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

Role of Animal Models

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9th Annual Bedside-to-Bench Conference:

Urinary Incontinence in the Elderly: A Translational Research Agenda for a Complex Geriatric Syndrome

U13 Conference Series, Bethesda, MD

October 17, 2016

Disclosures

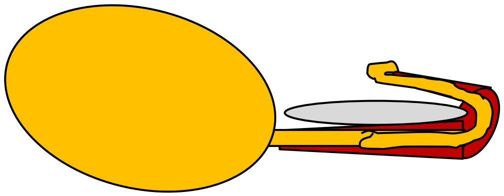
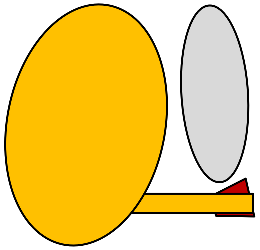
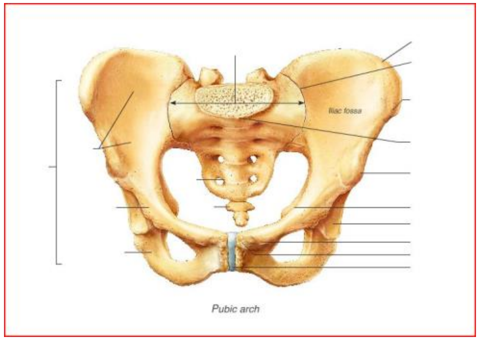
- **Current Funding**
  - NIDDK LURN Network
  - VA RR&D SPiRE
  - VA RR&D SPiRE
- **Other financial relationships**
  - SAB for Amphora Medical
  - Invited Speaker for Allergan
  - Consultant for Synergy Pharma and InVivo Pharma
  - Patent royalties from Lipella Pharma
- **Conflicts of interest**
  - None

Outline

- **Animal models for basic and translational research**
  - Species differences in LUT anatomy and physiology
  - Rodents as research models
  - Animal models of aging
  - Age-related changes in LUT function
- **Measurement of LUT function**
  - Cystometric Measurement of the Lower Urinary Tract
    - The Micturition Cycle
    - Open Cystometry
    - Closed Outlet
  - Metabolism Cage/VSOP
  - LPP
- **Conclusions**

Animal models for basic and translational research

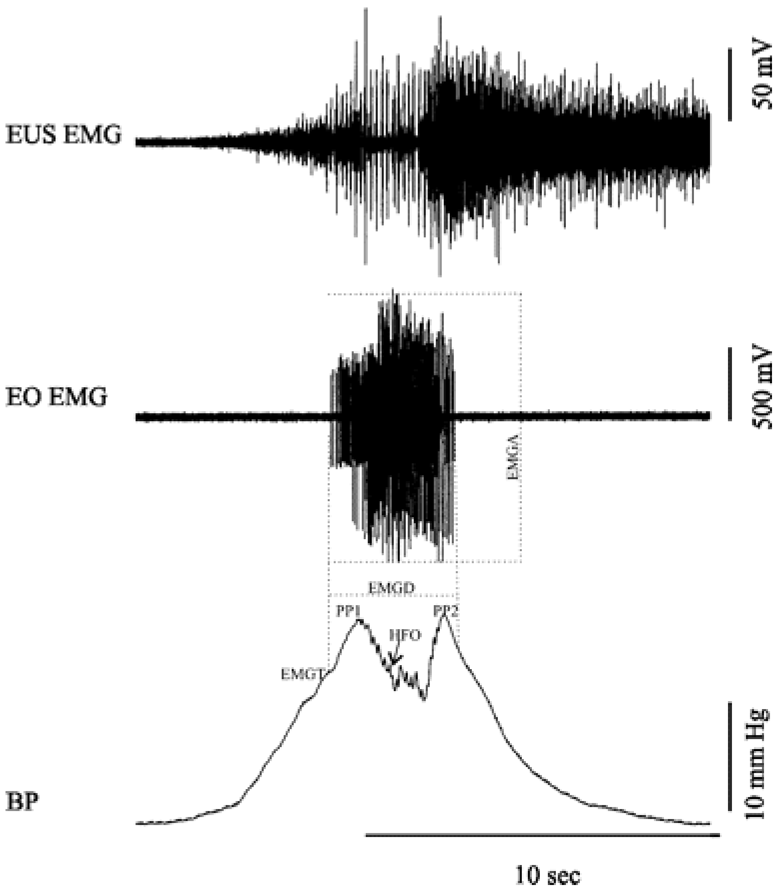
- **Species differences in LUT anatomy and physiology**

	Muriform Rodents	Humans
Female Urethra		
Pelvis		
Levator Muscles	Work the Tail	Form Pelvic Floor
Autonomic Ganglia	Extramural	Intramural
Sleep Patterns	Nocturnal	Diurnal

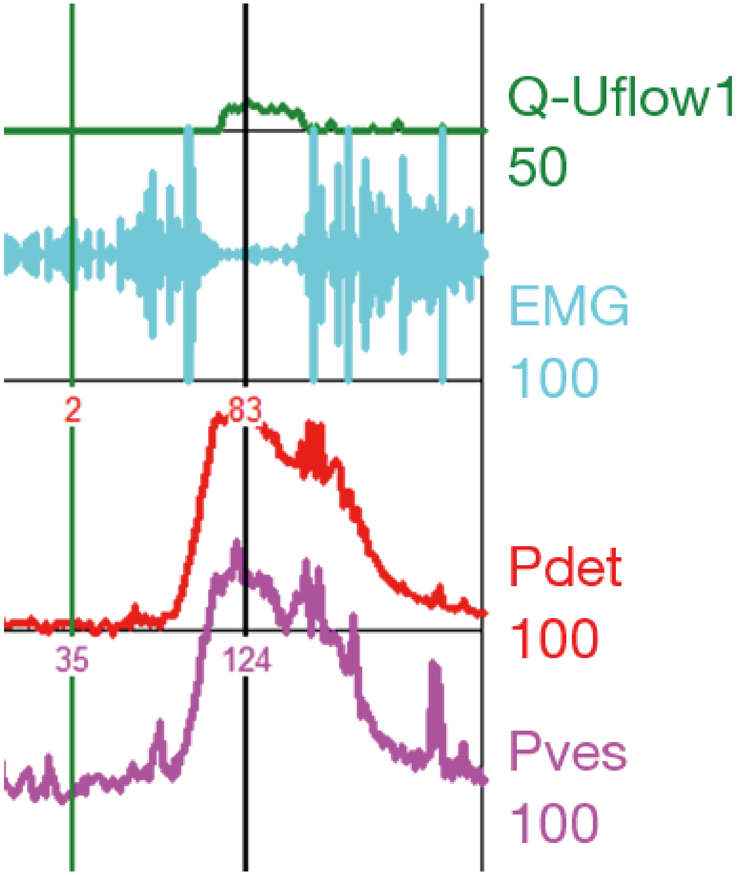
Animal models for basic and translational research

