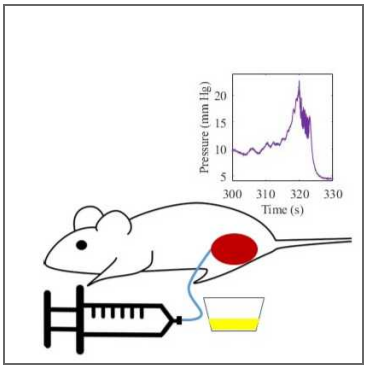

OCT 26, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj3xoblk5/v1

External link:
http://anil.fiu.edu

Protocol Citation: Daniel J Jaskowak, Zachary C Danziger 2023. Single-fill cystometry at varying bladder infusion rates in urethane anesthetized rats. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj3xoblk5/v1

MANUSCRIPT CITATION:
Jaskowak DJ, Danziger ZC. Reflex voiding in rat occurs at consistent bladder volume regardless of pressure or infusion rate. Neurourol Urodyn. 2023 Sep;42(7):1532-1546. doi: 10.1002/nau.25243. Epub 2023 Jul 9. PMID: 37583249.

Single-fill cystometry at varying bladder infusion rates in urethane anesthetized rats

Daniel J Jaskowak[1], Zachary C Danziger[1,2,3]

[1]Florida International University;
[2]Department of Rehabilitation Medicine - Division of Physical Therapy, Emory University;
[3]W.H. Coulter Department of Biomedical Engineering, Emory University

SPARC | BLADDER Consortium

Daniel J Jaskowak
Florida International University

ABSTRACT

Cystometry is a common method to observe the function of the lower urinary tract (LUT) in *in vivo* animal models where an infusion pump fills the bladder at a set rate with physiological saline until it evokes a bladder contraction. This protocol was created to determine if the rate of bladder infusion impacts the bladder volume or pressure that is coincident with the onset of cystometry induced bladder contractions. The effect of infusion rate on LUT reflex activation was investigated in 25 female Sprague-Dawley rats. Rats were anesthetized through subcutaneous injection of 1.2 g/kg urethane. A catheter in series with a pressure transducer and compter-controlled infusion pump was inserted into the dome of the bladder. Silver electrode wires were inserted into the external urethral sphincter percutaneously for electromyography. Each animal's bladder capacity was estimated prior to data collection with a series of 3-5 initial fill-to-void cycles. A subset of bladder infusion rates tested were computed as a percentage of this initial bladder capacity estimate (i.e., relative rates) to ensure the full infusion rate range was sampled across all bladder sizes. The remaining rates used were drawn from a predetermined set without reference to bladder size and selected to span a very broad range. The predetermined rates fell into three categories, 1) those similar to the average rate of fluid added from the ureters, 2) typical infusion rates used in prior rat cystometry studies, or 3) extremely high infusion rates. Infusion rates were sampled differently in different groups of animals to maximize the amount of overall infusion rates tested. A set of 10 infusion rates composed of relative and predefined rates were randomly presented during experiments such that each infusion rate was used at least 3 times.

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

Created: Jul 07, 2023

Last Modified: Oct 26, 2023

PROTOCOL integer ID: 84687

Keywords: lower urinary tract, cystometry

Funders Acknowledgement:
SPARC OT2
Grant ID: 3OT2OD030524-01S1
NIDDK R01
Grant ID: 1R01DK133605-01

IMAGE ATTRIBUTION

https://creazilla.com/nodes/19299-little-mouse-clipart; https://publicdomainvectors.org/en/free-clipart/Medical-syringe-icon-vector-clip-art/28666.html

MATERIALS

Harvard Apparatus: Pump 11 Elite (70-4504)
World Precision Instruments: Large FORT Force Transducer, 100g (FORT100)
Millar Mikro-Tip® solid state catheters SPR-1000 (841-0001)

SAFETY WARNINGS

Urethane is carcinogenic. Please read the Safety Data Sheet prior to use. Urethane waste is hazardous and categorized as a U list chemical by the EPA. Please dispose of excess urethane waste responsibly.

ETHICS STATEMENT

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Florida International University.

Animal Preparation

1 Urethane Anesthesia Protocol

1.1 Anesthetize rat via inhalation of isoflurane 10m

1.2 Weigh Animal

1.3 Use animal weight to determine urethane dosage (1.2 g of Urethane per kg of body weight [BW]). We use a 0.2 g/mL solution. Example calculation: 0.250 kg BW * 1.2 g/kg BW ÷ 0.2 g/mL urethane solution = 1.5 mL urethane dose.

1.4 Subcutaneous injection of urethane at the lower back towards to the midline of the body.

1.5 Monitor toe-pinch reflex every half hour to determine depth of anesthesia. 1h 45m

1.6 Supplementary subcutaneous doses of urethane (0.1 g/kg BW) were administered if needed in 30-minute intervals following initial injection.

