W7: Preclinical Urodynamics - Optimisation of Techniques, Measurements and Interpretation

Workshop Chair: Matthew O. Fraser, United States

13 September 2016 11:00 - 12:30

Start	End	Topic	Speakers
11:00	11:20	Lower Urinary Tract Physiology and Considerations for Urodynamic Study	Matthew O. Fraser
11:20	11:30	Questions	Matthew O. Fraser
11:30	11:50	Multichannel Urodynamics in Rodents	Phillip P. Smith
11:50	12:00	Questions	Phillip P. Smith
12:00	12:20	Pros and Cons of Anesthetized, Conscious and Decerebrate Preparations	Mitsuharu Yoshiyama
12:20	12:30	Questions	Mitsuharu Yoshiyama

Aims of course/workshop

This workshop will provide the physiological background and methodological considerations for urodynamic best practices in the preclinical setting. A better understanding of the underlying physiological principles will enable the participant to better chose the best assays for their purposes and the proper interpretation of the data. The strengths and weaknesses of various approaches will be explained by experts in the field, thereby enabling the participating basic and/or translational science researcher to maximize the quality of the information gathered from their preclinical urodynamic study designs and efforts. Proper interpretation of measurements and results will also be emphasized.

Learning Objectives

After this workshop participants should be able:

1. To understand the mechanics of lower urinary tract function
2. To understand the effects of different methodological approaches on proper interpretation
3. To provide the delegates with the conceptual tools to properly interpret previous results and to design future experiments

Learning Outcomes

In brief, this workshop will educate the delegates in both proper methodological design and subsequent interpretation of results, with emphasis on clinical correlates and translation. Attendees of this workshop will be further empowered to interpret basic science results in the context of their scientific and clinical interests.

Target Audience

Basic and translational researchers wishing to utilize or improve rodent urodynamics for understanding lower urinary tract physiology and pathophysiology. Particularly important for those interested in therapeutic development and model comparisons

Advanced/Basic

Advanced

Conditions for learning

This is a lecture course with expectations of discussion

Suggested Reading

- Yoshiyama M, deGroat WC, Fraser MO. Influences of external urethral sphincter relaxation induced by alpha-bungarotoxin, a neuromuscular junction blocking agent, on voiding dysfunction in the rat with spinal cord injury. Urology. 2000 Jun; 55(6):956-60
- Yang Z, Dolber PC, Fraser MO. Diabetic urethropathy compounds the effects of diabetic cystopathy. J Urol. 2007 Nov; 178(5):2213-219.
- Sorge RE, et al., Nature Methods 11: 629-632 (2014)
- Yoshiyama M, et al., American Journal of Physiology Renal Physiology 304: F390-F396 (2013)
- Yoshiyama M, et al., European Journal of Pharmacology 264: 417-425 (1994)
- Smith PP, Hurtado E, Smith CP, Boone TB, Somogyi GT. Comparison of cystometric methods in female rats. Neurourol Urodyn 2008;27:324-9.
- Smith PP, Kuchel GA. Continuous uroflow cystometry in the urethane-anesthetized mouse. Neurourol Urodyn 2010;29:1344-9

Matthew Fraser

Recent critiques of the use of urodynamics in animals for the preclinical development of therapeutics directed toward lower urinary tract dysfunctions have included claims that such studies are not translatable. Unfortunately, as performed and interpreted for decades in the majority of published reports, this is largely a valid critique - but generally not for the reasons that the critics believe. Rather, the issue of translatability stems more from the fact that classical physiological principles have not been embraced and included in the design of experiments or the interpretation of the results. In this session, classical physiological concepts will be applied to the measurement of lower urinary tract behavior during cystometric evaluation, and compared to those that currently drive research design and interpretation. Methodological and model specific considerations will be discussed in the context of the information that may be gained and that which may not be gained from different approaches. Common misconceptions and misinterpretations will be described. Additionally, novel insights in LUT physiology will be described and their impact on interpretation of urodynamic results discussed. Attendees of this workshop will be further empowered to properly interpret the basic science results published from any laboratory in the context of their scientific and clinical interests.

Phillip Smith

Multichannel Urodynamics in Rodents Urodynamic assessment includes measurements of urine storage volumes and pressure, and voiding expulsive pressures, volumes and flow rates. Clinical urodynamics distinguishes pressures attributable to the bladder wall (e.g. detrusor contraction) from those due to extrinsic pressures transmitted across the bladder wall (e.g. intra-abdominal abdominal pressure). Urine collection methods allow clinically sufficient precision to determine voided volumes and flow rates. Rat and Mouse urinary performance differ from human physiology in several important ways, including increasing pressure with filling, a necessity of abdominal wall contraction during voiding, and small voided volumes. For a full urodynamic assessment of Rat and Mouse lower urinary tract performance, techniques of multi-channel pressure/flow urodynamics and their interpretations must be adapted to these rodent systems. Some suggestions about how to address these concerns will be presented and discussion will be encouraged.

Mitsuharu Yoshiyama

Pros and Cons of Anesthetized, Conscious and Decerebrate Preparations Urodynamic studies in animals are commonly performed under either anesthetized or conscious conditions. Each of these preparations provides us with a different experimental state, which either represents reflex activity (anesthetized) or a behavioral response (conscious). This, by itself, is an important consideration regarding the suitability of these approaches for urodynamic study. Moreover, any anesthetic, as a neuroactive chemical, is likely to interfere/interact with both the normal physiology and the effects of any therapeutic approach (drug or device) that may be tested during an experiment. Awake animals, on the other hand, are easily affected by ambient circumstances and even individual experimenters. An alternative approach, precollicular decerebration (performed under inhaled anesthesia from which recovery is rapid), in which the reflex micturition circuit is preserved, can also be employed. This approach allows us to evaluate the reflex activity of an animal under unanesthetized conditions. This workshop will comprehensively discuss the pros and cons of anesthetized, conscious, and decerebrate unanesthetized animal preparations. Furthermore, expertise of the decerebration technique will be shared with all participants, so that they may add this approach to their preclinical urodynamic repertoire.

Division of Urology, Department of Surgery, Duke University Medical Center
Institute for Medical Research, Durham Veteran's Affairs Medical Center

Lower Urinary Tract Physiology and Considerations for Urodynamic Study

Matthew O. Fraser, Ph.D.

W7 Preclinical Urodynamics - Optimisation of Techniques, Measurements and Interpretation

ICS 2016, Tokyo, Japan
September 13, 2016

ICS

Affiliations to disclose:

Grants – Astellas, Medtronic, Pfizer
Consulting – Synergy Pharma, InVivo Pharma
SAB – Amphora Medical

Funding for speaker to attend:

- [X] **Self-funded**
- [ ] **Institution (non-industry) funded**
- [ ] **Sponsored by:**

Outline

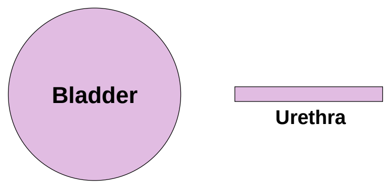
- **Functional Anatomy of the Lower Urinary Tract**
  - Gross Anatomy
  - Smooth Muscle Layers
  - Functional Compartmentalization
  - Neural Control
  - Non-neuronal Interactions
- **Cystometric Measurement of the Lower Urinary Tract**
  - The Micturition Cycle
  - Open Cystometry
  - Closed Outlet
- **Conclusions**

Functional Anatomy of the Lower Urinary Tract

Gross Anatomy

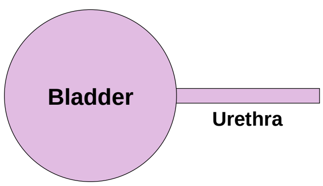
A Simplified Approach

- Lower Urinary Tract Anatomy – Sphere and tube models



Functional Anatomy of the Lower Urinary Tract

Gross Anatomy

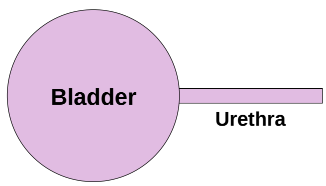


Many mathematical models assumed isotropy, homogeneity and incompressibility of bladder (and urethral) smooth muscle materials.