- **Species differences in LUT anatomy and physiology**

Muriform Rodents



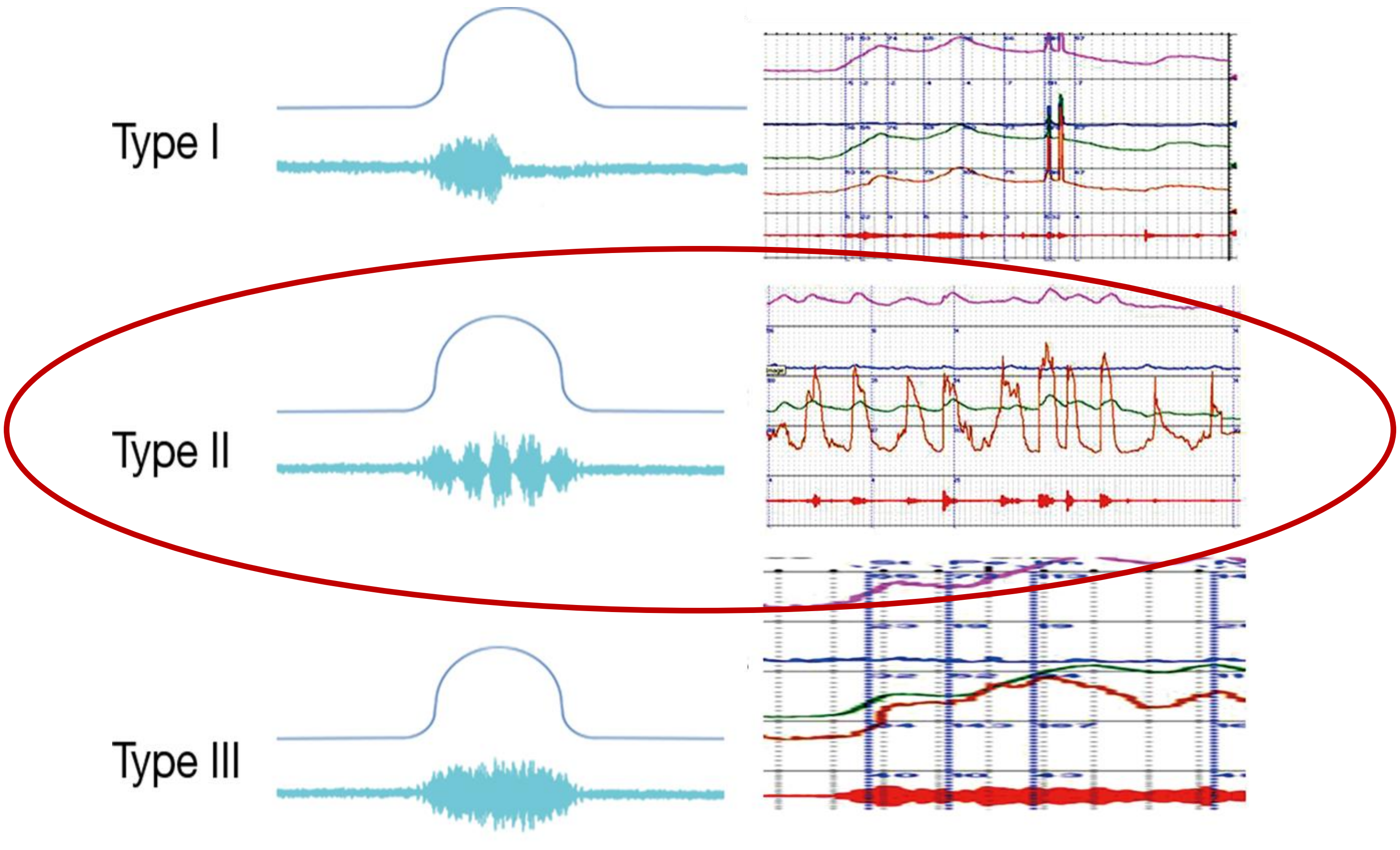
Humans



Animal models for basic and translational research

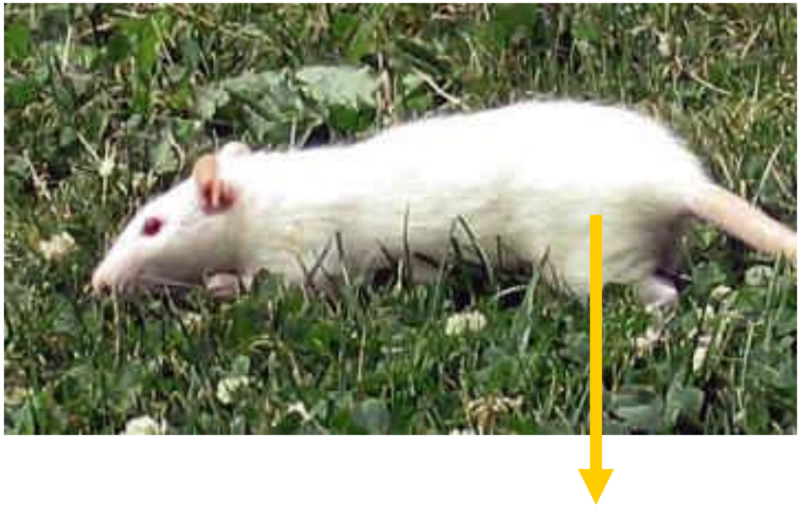
- **Species differences in LUT anatomy and physiology**

Special Circumstances - SCI



Animal models for basic and translational research

- **Species differences in LUT anatomy and physiology**



**In quadrupedal animals, gravity directs urine to the ventral abdominal wall.
In bipeds, gravity directs urine through the outlet.**

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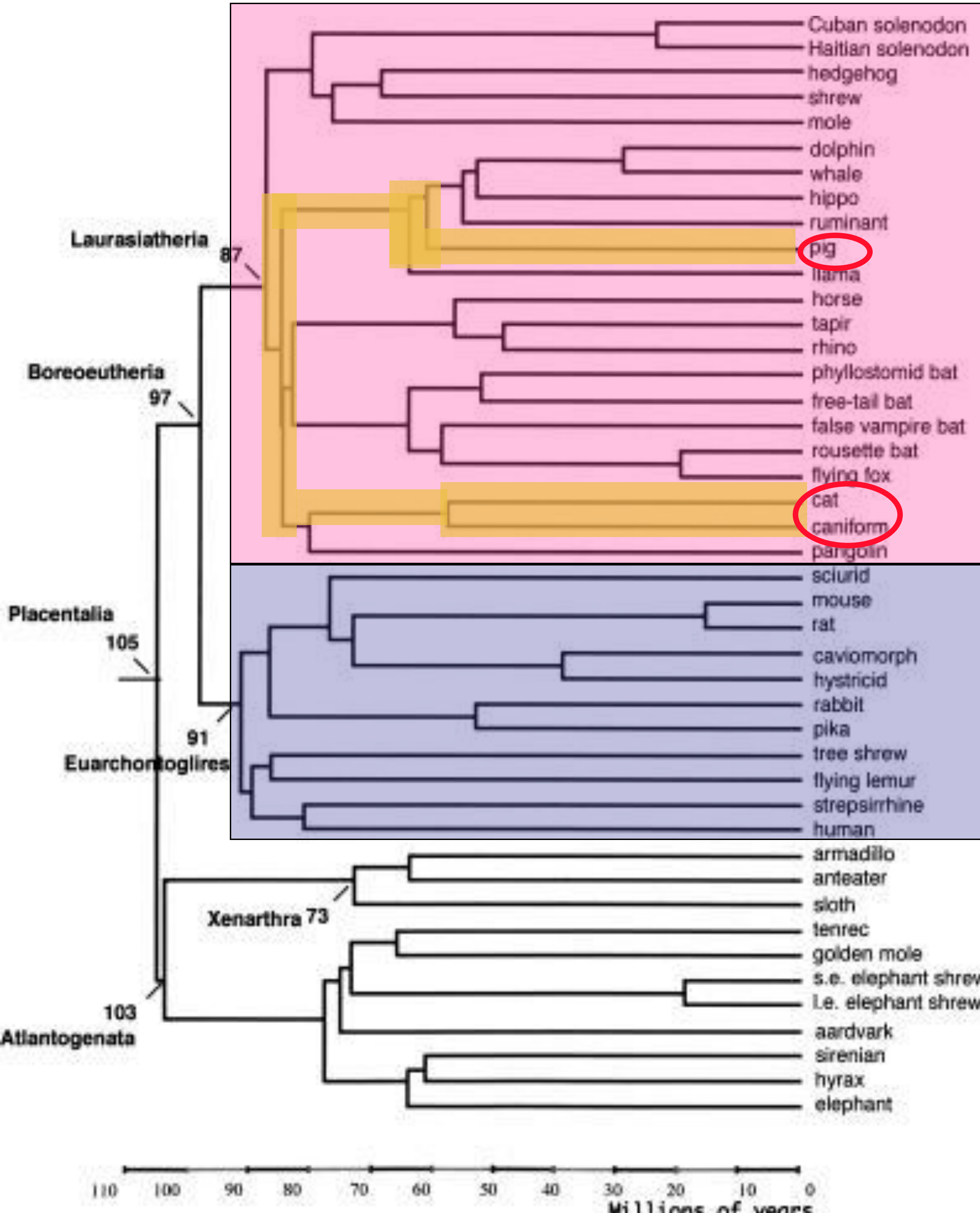
Animal models for basic and translational research

- **Rodents as research models**

  – Rodents are more closely related to humans than dogs, cats or pigs !!!


