged needle to make a purse string suture near dome. Insert a 25G 1x1/2 inch needle into the bladder in the center of the purse string suture. Pass the flared end of the cystostomy tube through this hole and into bladder. Tighten purse string suture and secure with square knots. Connect the other end of the tube to a blunt 23 gauge needle attached to a 1 ml syringe filled with saline and slowly instill saline into bladder to examine whether the cystostomy tube is installed properly (bladder should distend, no saline should leak around tube, and saline should exit urethra).
9. The tubing is passed across the body wall and tunneled to the subcutaneous tissue of the back of the neck taking care to leave sufficient slack within the abdomen to prevent the tubing from tugging on the bladder. The abdominal wall is closed with 4-0 absorbable suture. Skin wounds are closed with suture or wound clips. The skin of the back of the neck is prepared for sterile surgery, and an incision is made on the midline cranial to the shoulder blades to exteriorize the tubing. The tubing is trimmed to the appropriate length, sealed by heating, and secured to the skin. We typically leave a short (<1 cm) length of tubing exposed, but the tubing can also be coiled beneath the skin in the subcutaneous space. The skin wound is closed with suture or wound clips.
10. Mice receive buprenorphine (0.1 mg/kg, sc) or carprofen (5 mg/kg, sc) prior to recovery from anesthesia and on the day after surgery to provide analgesia.
11. On the day on which cystometry will be performed, flush cystometry apparatus system with saline to remove all air bubbles in the system. Load 15 ml saline to a BD syringe (diameter 21.6 mm, 30 ml size) and place it into the infusion pump that is connected to pressure transducer via a 3-way stopcock. Start infusion pump at a rate of 0.8 ml/hour for 5 minutes before performing the calibration (leave the end of 3-way stopcock that is to be connected to the cystostomy tube open).
Be sure to carefully remove all air from the lines.
12. Stop infusion, and close the 3-way stopcock. Connect a sphygmomanometer to the cystometry apparatus. Apply 100 mmHg pressure to the system and record for 1 minute or calibrate and balance transducer by auto-function, if available. Infusion pump, pressure transducer, and cystostomy are connected to computer running LabChart version 8 or proprietary program (Catamount) for recording data. Create file for data storage for this experiment.
13. When ready to perform awake cystometry, weigh mouse.
14. Briefly anesthetize mouse with isoflurane. If the tubing is in the subcutaneous space, make a small incision to extract the tubing. Place at least 1 suture to close the wound and secure the tubing to the skin.
15. Cut the tubing below the seal in the end and connect to the infusion line. Connect the cystostomy tube to a blunt 23 gauge needle attached to a 1 ml syringe filled with saline and slowly instill saline into bladder.



16. Place mouse in small (approx. 10 (W) x 20 (L) x 10 (H) cm) enclosure with grated bottom. Top should have a slot to accommodate entry of tubing into enclosure to connect to cystostomy tube but should prevent mouse from escaping. **Note: if mice are very active, tubing may become twisted. It may be helpful to place mice in enclosure for up to 30 minutes prior to connecting to infusion/recording line to allow them to calm down.** Alternatively, mice can be place in a restrainer that allows free flow of urine (Bowman style restrainers are popular for this purpose). If restrainers are used, it is vital that mice are acclimated to placement in the restrainer prior to testing.
17. Infuse saline into the bladder at a rate of 0.8 – 1.5 ml/hour to elicit repeated voiding cycles for about 30 minutes. Continue recording to obtain at least 3-4 consistent voiding cycles. Stop infusion and save file before close the software.
18. Mouse should be euthanized at the end of the experiment (via methods specified by your approved animal protocol), and tissues collected as appropriate.
19. The following cystometric parameters are recorded in each animal: baseline pressure (BP; pressure at the beginning of the bladder filling), threshold pressure (TP; bladder pressure immediately prior to micturition), peak micturition pressure (MP), intercontraction interval (ICI; time between micturition events), bladder capacity (BC) that is measured as the amount of saline infused in the bladder to induce micturition, void volume (VV), and nonvoiding bladder contractions (NVCs) that are defined as rhythmic intravesical pressure increases during the filling phase, without the release of fluid from the urethra. Voiding efficiency (% VE) that is determined as voided volume/bladder capacity x 100.
20. The most common difficulties with obtaining good data from awake cystometry include activity of the mice (movement, grooming, etc.) that affects intraabdominal pressure, preventing accurate assessment of baseline pressures and potentially affecting pressures associated with voiding. Kinking or twisting of the tubing may occur in active mice, and some mice may chew on tubing. Obtaining consistent readings is particularly difficult in active strains of mice and males in general of any strains.

End protocol

MATERIALS (We use the Catamount Cystometry Units)

PE 50 tubing (vendor information and Catalog # would be helpful since there are so many types)

6-0 suture with swaged needle

Suture (4-0 absorbable) to close abdominal incision



Suture (3-0 absorbable) or wound clips to close skin incision

Pressure transducers

Mouse enclosure with grated floor (to allow urine to fall through to be weighed)

Mettler Balance or Grass FT03 force transducers to weigh urine

Syringe pumps (Harvard Apparatus)

AD Instruments:

- PowerLab 8/35 (8 channel A-D converter)
- Bridge Amp (FE224-4 channel or FE228-8 channel)
- MLT844 pressure transducers
- MLAC11 adapter cable for Grass force transducers

OR

Catamount Cystometry Units (Catamount Research and Development, Inc., St. Albans, VT)

Includes:

- Interface Cabinet
- PCI Card - Data Acquisition
- Physiological Interface Card
- Cystometry Data Acquisition Software
- Cystometry Data Analysis Software
- Power Supply
- Pressure Transducer Calibration Kit
- 4+1 USB Hub
- Computer Package with Dual Video Card and Monitor
- Sound Attenuating Cubicle
- Precision Mettler Toledo Balance
- Razel Infusion Pump
- Mouse Cystometry Cage
- Pressure Transducer
- Signal Conditioner

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