Many also treat the bladders as spheres and the urethras as tubes. It allows for simple mathematical models to attempt description of observed responses.

Functional Anatomy of the Lower Urinary Tract

Gross Anatomy

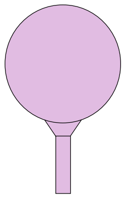


As with all other aspects of life, however, nothing is ever as simple as we might like.

The Lower Urinary Tract is no exception …

Functional Anatomy of the Lower Urinary Tract
Smooth Muscle Layers

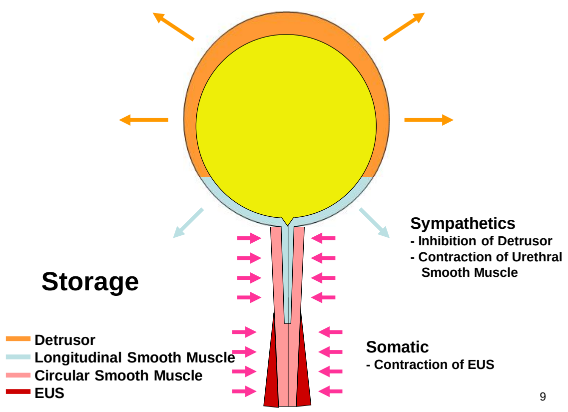
- **Bladder Smooth Muscle Anatomy**
  - Variably Defined as having 1 layer with intermeshed multi-oriented muscle fibers to 3 somewhat defined layers (inner + outer longitudinal and middle circular)
    - Depends on species, investigator and region examined



  - Many agree that orientations become more distinct as approach the urethra, especially the longitudinal smooth muscle systems
  - Further, that the inner longitudinal layer continues into the urethra – 1 organ, not 2!
    - Described as extending to mid-urethra or even more posterior
    - Not 2 organs, but 1 – the **Vesicourethral muscularis**

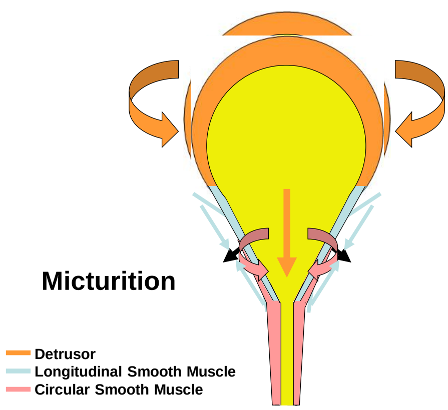
Functional Anatomy of the Lower Urinary Tract
Functional Compartmentalization

- **Bladder Smooth Muscle Anatomy**
  - The bladder demonstrates functional compartmentalization depending on the role at the time.
    - **During filling, it is at least a 2 compartment system, the bladder base and dome (open lumen) and the urethra (closed lumen)**

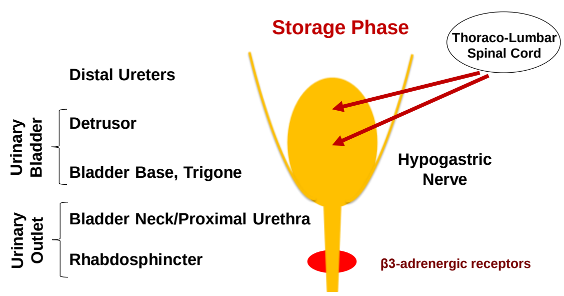


Functional Anatomy of the Lower Urinary Tract
Functional Compartmentalization

- **Bladder Smooth Muscle Anatomy**
  - The bladder demonstrates functional compartmentalization depending on the role at the time.
    - **During filling, it is at least a 2 compartment system, the bladder base and dome (open lumen) and the urethra (closed lumen)**
    - **Upon micturition, it becomes a 1 compartment system with a single lumen**



Functional Anatomy of the Lower Urinary Tract
Neural Control



Functional Anatomy of the Lower Urinary Tract
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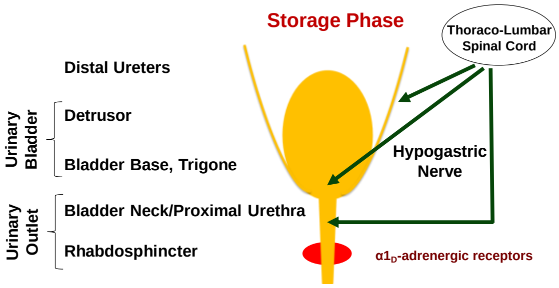
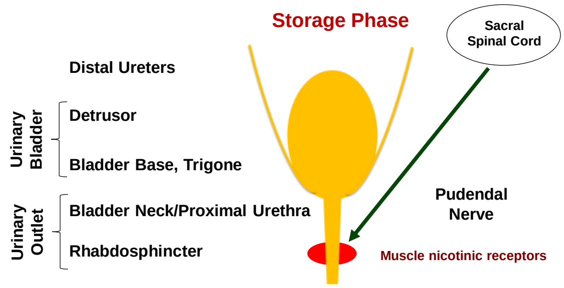


Functional Anatomy of the Lower Urinary Tract
Neural Control



Functional Anatomy of the Lower Urinary Tract
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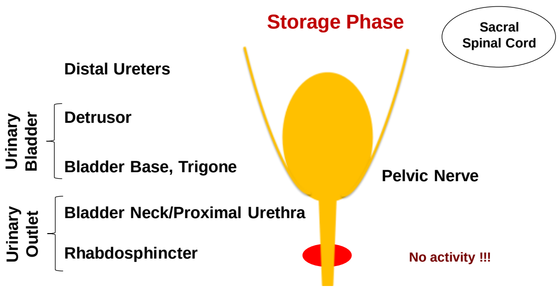
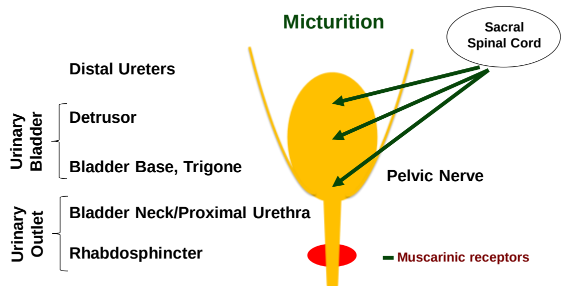