Cuban solenodon
Haitian solenodon
hedgehog
shrew
mole
dolphin
whale
hippo
ruminant
pig
llama
horse
tapir
rhino
phyllostomid bat
free-tail bat
false vampire bat
rousette bat
flying fox
cat
caniform
pangolin
sciurid
mouse
rat
caviomorph
hystricid
rabbit
pika
tree shrew
flying lemur
strepsirrhine
human
armadillo
anteater
sloth
tenrec
golden mole
s.e. elephant shrew
l.e. elephant shrew
aardvark
sirenian
hyrax
elephant
Laurasiatheria
87
Boreoeutheria
97
Placentalia
105
91
Euarchontoglires
Xenarthra 73
103
Atlantogenata
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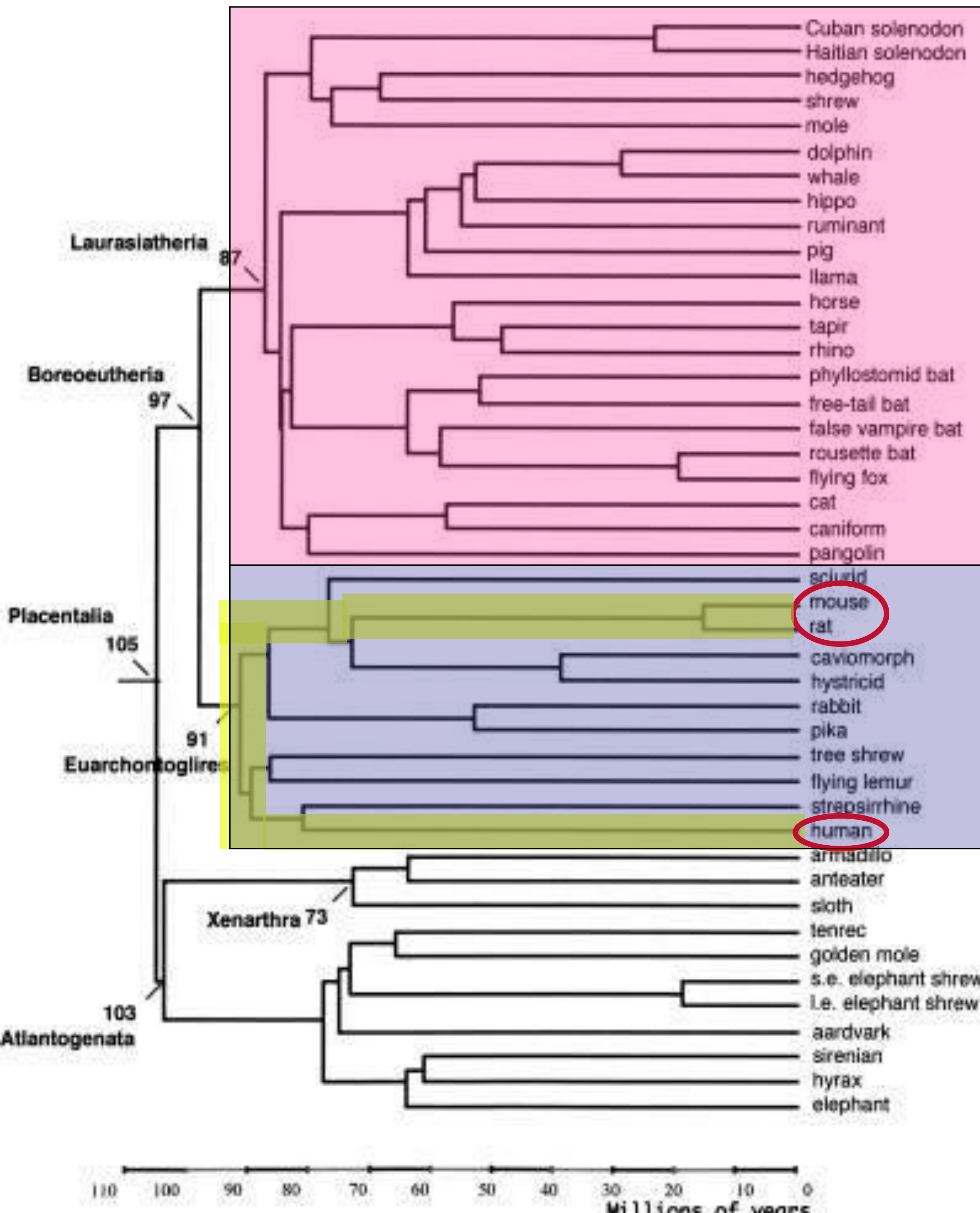
Millions of years



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phyllostomid bat
free-tail bat
false vampire bat
rousette bat
flying fox
cat
caniform
pangolin
sciurid
mouse
rat
caviomorph
hystricid
rabbit
pika
tree shrew
flying lemur
strepsirrhine
human
armadillo
anteater
sloth
tenrec
golden mole
s.e. elephant shrew
l.e. elephant shrew
aardvark
sirenian
hyrax
elephant
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87
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Placentalia
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Xenarthra 73
103
Atlantogenata
110
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30
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10
0
Millions of years

Animal models for basic and translational research

- **Rodents as research models**
  - Rodents are more closely related to humans than dogs, cats or pigs
  - However, rodents are not always reliable as preclinical models for human disease and the scientific literature is littered with examples of drugs that worked well in animals but turned out to be ineffective in clinical trials on humans.
  - This is in part due to differences in
    - Anatomy and physiology
    - Drug metabolism
    - Structure Activity Relationships (SAR) between species-specific receptor modifications and a constant structure drug candidate
    - Off-target effects that may contribute to species-specific outcomes
  - It is also due, in part, to methodology and interpretation of results failing to account for species differences

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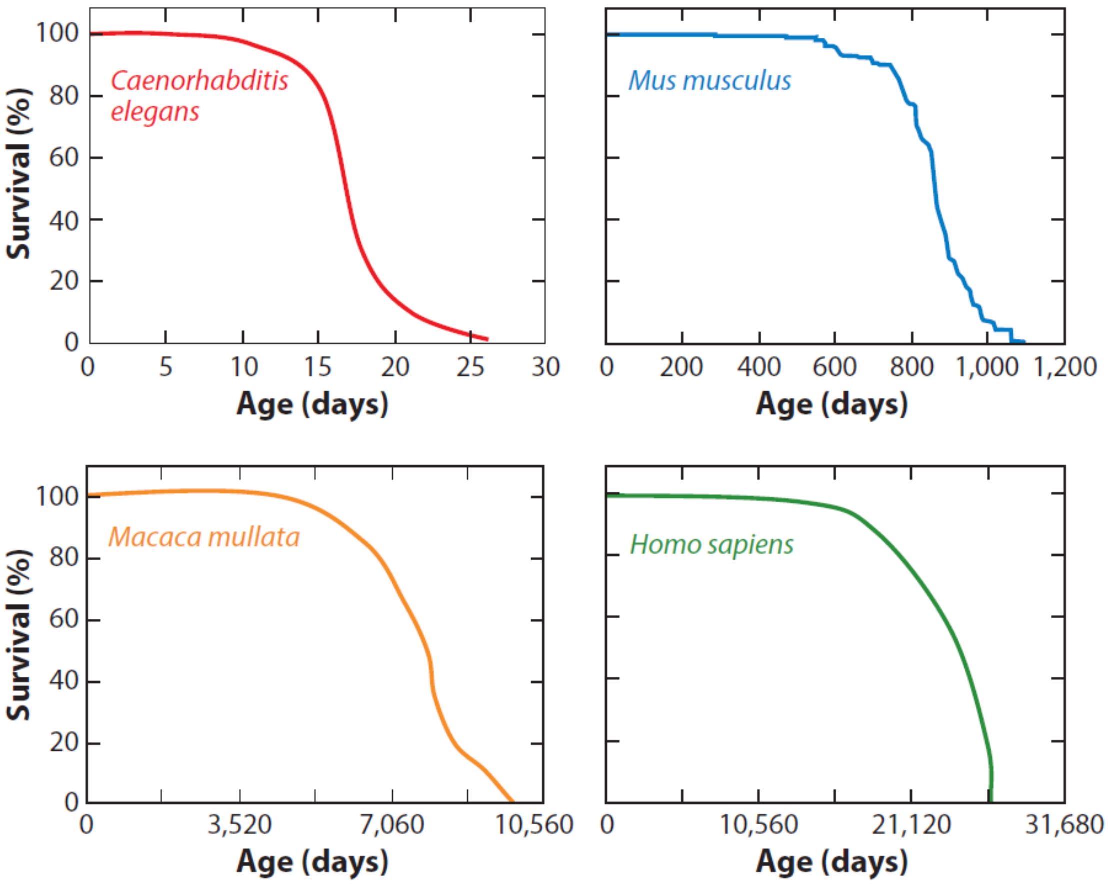
Animal models for basic and translational research

- **Animal models of aging**
  - **Aging (health span) is similar across species when normalized to life span**



Mitchell et al., 2015. Ann Rev Anim Biosci 3:283-303

Animal models for basic and translational research

- **Animal models of aging**
  - **NIA Rodents Available for Aging Research**

	% Survival	90%	75%	50%	25%	10%
Rats	F344-cdf Males	14 mo.	19 mo.	22 mo.	25 mo.	27 mo.
Rats	F344-cdf Females	16 mo.	21 mo.	25 mo.	29 mo.	31 mo.
Rats	F344BN Males	25 mo.	29 mo.	34 mo.	37 mo.	38 mo.
Rats	F344BN Females	23 mo.	26 mo.	30 mo.	34 mo.	36 mo.
Rats	BN Males	22 mo.	27 mo.	32 mo.	34 mo.	36 mo.
Rats	BN Females	22 mo.	27 mo.	32 mo.	35 mo.	38 mo.
Mice	C57BL/6 Males	19 mo.	24 mo.	27 mo.	30 mo.	32 mo.
Mice	C57BL/6 Females	18 mo.	22 mo.	25 mo.	28 mo.	30 mo.
Mice	DBA/2 Males	16 mo.	22 mo.	25 mo.	28 mo.	29 mo.
Mice	DBA/2 Females	8 mo.	16 mo.	23 mo.	26 mo.	29 mo.