2 Suprapubic bladder catheterization 20m

2.1 Make a midline incision at the lower abdomen of the rat to open the pelvic cavity.

2.2 Make a small incision in the dome of the bladder.

2.3 Insert catheter (polyethylene tubing, PE-90) with a flared tip into the bladder dome.

2.4 Secure catheter with purse string suture to the bladder dome grabbing a minimal amount of bladder tissue.

2.5 Fill bladder with infusion pump to ensure that there is a water tight seal around the catheter.

2.6 Suture the rat's abdomen together.

3 Place electrode wires percutaneously into the external urethral sphincter

4 Lay rat prone with urethral meatus positioned over voided volume collection cup.

Bladder Capacity Estimation

5 Perform 3 to 5 single cystometrograms.

5.1 Connect infusion pump to MATLAB. Pump commands (on, off, and setting the infusion rate) were executed from a computer.

5.2 Empty the bladder.

5.3 Set infusion pump rate to 5 ml/hr and fill until a voiding contraction occurs.

5.4 Turn the pump off, measure the residual volume and voided volume.

5.5 Rest for 20 minutes between cystometrograms.

Determination of Infusion rates

6 Relative Infusion Rates (Qi_rel)

6.1 Calculate the average bladder capacity (BC_avg) from the bladder capacity estimation procedure.

6.2 Add 0.19 mL to average bladder capacity (BC_avg'=BC_avg+0.19)to account for bladder capacity drift in preliminary experiments

6.3 The relative infusion rates (Qi_rel) used in the study were calculated by the following equation: Qi_rel=BC_avg'/300*R, where 300 is a fill duration in seconds and R is a 1x5 matrix written as [0.25 0.75 1.25 1.75 2.25].

6.4 There were group differences in the infusion rates used during an experiment which allowed us to expand sampling of infusion rates. We show here how the matrix R changed with each group.
subject experimental group-G1: R=[0.25 0.75 1.25 1.75 2.25]
subject experimental group-G2: R=[0.75 1.25 1.75 2.25]
subject experimental group-G3: R=[1.25 1.75 2.25]
subject experimental group-G4: R=[1.25 1.75 2.25]

7 Predefined Infusion Rates (Qi_pre)

7.1 We wanted to sample the infusion rates that are suggested for cystometry in rats.[1,2] The range includes infusion rates of 2.4-10.8 ml/hr. We sampled infusion rates of 2.4, 3.93, 5, 6.86, and 10.8 ml/hr.

7.2 Larger infusion rates were selected to ensure that there was no "upper bound" where the bladder capacity would start to decrease. We sampled infusion rates of 16.2, 21.6, 32.7, and 65.5 ml/hr.

7.3 The rates in steps 7.1 and 7.2 are larger than the average addition of fluid from the ureters per hour. Therefore, rates of 0.92 and 1.3 ml/hr were sampled.

7.4 Let Qi_pre be a matrix of predefined infusion rates. Qi_pre changed with each group to expand the sampling of infusion rates.
subject experimental group-G1: Qi_pre=[2.4 3.93 5 6.86 10.8]
subject experimental group-G2: Qi_pre=[3.93 5 6.86 10.8 16.2 21.6]
subject experimental group-G3: Qi_pre=[0.92 1.3 10.8 16.2 21.3 32.7 65.5]
subject experimental group-G4: Qi_pre=[2.4 3.93 5 6.86 10.8 32.7 65.5]

8 Pseudorandom order of experiment infusion rates (Qi_exp)

8.1 Let Qi_exp be a 1x10 matrix of the infusion rates used in the experiment. Each group had 10 infusion rates composed of Qi_rel and Qi_pre.
subject experimental group-G1: Qi_exp=[2.4 3.93 5 6.86 10.8 0.25*Qi_rel 0.75*Qi_rel 1.25*Qi_rel 1.75*Qi_rel 2.25*Qi_rel]
subject experimental group-G2: Qi_exp=[3.93 5 6.86 10.8 16.2 21.6 0.75*Qi_rel 1.25*Qi_rel 1.75*Qi_rel 2.25*Qi_rel]
subject experimental group-G3: Qi_exp=[0.92 1.3 10.8 16.2 21.3 32.7 65.5 1.25*Qi_rel 1.75*Qi_rel 2.25*Qi_rel]
subject experimental group-G4: Qi_exp=[2.4 3.93 5 6.86 10.8 32.7 65.5 1.25*Qi_rel 1.75*Qi_rel 2.25*Qi_rel]

8.2 The matrix Qi_exp was replicated 7 times. Each replicate of Qi_exp was pseudorandomly ordered. The pseudorandomly ordered replicates of Qi_exp were appended together into a 1x70 matrix. Infusion rates were presented according to the index in the 1x70 matrix.

Data Collection

9 Experiment Sessions

9.1 A session was a set of five cystometrograms. For each cystometrogram, the bladder was emptied prior to the infusion pump turning on. The infusion pump was turned on and the bladder filled until a reflex voiding contraction occurred. The pump was turned off and the residual volume was collected. Between cystometrograms of the same session the rest was ~1 minute.

9.2 Between sessions there was a 15 minute rest period.

9.3 Sessions were continued until there were at least 3 cystometrograms for every infusion rate tested for an individual animal (at least 30 cystometrograms).