Functional Anatomy of the Lower Urinary Tract
Neural Control



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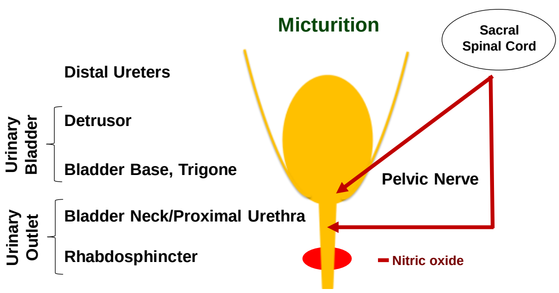
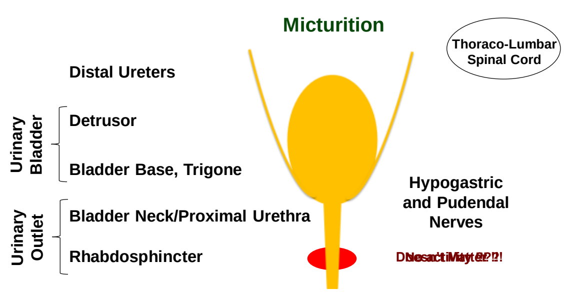
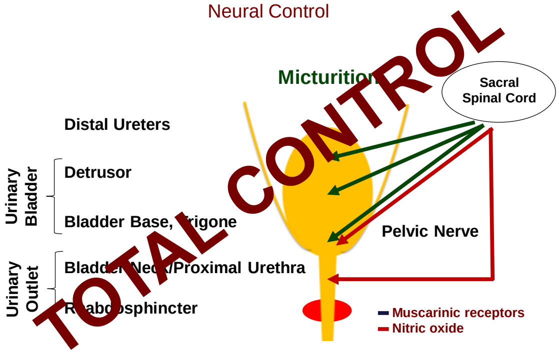


Functional Anatomy of the Lower Urinary Tract
Neural Control



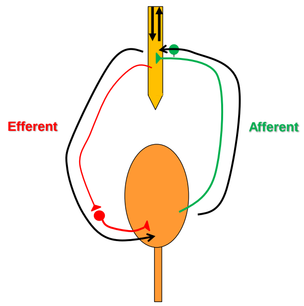
Functional Anatomy of the Lower Urinary Tract
Neural Control



Functional Anatomy of the Lower Urinary Tract
Non-neuronal Interactions

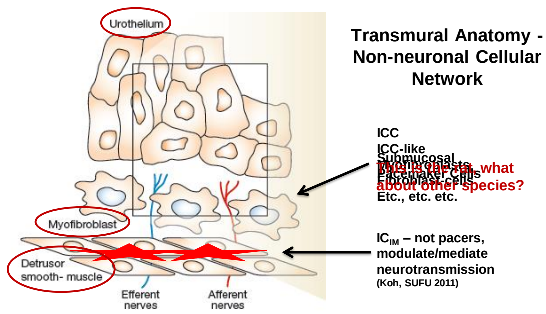
- As with all things, nothing is that easy. Other important factors include
  - Extracellular matrix
  - Non-neural signaling cells
  - Interactions of the whole with local and distant neuronal circuitry

Functional Anatomy of the Lower Urinary Tract
Non-neuronal Interactions



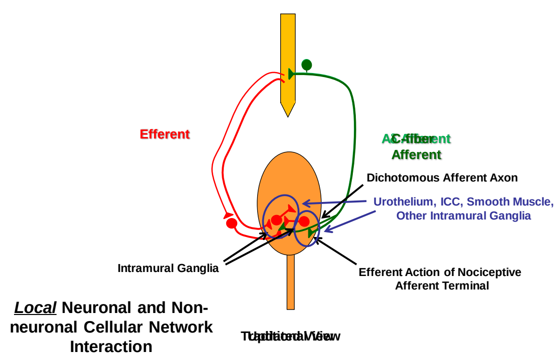
Traditional View

Functional Anatomy of the Lower Urinary Tract
Non-neuronal Interactions



Birder and de Groat. 2007. Nat Clin Pract Urol. 4:46-54.

Functional Anatomy of the Lower Urinary Tract
Non-neuronal Interactions



Outline

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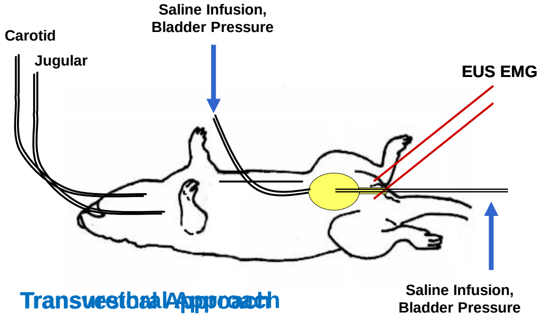
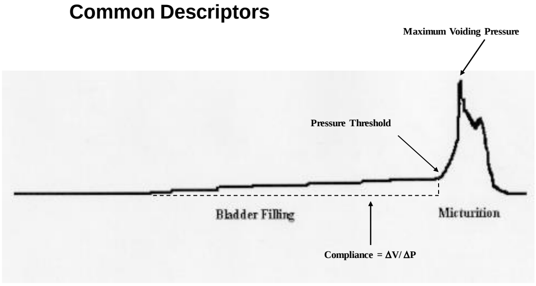
Open Cystometry Protocol



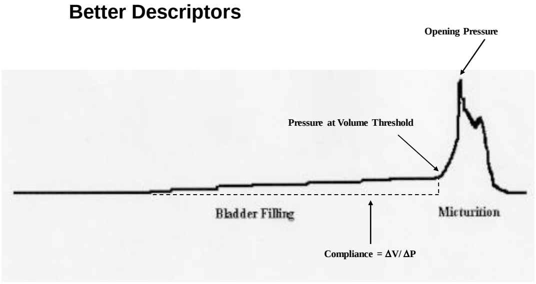
Cystometry - The Micturition Cycle

Common Descriptors

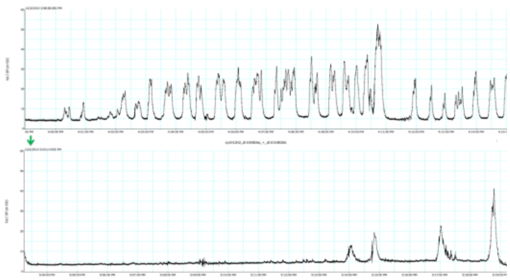


Cystometry - The Micturition Cycle

Better Descriptors

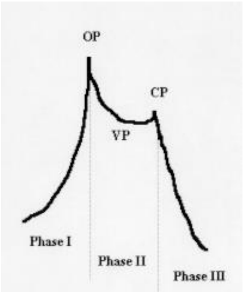


Where is Pressure Threshold?



Cystometric traces during conscious, restrained cystometry in a chronic SCI rat – The top trace is from the vehicle control period, while the bottom trace is from the period following 100 µg/kg of CL-316,243.

What is Maximal Voiding Pressure?



Conclusions about the actual voiding contraction are not so straightforward.