NIA Website. https://www.nia.nih.gov/research/dab/aged-rodent-colonies-handbook/strain-survival-information

Animal models for basic and translational research

- **Animal models of aging**
  - **Comparison of results of interventions in mice and humans**

Interventions/genetic modifications	Mean lifespan extension (%) in the mouse	Effects on age parameters and age-related disease in the mouse	ref mouse	Reports in humans	ref humans
Caloric restriction	35–65 both sexes	Delays the onset and/or slows the progression of most age-associated diseases including neoplastic, degenerative and immune diseases.	(Weindruch, 1996; Weindruch et al., 1986) (Liao et al., 2010)	No definite data that CR prolongs life span but data on the health benifits are promising.	(Roth and Polotsky, 2012)
Methionine restriction	30 females 17 males	Lower rate of age-related change in T-cell subsets and slower development of cataracts.	(Miller et al., 2005) (Sun et al., 2009)	Vegetal-based low methionine diets can increase life expectancy up to 10 years compared to people consuming typical Western diets.	(Singh et al., 2003) (Tognon et al., 2011)
Resveratrol	None	Shifts the physiology of middle-aged mice on a high-caloric diet towards that of mice on a standard diet and prevents their early mortality. Delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending lifespan.	(Baur et al., 2006) (Miller et al., 2011) (Pearson et al., 2008)	Reduced risk for coronary hart disease and a possible extension of life span was found in populations consuming wine with a higher amount of resveratrol. Reseveratrol-like compounds promote beneficial changes in health.	(Corder et al., 2006) (Chachay et al., 2011) (Smoliga et al., 2011)
Aspirin	8 for males, no extension females		(Strong et al., 2008)	Aspirin use was associated with lower risks of cancer incidence and mortality.	(Bardia et al., 2007)
Rapamycin	9 males and 14 females 10 males and 18 females		(Harrison et al., 2009) (Miller et al., 2011)	Possible anti-cancer effect.	(Sharp and Strong, 2010)

Vanhooren and Libert, 2013. Age Res Rev 12:8-21

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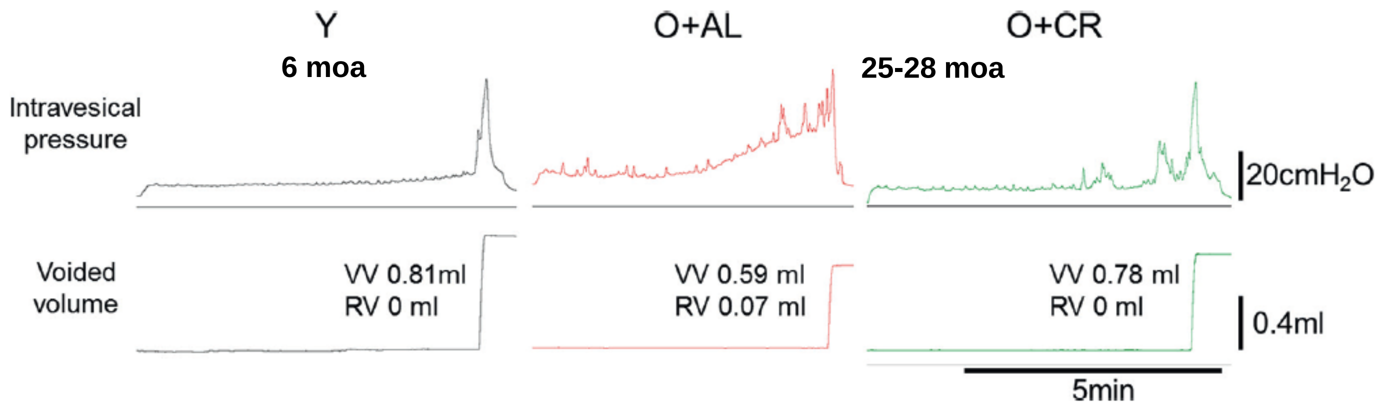
Animal models for basic and translational research

- **Animal models of aging**
  - **Affects of Aging on LUT Function – Conscious cystometry in F344 rats**



		O+AL		O+CR		
	Mean ± SEM Y	Mean ± SEM	p Value vs Y	Mean ± SEM	p Value vs Y	p Value O+AL
Pressure (cm H_2O):						
Basal	5.20 ± 0.39	7.02 ± 1.56	0.547	5.29 ± 1.24	0.999	0.557
Threshold	10.43 ± 0.94	21.15 ± 3.57	0.018*	16.55 ± 2.14	0.199	0.389
Max	47.50 ± 2.94	52.32 ± 4.62	0.724	43.16 ± 4.86	0.755	0.305
Vol (ml):						
Voided	0.71 ± 0.03	0.67 ± 0.068	0.869	0.77 ± 0.07	0.764	0.452
Residual	0.022 ± 0.007	0.11 ± 0.025	0.009†	0.035 ± 0.018	0.870	0.022*
Bladder capacity (ml)	0.74 ± 0.03	0.78 ± 0.06	0.864	0.81 ± 0.07	0.677	0.947
Mean flow rate (ml/sec)	0.130 ± 0.008	0.126 ± 0.008	0.523	0.128 ± 0.007	0.685	0.862
Voiding efficiency (%)	97.21 ± 0.74	85.38 ± 3.11	0.014*	95.51 ± 2.66	0.984	0.047*
Bladder compliance (ml/cm H_2O)	0.22 ± 0.05	0.08 ± 0.02	0.044*	0.11 ± 0.03	0.123	0.814
NVCs:						
No./min	0.27 ± 0.11	1.20 ± 0.36	0.034*	0.73 ± 0.18	0.369	0.344
Amplitude (cm H_2O)	5.57 ± 0.30‡	5.89 ± 0.65	0.950	6.32 ± 0.78	0.747	0.890

Ito et al., 2016. J Urol 196:1575-1583

Animal models for basic and translational research

- **Animal models of aging**
  - **Affects of Aging on LUT Function – Conscious cystometry in F344 rats**
  - **Combined results suggest more of an overactive bladder condition, as functional bladder capacity would be expected to be decreased, NVC are increased and compliance is low**
  - **This is consistent with their gene expression studies which demonstrated increases immune and inflammation pathways in the bladder and DRG**

		O+AL		O+CR		
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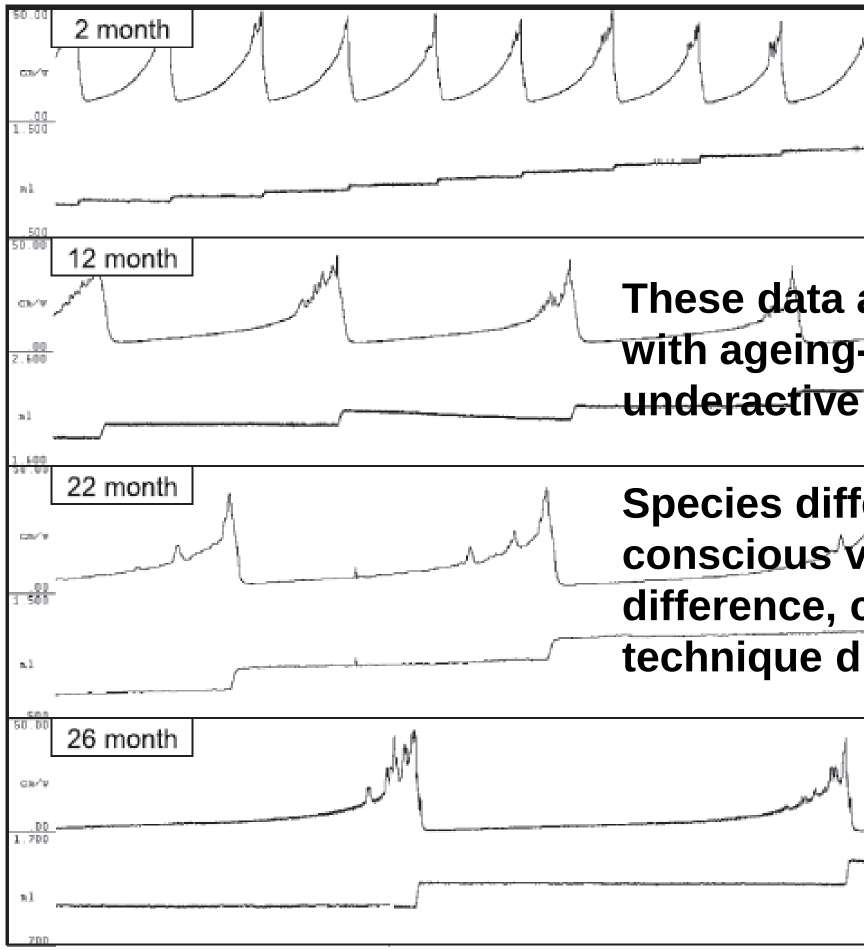
Animal models for basic and translational research

- **Animal models of aging**
  - **Affects of Aging on LUT Function – Anesthetized cystometry in C57BL6 mice**



