W7: Preclinical Urodynamics - Optimisation of Techniques, Measurements and Interpretation
Workshop Chair: Matthew O. Fraser, United States
13 September 2016 11:00 - 12:30

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

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

A Simplified Approach

- Lower Urinary Tract Anatomy – Sphere and tube models

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.

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

The Lower Urinary Tract is no exception ...

Affiliations to disclose:
Grants – Astellas, Medtronic, Pfizer
Consulting – Synergy Pharma, InVivo Pharma
SAB – Amphora Medical

Funding for speaker to attend:
- Self-funded
- Institution (non-industry) funded
- Sponsored

by:
**Functional Anatomy of the Lower Urinary Tract**

**Smooth Muscle Layers**

- **Bladder Smooth Muscle Anatomy**
  - Varially 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

**Detrusor**

- **Longitudinal Smooth Muscle**
- **Circular Smooth Muscle**

**Sympathetics**

- Inhibition of Detrusor
- Contraction of Urethral Smooth Muscle

**Somatic**

- Contraction of EUS

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**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

**Storage Phase**

- Distal Ureters
- Detrusor
- Bladder Base, Trigone
- Bladder Neck/Proximal Urethra
- Rhabdosphincter

**Storage**

- Thoro-Lumbal Spinal Cord
- Hypogastric Nerve

**Micturition**

- Detrusor
- Longitudinal Smooth Muscle
- Circular Smooth Muscle

**Neural Control**

- β3-adrenergic receptors
Functional Anatomy of the Lower Urinary Tract

**Neural Control**

**Storage Phase**

- Distal Ureters
- Detrusor
- Bladder Base, Trigone
- Bladder Neck/Proximal Urethra
- Rhabdosphincter

- Hypogastric Nerve
- α1-adrenergic receptors

**Sacral Spinal Cord**

**Thoraco-Lumbar Spinal Cord**

**Muscle nicotinic receptors**

**Muscarinic receptors**

**No activity ???**

**Micturition**

- Distal Ureters
- Detrusor
- Bladder Base, Trigone
- Bladder Neck/Proximal Urethra
- Rhabdosphincter

- Pelvic Nerve
- Nitric oxide

- Muscarinic receptors

- Doesn't Matter !!!

No activity ???
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

Open Cystometry Protocol

Cystometry - The Micturition Cycle

Common Descriptors

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.

Where is Pressure Threshold?

What is Maximal Voiding Pressure?

Conclusions about the actual voiding contraction are not so straightforward.

Need to understand the anatomy of the voiding contraction:

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

Pressure-Flow relationships can be explored

Different Phases first defined by Maggi et al. 1986
Continuously 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.

Ambiguous Bladder Contraction – Tonic EUS gives False OP

- FBC decreases with atropine
- TBC increases !!! Decreased FBC due to decreased voiding efficiency.
It had only performed continuous open cystometry, might misinterpret effect as
mild irritation or sensitization reflex voiding !!!

**What is Bladder Capacity**

**Continuous vs. Single Fill Cystometry**

<table>
<thead>
<tr>
<th>Continuous open cystometry</th>
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| By combining the approaches, as shown above, one can determine voiding
efficiency easily by the equation: %VE = mean FBC/TBC x 100.

**Response to Drugs**

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

**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!

**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.
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

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
Simultaneous Isolated Bladder and Urethra


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)

Outline

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  - Neural Control
  - Non-neuronal Interactions
- Cystometric Measurement of the Lower Urinary Tract
  - The Micturition Cycle
  - Open Cystometry
  - Closed Outlet
- Conclusions

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

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

Affiliations to disclose*: None for author
None for presenter/speaker

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☐ Sponsored by:

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 \( T = \frac{PR}{2} \)

RODENT CYSTOMETRY

• Data that Can Be Obtained
  — Pressures
  — Volumes
  — Flows
  — EMG
  — Afferent/afferent 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
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

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

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

Analyses of potential interest

• Non Voiding Contractions
  – Frequency
  – Amplitude over compliance curve
• IPHFO
  – Frequency
  – Amplitude
• Pseudoaffective responses
• Power Spectral Analysis

REFERENCES


Introduction

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

Laboratory → Bedside

Pros and Cons of Anesthetized, Conscious, and Decerebrate Preparations

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

Animals = Humans or Animals ≠ Humans

Impacts of species difference, experimental design, and data analysis/interpretation