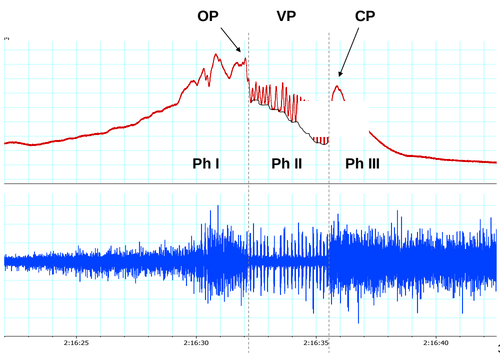
Need to understand the anatomy of the voiding contraction:

<u>Phase I</u> – Isovolumetric Contraction
<u>Phase II</u> – Entire LUT open to external environment during peak detrusor contraction
<u>Phase III</u> – Isovolumetric Relaxation

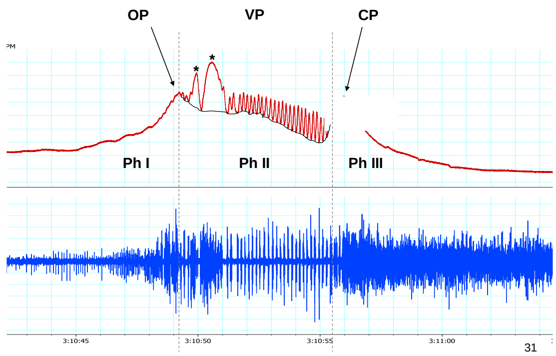
Pressure-Flow relationships can be explored

Different Phases first defined by Maggi et al, 1986

Easy Bladder Contraction



Ambiguous Bladder Contraction – Tonic EUS gives False OP*



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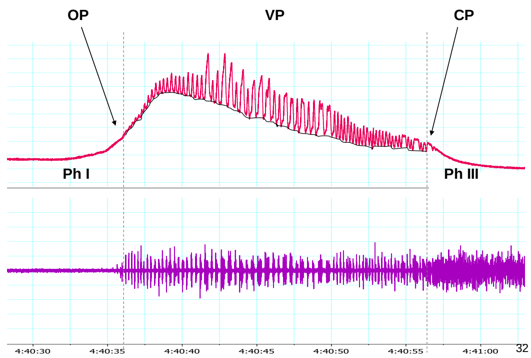
Ambiguous Bladder Contraction – "Missing" OP



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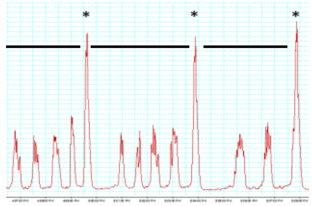
What is Bladder Capacity

Continuous vs. Single Fill Cystometry



- **Continuous open cystometry is the current method of choice by many researchers**
- **Allows for the determination of functional bladder capacity (FBC), as defined as infusion flow rate x ICI or IMI**
- **However, it often underestimates true bladder capacity (TBC), which is best determined by single fill cystometrograms**
- **By combining the approaches, as shown above, one can determine voiding efficiency easily by the equation: %VE = mean FBC/TBC x 100**

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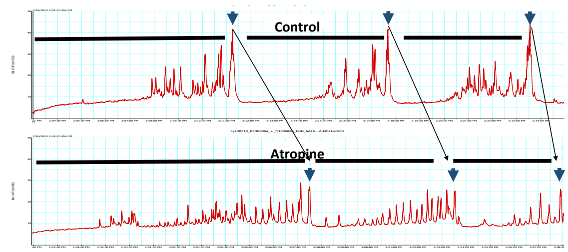
Response to Drugs



- **FBC decreases with atropine**
- **TBC increases !!! Decreased FBC due to decreased voiding efficiency.**

If had only performed continuous open cystometry, might misinterpret effect as mild irritation or sensitization reflex voiding !!!

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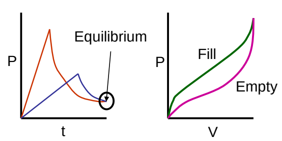
Response of the Bladder to Filling: Biomechanical Considerations

- **Rate dependency** – slow strain causes lesser increase in force than fast strain – or - rapid filling results in decreased compliance
- **Time dependency** – It takes longer to reach equilibrium pressure if strain is faster
- **Hysteresis** – the pressure-volume relationship (force curve) is different – Viscoelasticity!



Flow rate affects the compliance measurements!

35

Coolsaet 1985

Response of the Bladder to Filling:

Measurement System Considerations

- Flow rates matter not only to tissue biomechanics, but also to recordings
  - Resistance of the filling and recording catheter affects the pressure baseline as well as the fidelity of recording during filling
  - Effects become worse with increased fill rate

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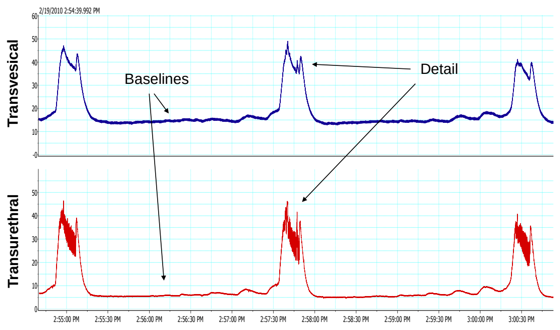
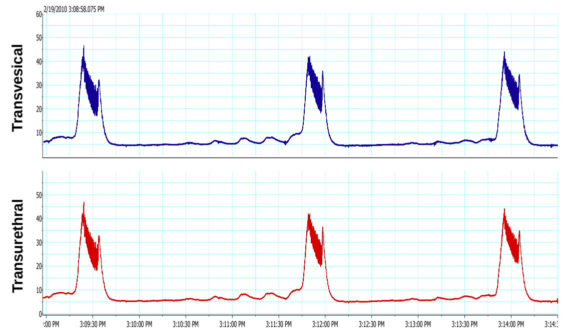
Transvesical Filling



Transureteral Filling



Response of the Bladder to Filling:
Measurement System Considerations

- Placement of catheters may affect dynamic active measurements
  - The top-down contraction of the dome may occlude the catheter tip in transvesical filling and recording

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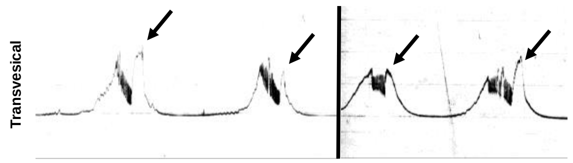
Transvesical Filling

Traces are from transvesical double-lumen catheters with a static internal lumen for pressure recording.



Arrows Point to Apparent Closing Pressures

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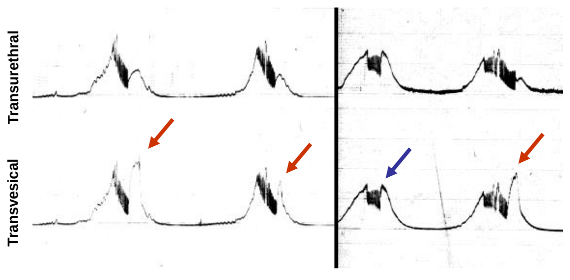
Transvesical Filling – False CP

False closing pressures (red arrows) may be due to bladder contraction from top-down, creating transient seal around transvesical filling/recording catheter tip



Cystometric Evaluation
of Lower Urinary Tract Function

- Cystometric techniques in animals
  - Closed outlet cystometry
    - Traditional
      - Single filling cystometrograms
      - Isovolumetric recordings
      - Combined closed methods (closed outlet single fill cystometrogram to trigger volumes followed by isovolumetric)
    - Simultaneous bladder and urethral recording
      - Open cystometry with urethral pressure measurement
      - Isolated bladder-urethra preparations
        - » Closed cystometry
        - » Open cystometry with vent catheter