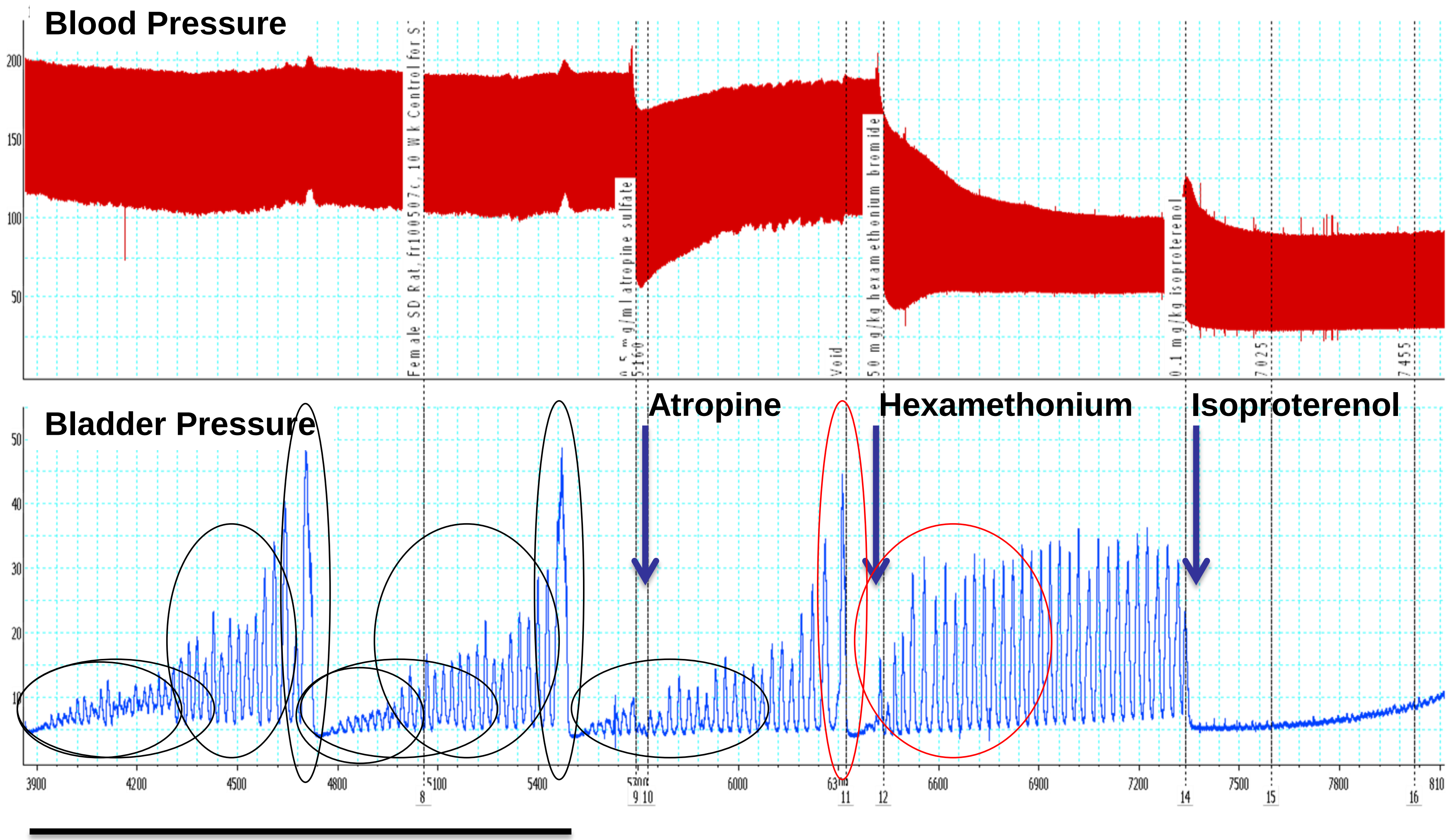
These data are consistent with ageing-induced underactive bladder !!!

Species difference, conscious vs. anesthesia difference, cystometric technique difference ???

Smith et al., 2012. Am J Phys Reg Int Comp Phys 302:R577-R586

Animal models for basic and translational research

- **Affects of Aging on LUT Function – Anesthetized cystometry in 18 moa SD rats**



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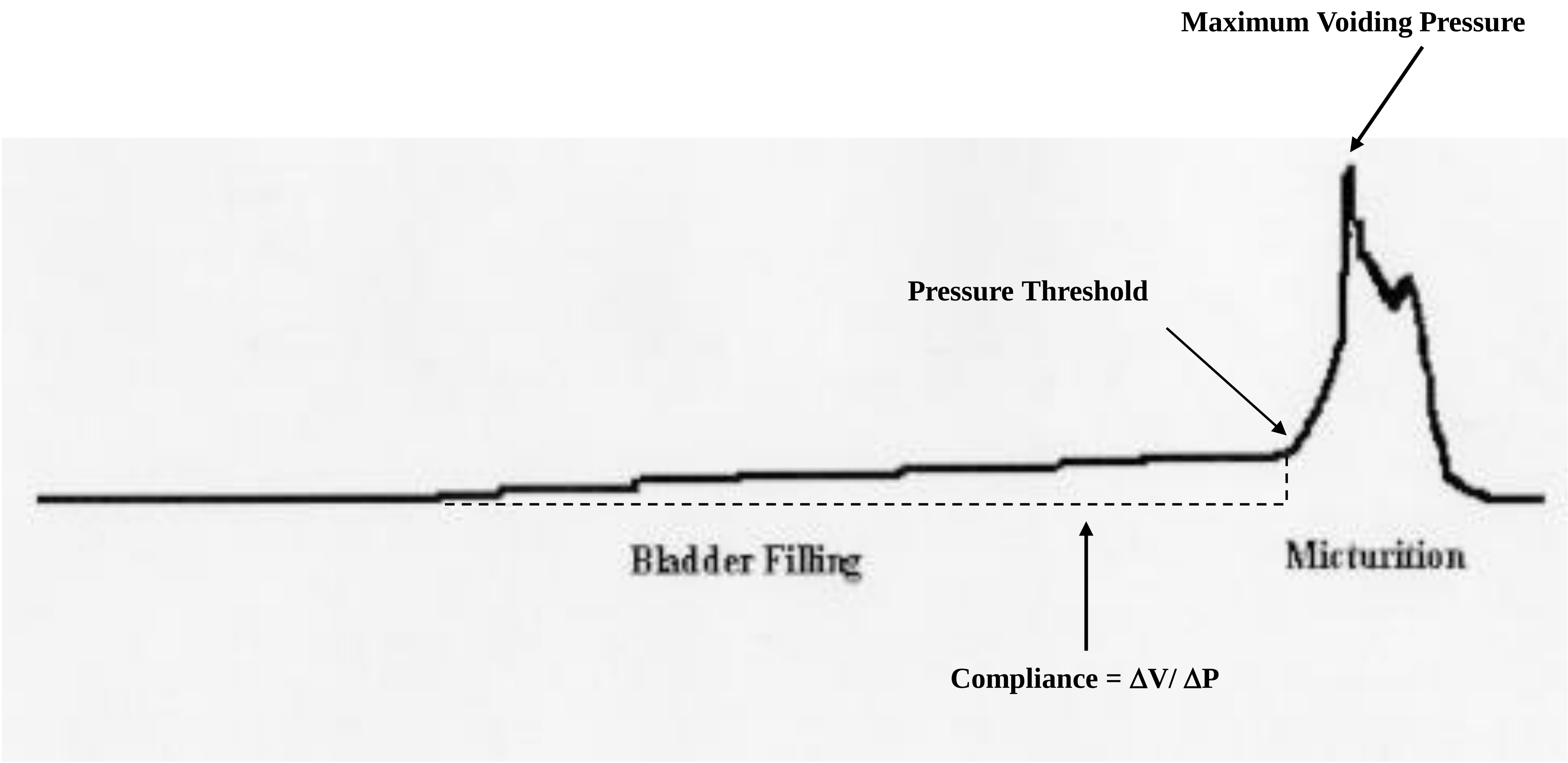
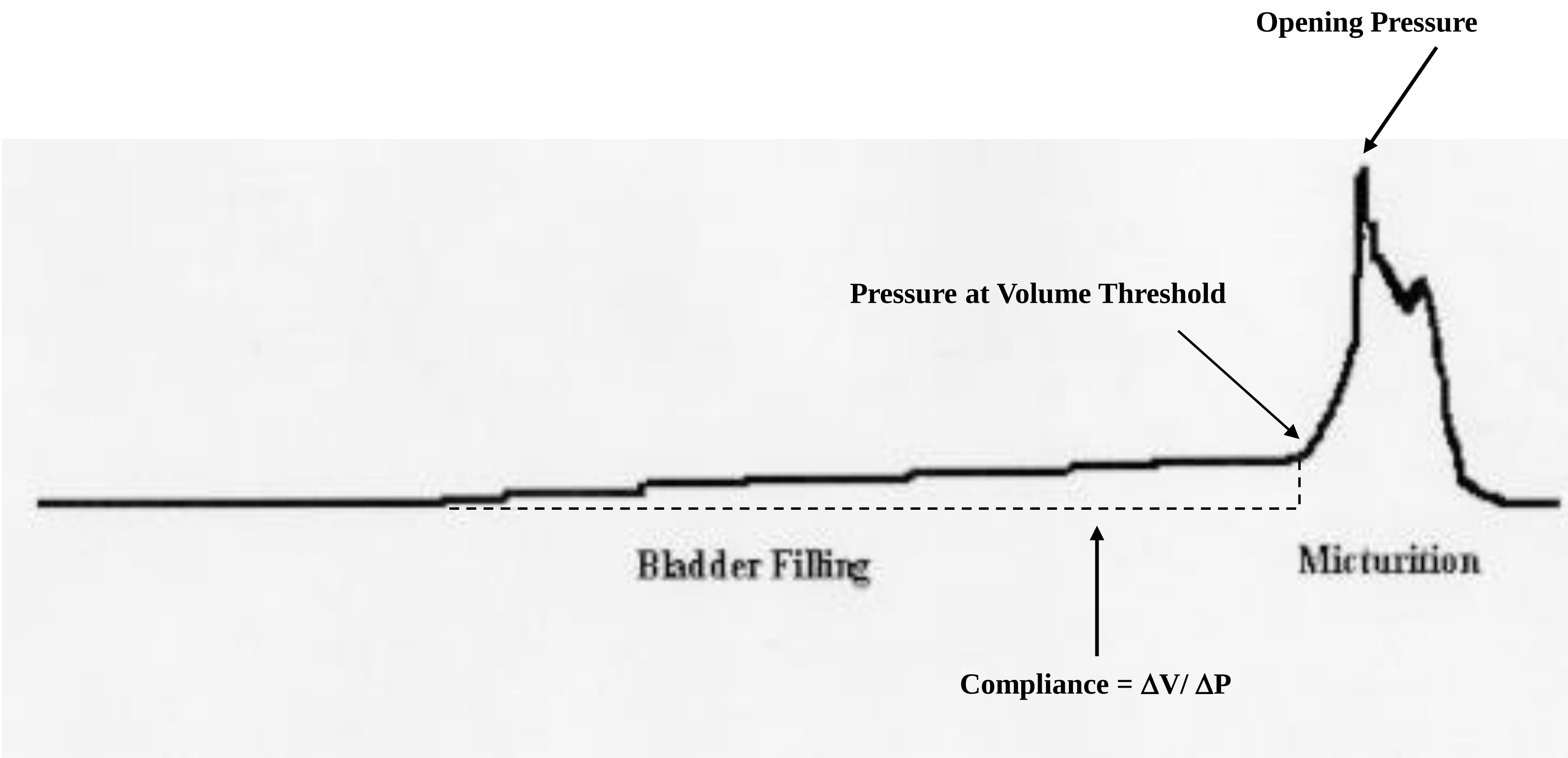
Cystometry - The Micturition Cycle