Laboratory → Bedside

Frequency-Volume Chart (FVC)

<table>
<thead>
<tr>
<th>Time</th>
<th>Input (ml)</th>
<th>Output (ml)</th>
<th>Leak</th>
<th>Urgency</th>
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Metabolic Cage (MC)

Urine output (µl)

Water intake

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<tr>
<th>Time (xx:yy)</th>
<th>Input (µl)</th>
<th>Output (µl)</th>
<th>Frequency (voids/day)</th>
<th>Voiding duration (s)</th>
<th>Flow rate (µl/s)</th>
<th>Leak</th>
<th>Urgency</th>
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**Human FVC vs. Rodent MC**

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<th>Output</th>
<th>Leak</th>
<th>Urgency</th>
<th>Frequency</th>
<th>Flow rate</th>
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<tr>
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<td>✓</td>
<td>✓</td>
<td>?</td>
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- Leakage frequency
- Flow rate
- Post-void residual volume
- Intravesical pressure change
- Uroflowmetry
- Echographic examination

**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**

<table>
<thead>
<tr>
<th></th>
<th>PT</th>
<th>MVP</th>
<th>BCD</th>
<th>BCP</th>
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<th>VV</th>
<th>VE</th>
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</tbody>
</table>

- First desire to void
- Normal desire to void
- Strong desire to void
- VT / Bladder capacity

**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. Sorge1,2,3, Lena Martini2,5, Kelsey A. Blenner1,4, Sanaa G. Elabd2, Sarah Reiser4, Alexander H. Talley4, Jeffrey J. Rybak4, Tiziana Diosi1,5, Baptiste Frey1,5, Philipp Legler5, Joseph W. Maggio1,5, Martin M. Rehkugler4, John Delaney2, Carlos Whitaker1,4, Alex P. Runnels1, Tiziana Diosi1,5, Johanne Fraden1,4, Carissa L. Hennessey1,4, 'Nancy F. Condie'1,4, & Jeffrey J. Rybak4,5,7

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 -

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

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

Approx. 1.0 mm anterior part to the lambdoid suture should be left.

Cutting line (cut using a scalpel)

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.

Superior colliculus
Sutured
Cystometry
in Decerebrate, Unanesthetized Mouse

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

TRP channel-deficient
mouse model

2016/9/20

Cystometry

**Comparisons between WT, TRPV1-KO and TRPV4-KO**

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

**Comparison in non-voiding contractions**

<table>
<thead>
<tr>
<th></th>
<th>p1-1st NVC (mmHg)</th>
<th>v1-1st NVC (μl)</th>
<th>peak pressure of 1st NVC (mmHg)</th>
<th>mean peak pressure of NVCs (mmHg)</th>
<th>number of NVCs/micturition cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV1-KO</td>
<td>3.3 ± 0.3</td>
<td>98.1 ± 17.1</td>
<td>11.3 ± 0.7</td>
<td>14.6 ± 1.5</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>TRPV4-KO</td>
<td>2.8 ± 0.2</td>
<td>106.6 ± 10.4</td>
<td>12.8 ± 0.9</td>
<td>13.4 ± 0.7</td>
<td>2.8 ± 0.7</td>
</tr>
</tbody>
</table>

Metabolic cage
Reflex micturition vs. Conscious voiding

Cystometry
Metabolic cage

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

Animal conditions during cystometry

<table>
<thead>
<tr>
<th></th>
<th>Awake</th>
<th>Anesthetized</th>
<th>Decerebrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pros</strong></td>
<td>• Little influence of anesthesia</td>
<td>• Easy to handle an animal and catheters during experiment</td>
<td>• Little influence of anesthesia</td>
</tr>
<tr>
<td></td>
<td>• No influence of emotion</td>
<td>• No influence of emotion</td>
<td>• Little influence of anesthesia</td>
</tr>
<tr>
<td></td>
<td>• Little influence from circumstances</td>
<td>• Generally in experiment design</td>
<td>• Little influence from circumstances</td>
</tr>
</tbody>
</table>

| **Cons**       | • Influence from circumstances | • Pharmacological and physiological intervention | • Long-term training and experience |
|                | • Emotion changes | • Long time for surgery | • Longer time for surgery |
|                | • Hard to handle an animal and catheters during experiment | | |

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