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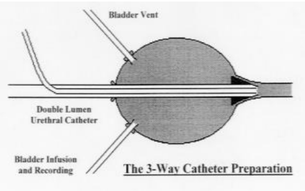
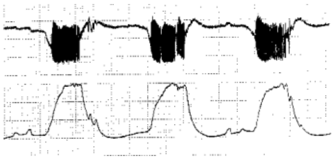
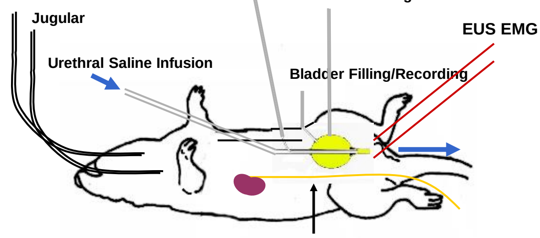
Simultaneous Isolated Bladder and Urethra



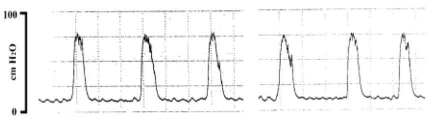
- Fraser MO, Flood HD, de Groat WC, 1995, *Journal of Urology*, 153: 461A.
- Jung SY, Fraser MO, et al., 1999, *Journal of Urology*, 162: 204.
- Kakizaki H, Fraser MO, de Groat WC, 1997, *American Journal of Physiology*, 272: R1647.



Rat UPP (3-Way System)

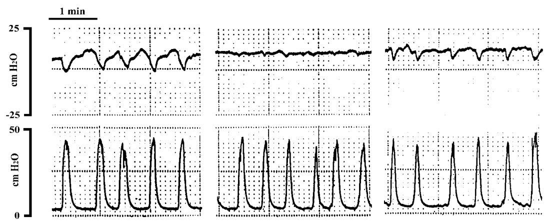


Isovolumetric IVP and UPP



- **Allows for pharmacological dissection of Active State players in the physiology of LUT function – External Urethral Sphincter contribution**
- **Note no change in the dynamic active responses of the bladder to isovolumetric conditions (constant volume distension)**

NO-Mediated Relaxation



- **Allows for pharmacological dissection of active players in the physiology of LUT function – Parasympathetic NO relaxation of urethral smooth muscle.**
- **Note no change in the dynamic active responses of the bladder to isovolumetric conditions (constant volume distension)**

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Conclusions

- LUT anatomy is not as simple as a sphere and tube
- Many of the measurements used in the literature are either incorrect or less than optimal
- Studying physiologically isolated components of the LUT provides a better understanding of the effects of treatments or disease

End

Preclinical Urodynamics: Multichannel Urodynamics in Rodents

ICS 2016 TOKYO

Phillip P. Smith MD

Associate Professor of Surgery
Research Associate, Center on Aging
Associate, CT Institute on Brain and Cognitive Science

Presented by

George A. Kuchel MD

Professor of Medicine
Citicorp Chair in Geriatrics and Gerontology
Director, UConn Center on Aging
Chief, Division of Geriatric Medicine

University of Connecticut College of Medicine
Farmington CT USA

George A. Kuchel (for Phillip P. Smith)

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Affiliations to disclose†:

None for author

None for presenter/speaker

† All financial ties (over the last year) that you may have with any business organisation with respect to the subjects mentioned during your presentation

Funding for speaker to attend:

- [X] Self-funded
- [ ] Institution (non-industry) funded
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CYSTOMETRY

Functional assessment of

Bladder

- Store urine
- Generate expulsive pressure
- Signal content to CNS

And/or

Urethra

- Closure characteristics
- Distensibility/flow characteristics

GOALS

Fundamental functional features of LUT are:

- urine storage
- urine expulsion
- generation of sensory information about content

Tools to measure:

- Pressures
- Volumes
- Flow rates (dP/dt)
- (EMG, nerve recordings, brain imaging, fluoro)

PRINCIPLES

Pressure
- total intravesical
- detrusor-generated

Volumes
- voided volume
- post-void residual volume

Flow rate
- average vs peak
- pattern

PRINCIPLES

- Transduction:
  - Fluid transmission of visceral pressure to transducer
  - Weighing voided volumes
- Amplification, filtration
- Digitization
- Data acquisition, storage, and display

HUMAN CYSTOMETRY
Urodynamics

- Goals:
  1. Ensure low pressure urine storage
  2. Characterize size and extent of reservoir
  3. Assess emptying function
  4. Locate (as possible) control deficits

HUMAN CYSTOMETRY
Urodynamics

- Pre-built commercial “kits”
- Large-diameter tubing remains small compared to bladder volume
- Separate ports/channels for infusion and pressure measurement
  - Allows use of peristaltic pumps
- Integrated electronics with little adjustability

HUMANS vs. RODENTS

- Rodents do not report sensations *(modelling OAB and UAB in rodents make no sense).*
- Catheterization
- Anesthesia/sedation
- Tubing size – large compared to bladder
- Infusion rates (and pumps)
- Voiding mechanism
- Tension vs. Pressure ($T = PR/2$)

RODENT CYSTOMETRY

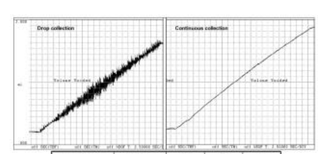
- Data that Can Be Obtained
  - Pressures
  - Volumes
  - Flows
  - EMG
  - Afferent/efferent nerve recordings
  - Estimates of system sensitivity
  - Sphincteric adequacy (maybe)
- Data that Cannot Obtained
  - Sensations
  - Human-like stress-testing

RODENT CMG -Catheters

- PE 10 – PE 60
  - Stiffness
  - Damping of signal
    - Length
    - Diameter
- Placement
  - Trans-bladder
  - Trans-urethral

RODENT CMG – Urine Collection

- When is it needed?
- The Drop Problem
- Our Solution



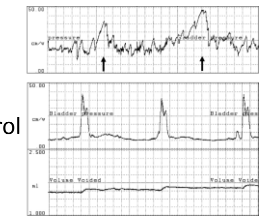
nominal pump rate	ml/hr	80	100	120
	ml/sec	*0.022*	*0.028*	*0.033*
drop collection, ml/sec		0.0213	0.0266	0.0322
error vs pump		*-4.1%*	*-4.2%*	*-3.4%*
continuous collection, ml/sec		0.0217	0.0274	0.0321
error vs pump		*-2.3%*	*-1.4%*	*-3.7%*

RODENT CMG: Electronics

- Transducers
  - Pressure
  - Volume (weight)
- Amplifier / filters
- Digitization
  - Sampling rate – pressure
  - Sampling rate – volume (min 30 Hz)
  - Sampling rate – EMG (typical 4000 Hz)
- Data Acquisition/display/storage

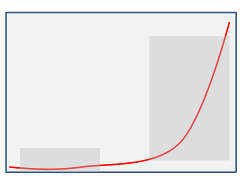
Rodent CMG: Conduct

- Anesthesia
- Surgery
- Positioning
- Run-in and quality control
- PVR measurement
- Data acquisition
- terminate