Common Descriptors

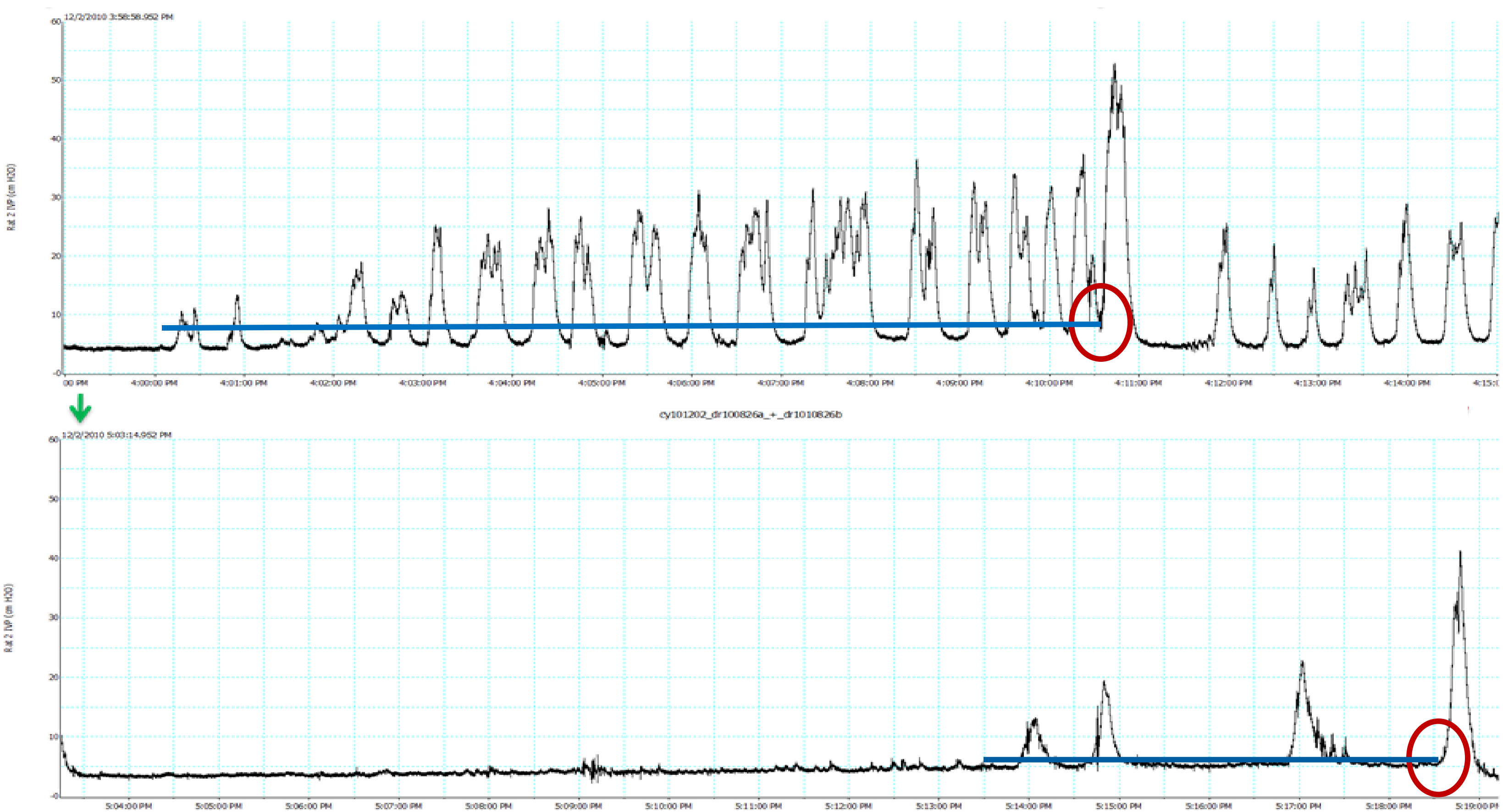


Cystometry - The Micturition Cycle

Better Descriptors

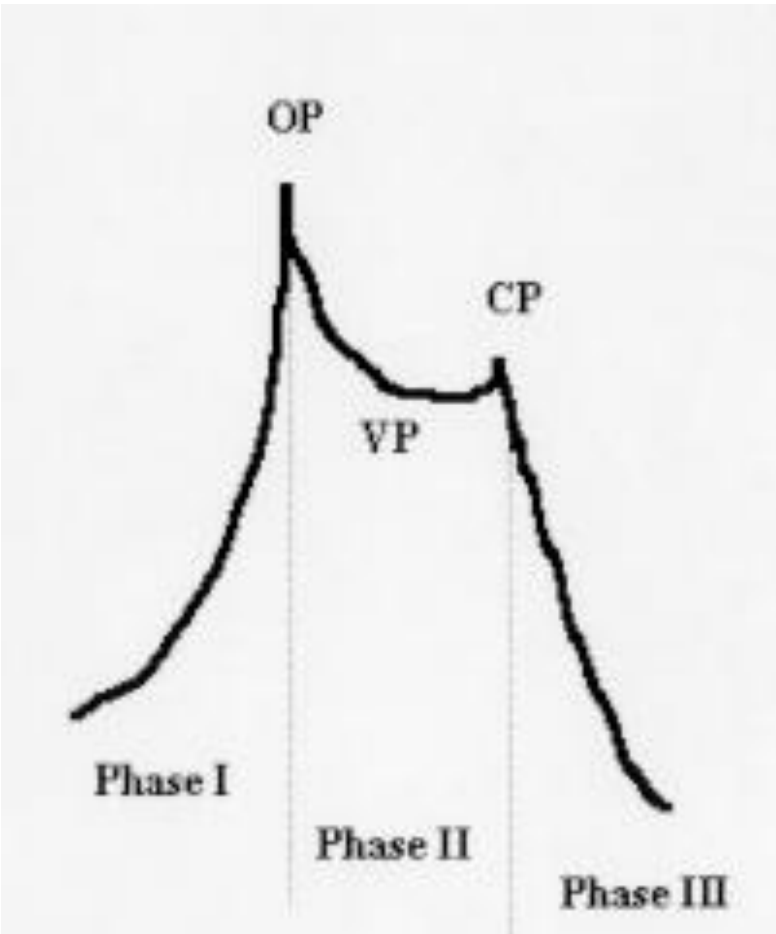


Where is “Pressure” Threshold?



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.

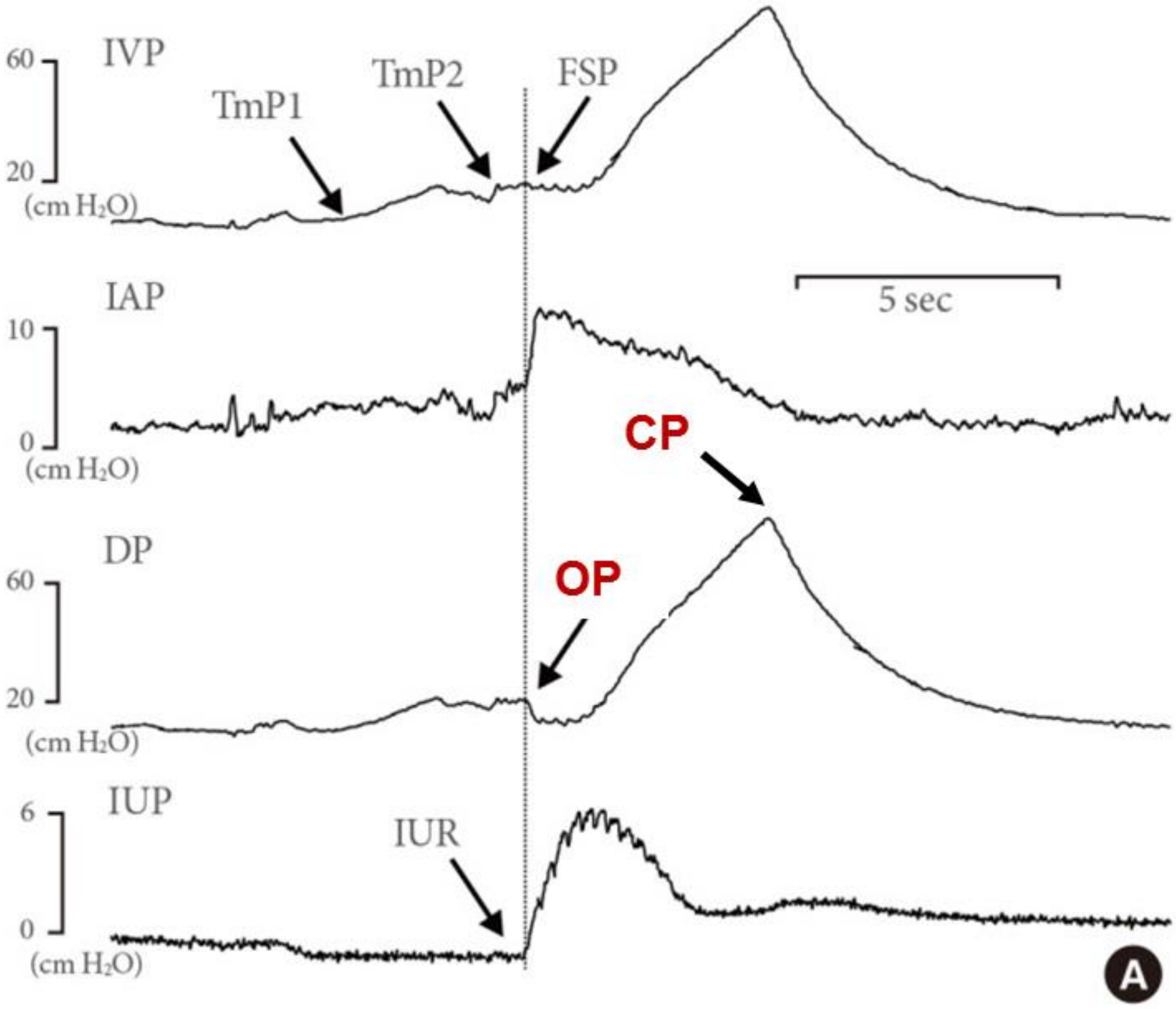
Need to understand the anatomy of the voiding contraction:

<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

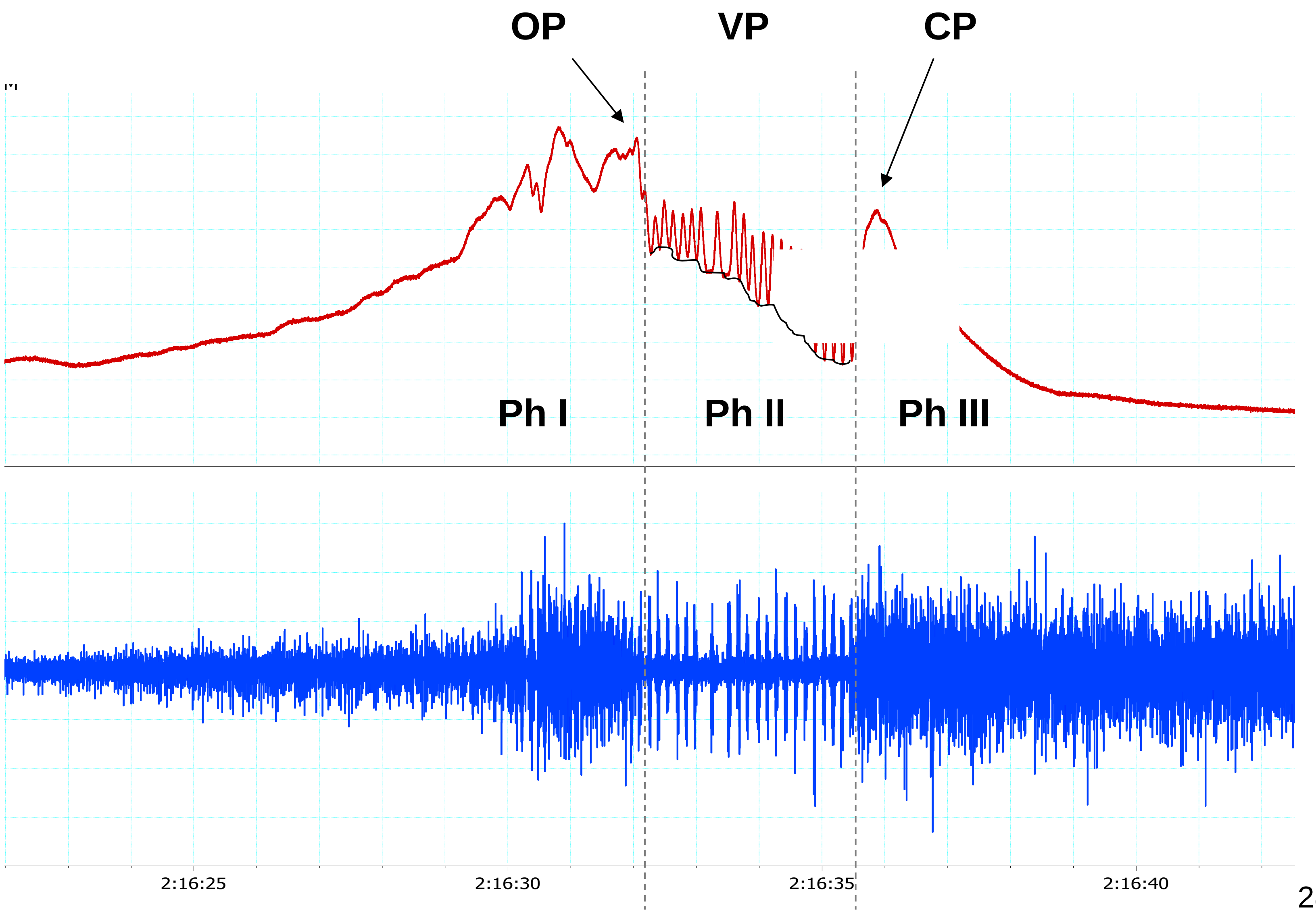
Pressure-Flow relationships can be explored during Phase II