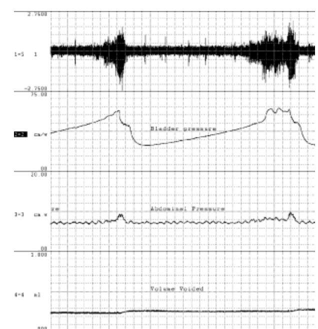


RODENT CMG: Conduct / PVR

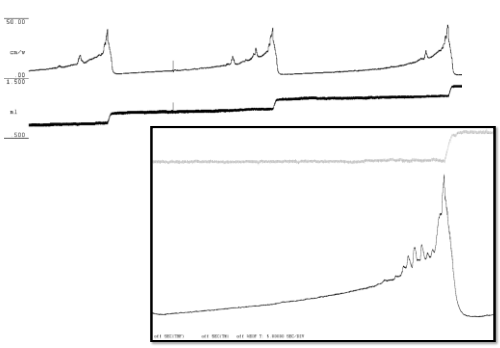
- PVR
- Non-linear pressure/volume filling (compliance) means operational volume range contributes to “compliance” measure
- Post-void suction
- Out vs. In
- Calculated models



Multichannel Rodent CMG: Output



RODENT CMG: Analysis



RODENT CMG: Analysis

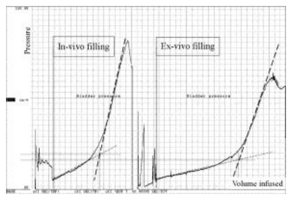
Proposed minimum analysis

- Pressure:
  - Basal
  - Voiding Threshold
  - Peak pre-flow
  - (estimated pre-flow peak compliance pressure)
  - End flow
  - (estimated end-flow peak compliance pressure)
- Volume:
  - Voiding Threshold
  - Voided Volume
  - Estimated/measured PVR

RODENT CMG: Analysis

Proposed minimal analysis

- Intercontraction interval
- Compliance (1/stiffness)
  - First 10%ile
  - Last 10%ile
  - Curve modelling

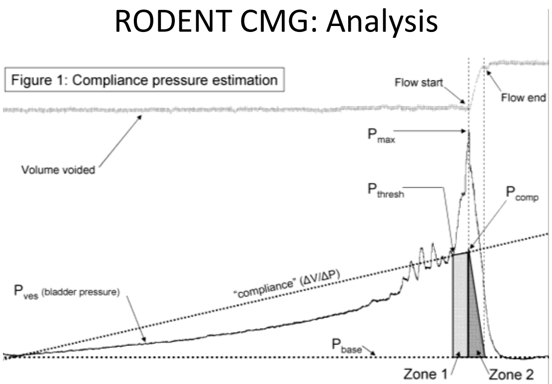


RODENT CMG: Analysis

Voiding analysis

- Pressure
  - Isovolumetric
  - Pressure vs. flow rate curves
- Area under pressure curve
  - Accounting for Compliance
  - Total curve vs. flow only
- Work
  - Measure of force x volume voided
- Power
  - Measure of force x voiding rate

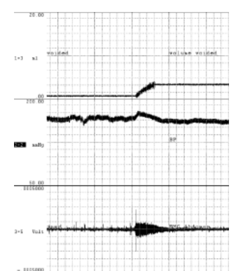
RODENT CMG: Analysis



RODENT CMG: Analysis

Analyses of potential interest

- NonVoiding Contractions
  - Frequency
  - Amplitude over compliance curve
- IPHFO
  - Frequency
  - Amplitude
- Pseudoaffective responses
- Power Spectral Analysis



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Smith PP, Deangelis AM, Kuchel GA. Evidence of central modulation of bladder compliance during filling phase. Neurourol Urodyn. 2012 Jan;31(1):30-5. doi: 10.1002/nau.21223. Epub 2011 Oct 28. PubMed PMID: 22038779; PubMed Central PMCID: PMC3265612.

Smith PP, Kuchel GA. Continuous uroflow cystometry in the urethane-anesthetized mouse. Neurourol Urodyn. 2010 Sep;29(7):1344-9. doi: 10.1002/nau.20850. PubMed PMID: 20127833; PubMed Central PMCID: PMC2892555.

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Smith PP, Smith CP, Boone TB, Somogyi GT. Is abdominal wall contraction important for normal voiding in the female rat? BMC Urol. 2007 Mar 7;7:5. PubMed PMID: 17343732; PubMed Central PMCID: PMC1831476.

Mitsuharu Yoshiyama, MD, PhD

ICS 2016 TOKYO

Affiliations to disclose†:

None

† All financial ties (over the last year) that you may have with any business organisation with respect to the subjects mentioned during your presentation

Funding for speaker to attend:

- [X] Self-funded
- [ ] Institution (non-industry) funded
- [ ] Sponsored by:

ICS 2016, Tokyo
Tokyo International Forum
September 13th, 2016

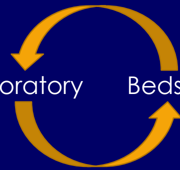
W7: Preclinical Urodynamics – Optimization of Techniques, Measures and Interpretation

Pros and Cons of Anesthetized, Conscious and Decerebrate Preparations

Mitsuharu Yoshiyama, MD, PhD
Department of Urology
University of Yamanashi Graduate School of Medical Science

Introduction

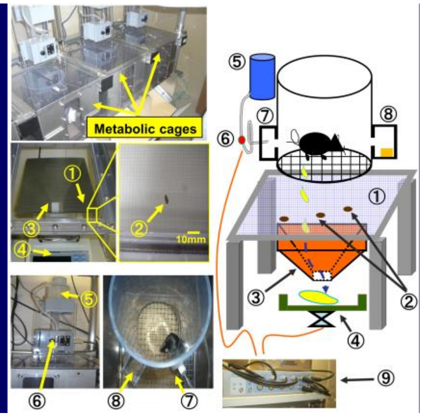
- Animals = Humans or Animals ≠ Humans
- Impacts of species difference, experimental design, and data analysis/interpretation



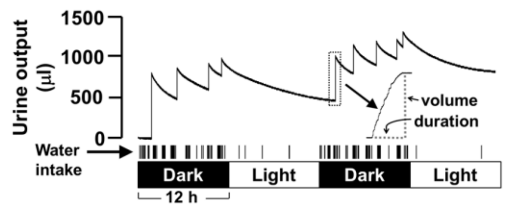
Frequency-Volume Chart (FVC)

Time	Input (ml)	Output (ml)	Leak	Urgency
6:00				
7:00		55		✓
8:00				
9:00			✓	✓
10:00	200			
11:00		220		
. . .				

Metabolic Cage (MC)



Metabolic Cage (MC)



Time (xx:yy)	Input (µl)	Output (µl)	Frequency (voids/day)	Voiding duration (s)	Flow rate (µl/s)	Leak	Urgency
✓	✓	✓	✓	✓	✓		

Human FVC vs. Rodent MC

	Time	Input	Output	Leak	Urgency	Frequency	Flow rate
Human	✓	✓	✓	✓	✓	✓	
Rodent	✓	✓	✓	?		✓	✓

Flow rate (Human) → Uroflowmetry
Urgency (Rodent) → ?

Post-void residual volume → Echographic examination
Intravesical pressure change → Cystometry

Urodynamic evaluation

- Intravesical pressure change (filling and voiding)
- First desire to void
- Normal desire to void
- Strong desire to void
- Uroflow rate

Cystometry - Human vs. Rodent

	PT	MVP	BCD	BCP	VV	RV	VT	VE	NVC
Human	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rodent	✓	✓	✓	✓	✓	✓	✓	✓	✓

First desire to void
Normal desire to void
Strong desire to void

= ?
≠ ?

VT / Bladder capacity

PT, pressure threshold; MVP, maximal voiding pressure; BCD, bladder contraction duration; BCP, bladder compliance; VV, voided volume; RV, post-void residual volume; VT, volume threshold; VE, voiding efficiency; NVC, non-voiding contraction

Animal conditions during cystometry

- Awake
- Anesthetized
- Decerebrate, unanesthetized

Awake

Pros
- Little influence of anesthesia

Cons
- Great influence from circumstances
- Emotional changes
- Hard to handle an animal and catheters during experiment

Rodents feel more stress when experimenters are males?!

Olfactory exposure to males, including men, causes stress and related analgesia in rodents

Robert E Sorge[1,2,8], Loren J Martin[1,8], Kelsey A Isbester[1], Susana G Sotocinal[1], Sarah Rosen[1], Alexander H Tuttle[1], Jeffrey S Wieskopf[1], Erinn L Acland[1], Anastassia Dokova[1], Basil Kadoura[1], Philip Leger[1], Josiane C S Mapplebeck[1], Martina McPhail[3], Ada Delaney[4], Gustaf Wigerblad[4], Alan P Schumann[2], Tammie Quinn[2], Johannes Frasnelli[5,6], Camilla I Svensson[4], Wendy F Sternberg[3] & Jeffrey S Mogil[1,7]

Sorge RE et al. Nature Methods 11: 629-632 (2014)

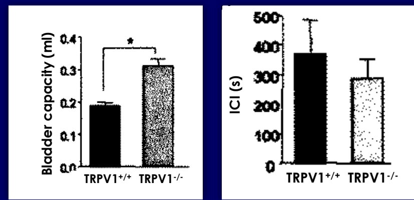
Anesthetized

Pros
•Easy to handle an animal and catheters during experiment
•No influence of emotion
•Little influence from circumstances
•Extensity in experimental design (e.g., abdomen opened, route of drug injection)

Cons
•Pharmacological and physiological interference

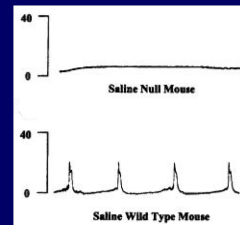
Influence of urethane - TRPV1 -

Urethane-anesthetized *Awake*



Birder *et al., Nat Neurosci* 5: 856-860 (2002)

Influence of urethane - Tachykinins -

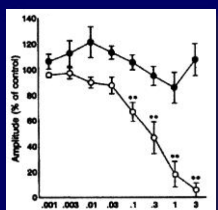


NK2-KO

WT

Kiss S, Yoshiyama M, et al., *Neurosci Lett* 313: 57-60 (2001)

Influence of urethane - NMDA glutamatergic antagonist -



MK-801 (mg/kg i.v.)

Decerebrate -unanesthetized

Urethane -anesthetized

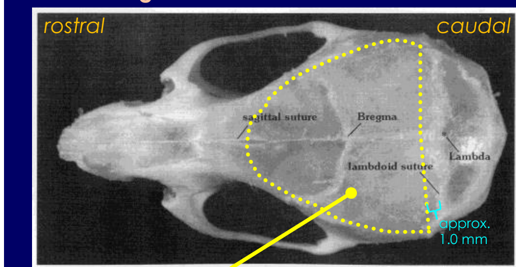
Yoshiyama M, et al. *Eur J Pharmacol* 264: 417-425 (1994)

Decerebrate, unanesthetized

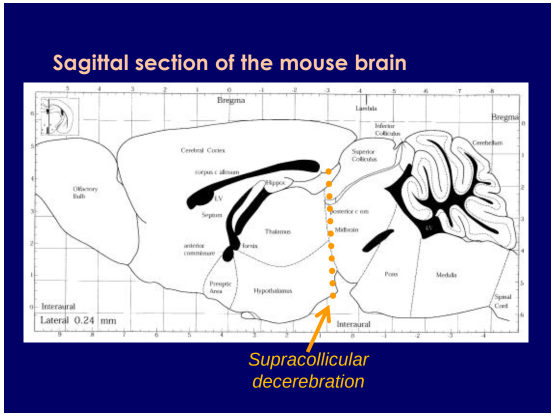
Pros
•Little influence of anesthesia
•Little influence from circumstances
•Easy to handle an animal and catheters during experiment
•No influence of emotion
•Extensity in experimental design (e.g., abdomen opened, route of drug injection)

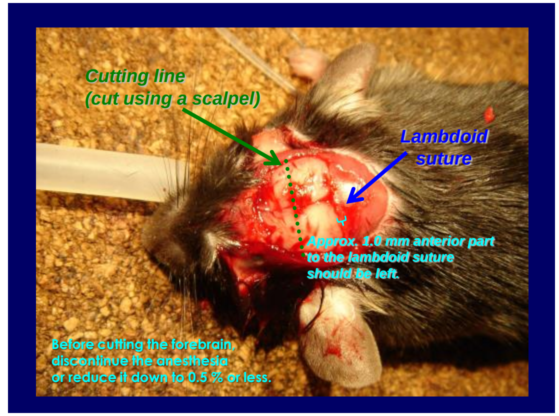
Cons
•Long-term training and experience
•Longer time for surgery

Skull diagram



Remove this part of the skull


Sagittal section of the mouse brain
Supracollicular
decerebration


Cutting line
(cut using a scalpel)
Lambdoid
suture
Approx. 1.0 mm anterior part
to the lambdoid suture
should be left.
Before cutting the forebrain,
discontinue the anesthesia
or reduce it down to 0.5 % or less.


Flip the remaining cortex over
to expose the brainstem


Cutting line
Superior colliculus

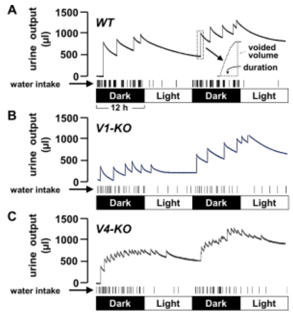
Cystometry
in Decerebrate, Unanesthetized Mouse

Dual Analysis of
Voiding Behavior (i.e., Metabolic Cage)
and
Reflex Micturition (i.e., Cystometry)

Yoshiyama M et al. *Am J Physiol Renal Physiol* (2015)

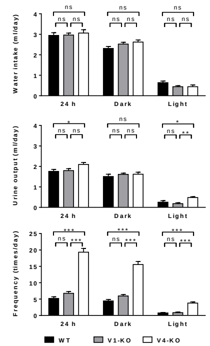
TRP channel
-deficient
mouse model

Metabolic cage



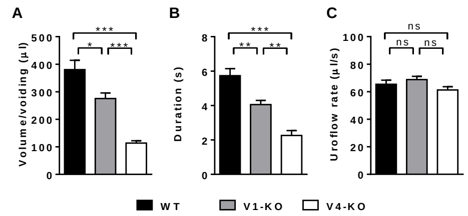
Yoshiyama M et al. Am J Physiol Renal Physiol (2015)

Metabolic cage



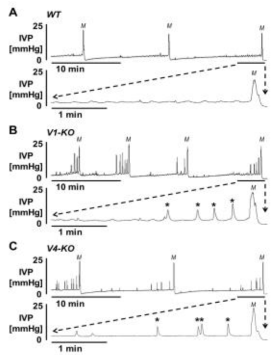
Yoshiyama M et al. Am J Physiol Renal Physiol (2015)

Metabolic cage



Yoshiyama M et al. Am J Physiol Renal Physiol (2015)

Cystometry



Yoshiyama M et al. Am J Physiol Renal Physiol (2015)

Cystometry

Comparisons between WT, TRPV1-KO and TRPV4-KO

	Pressure threshold (mmHg)	Maximal voiding pressure (mmHg)	Bladder compliance (μl/mmHg)	Voided volume (μl)	Post-void residual (μl)	Bladder capacity (μl)	Voiding efficiency (%)
WT	3.8 ± 0.4	23.0 ± 2.2	35.2 ± 2.3	131 ± 11	13.6 ± 3.3	144 ± 10	90 ± 2
V1-KO	3.4 ± 0.3	21.9 ± 2.1	28.2 ± 5.1	118 ± 27	17.8 ± 3.6	136 ± 27	83 ± 4
V4-KO	3.0 ± 0.4	20.3 ± 1.2	43.5 ± 9.6	135 ± 23	12.4 ± 3.6	148 ± 23	91 ± 2

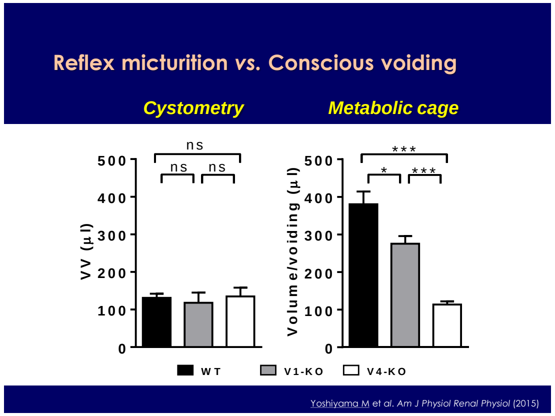
Yoshiyama M et al. Am J Physiol Renal Physiol (2015)

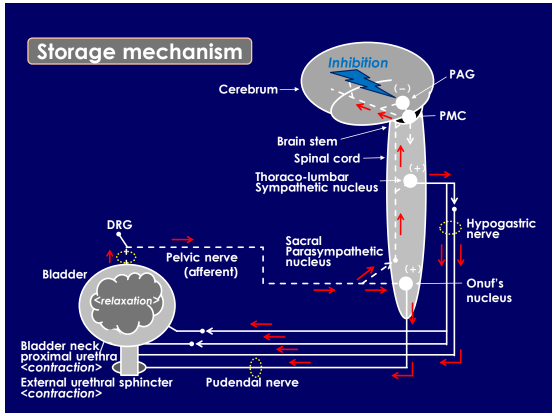
Cystometry

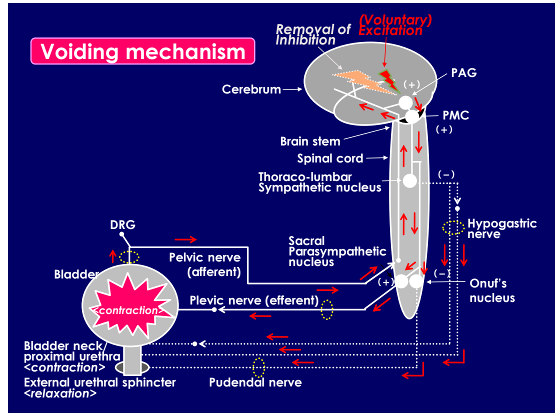
Comparison in non-voiding contractions

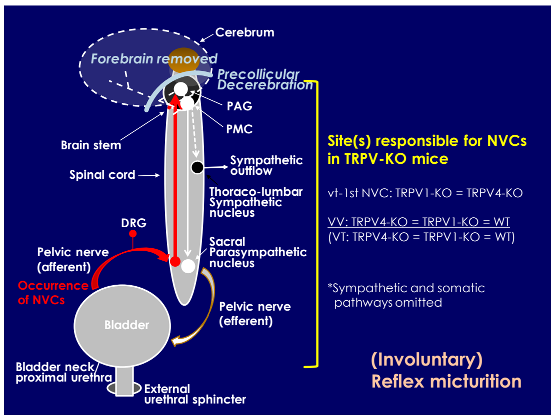
	pt-1st NVC (mmHg)	vt-1st NVC (μl)	peak pressure of 1st NVC (mmHg)	mean peak pressure of NVCs (mmHg)	number of NVCs/micturition cycle
TRPV1-KO	3.3 ± 0.5	98.1 ± 17.1	11.3 ± 0.7	14.8 ± 1.5	3.5 ± 0.9
TRPV4-KO	2.8 ± 0.2	106.6 ± 10.4	12.8 ± 0.9	13.4 ± 0.7	2.8 ± 0.7

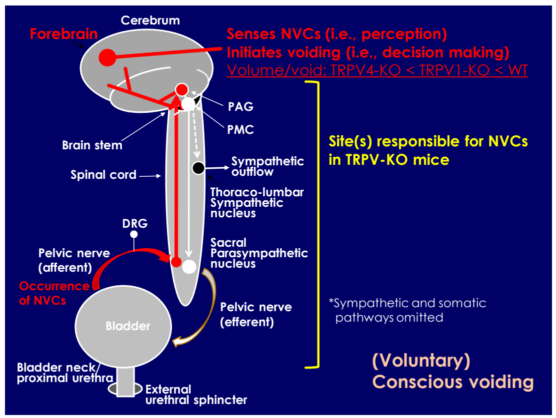
Yoshiyama M et al. Am J Physiol Renal Physiol (2015)











Data Interpretations
- Function of TRPV1 and TRPV4 -

Decerebrate CMG

- Brainstem, spinal cord, dorsal root ganglion, and/or bladder
- Stabilizing the bladder during filling

MC + CMG

- Forebrain
- Influence on decision-making in the timing of urine release

Pflugers Arch - Eur J Physiol (2016) 468:1573–1585
DOI 10.1007/s00424-016-1863-0

MUSCLE PHYSIOLOGY

Functional coupling of TRPV4 channels and BK channels in regulating spontaneous contractions of the guinea pig urinary bladder

Ayu Isogai[1] · Ken Lee[1,2] · Retsu Mitsui[1] · Hikaru Hashitani[1]

Isogai A et al. Pflugers Arch – Eur J Physiol 468: 1573-1585 (2016)

TRPV4 is functionally coupled with BK channels to stabilize the detrusor smooth muscle excitability upon bladder-filling.

Animal conditions during cystometry

	Awake	Anesthetized	Decerebrate
	Voluntary voiding	**Reflex micturition**	
Pros	•Little influence of anesthesia	•Easy to handle an animal and catheters during experiment •No influence of emotion •Little influence from circumstances •Extensity in experiment design	•Little influence of anesthesia •Little influence from circumstances •Easy to handle an animal and catheters during experiment •No influence of emotion •Extensity in experiment design
Cons	•Influence from circumstances •Emotion changes •Hard to handle an animal and catheters during experiment	•Pharmacological and physiological intervention	•Long-term training and experience •Longer time for surgery

Intact "micturition reflex" pathway

Conclusions

- Designing experiments with knowledge of the pros and cons of each animal model and interpreting the results considering them carefully
- Conducting multiple types of different experimental models and integrating the results at the interpretation