Maggi et al, 1986

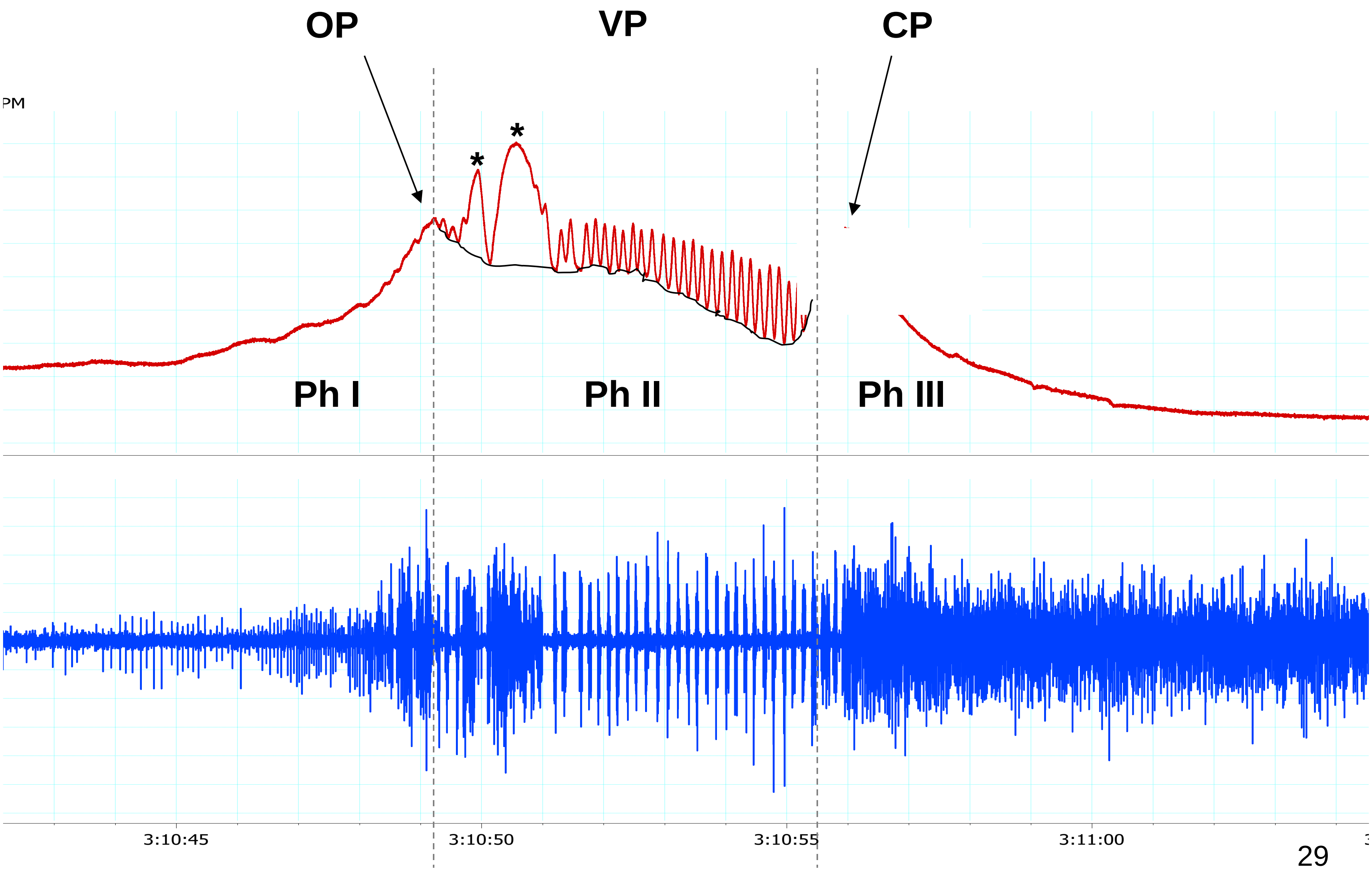
Human OP and CP also Discernable



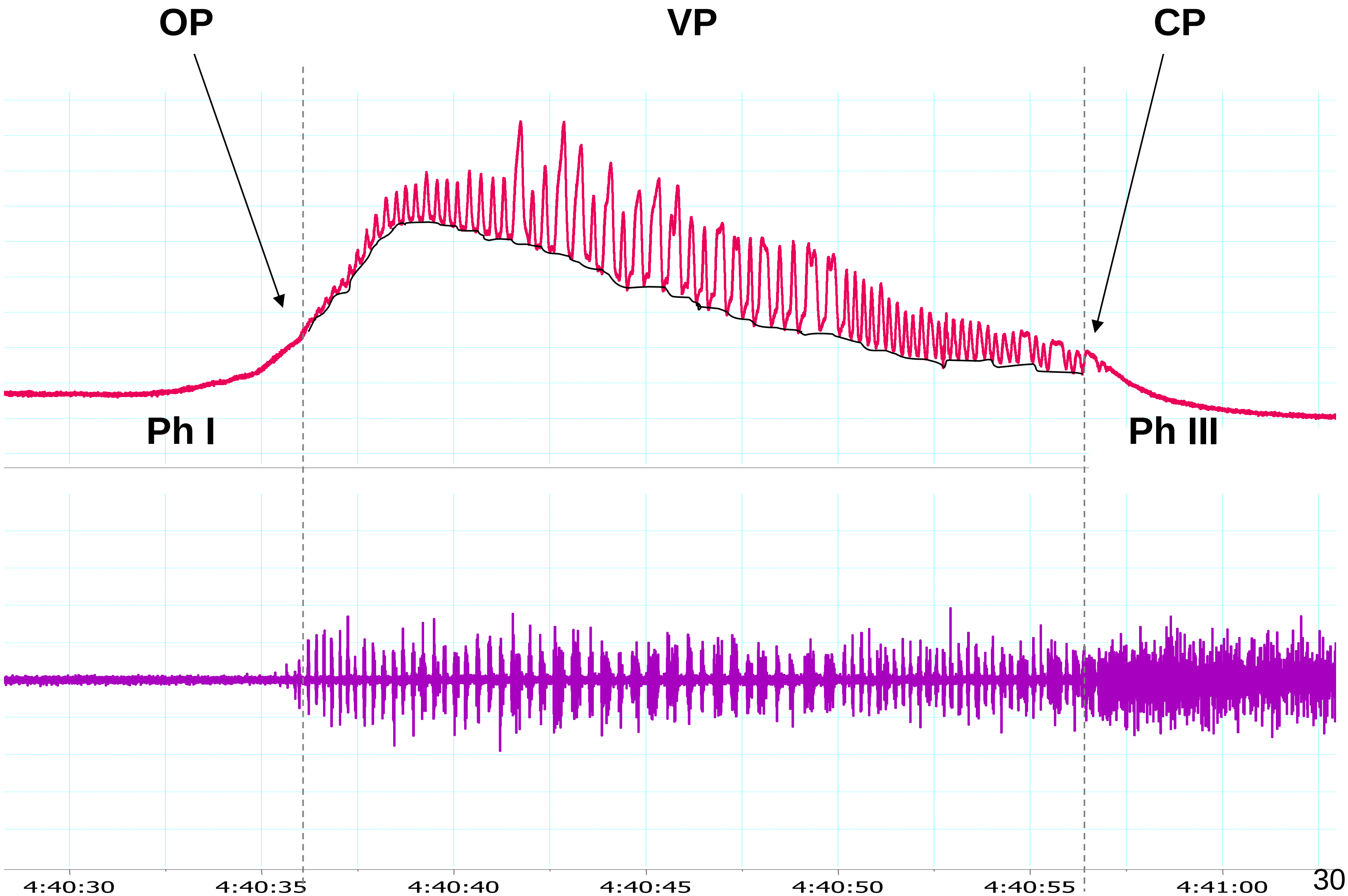
Easy Bladder Contraction



Ambiguous Bladder Contraction – Tonic EUS gives False OP*



Ambiguous Bladder Contraction – “Missing” OP



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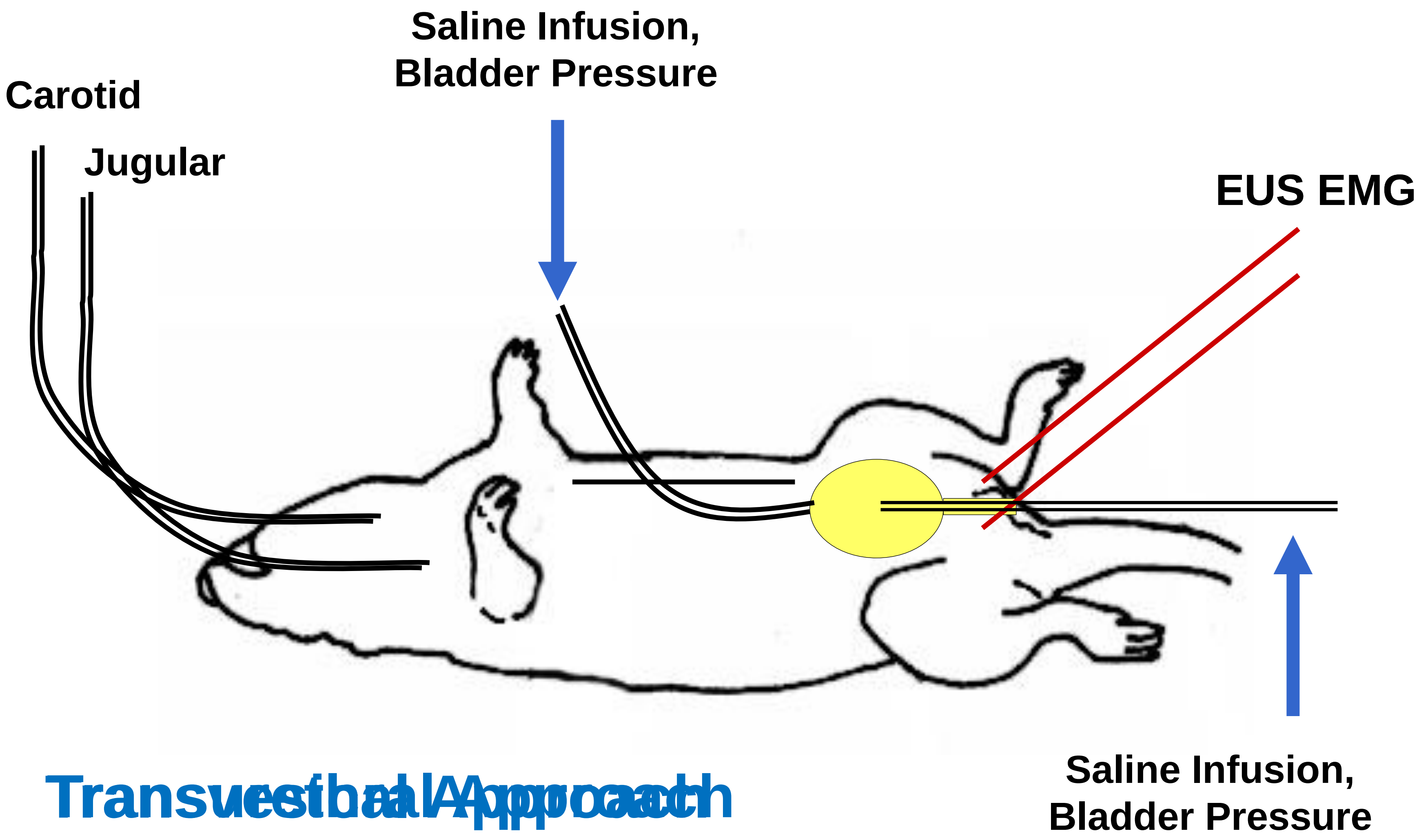
Open Cystometry Protocol

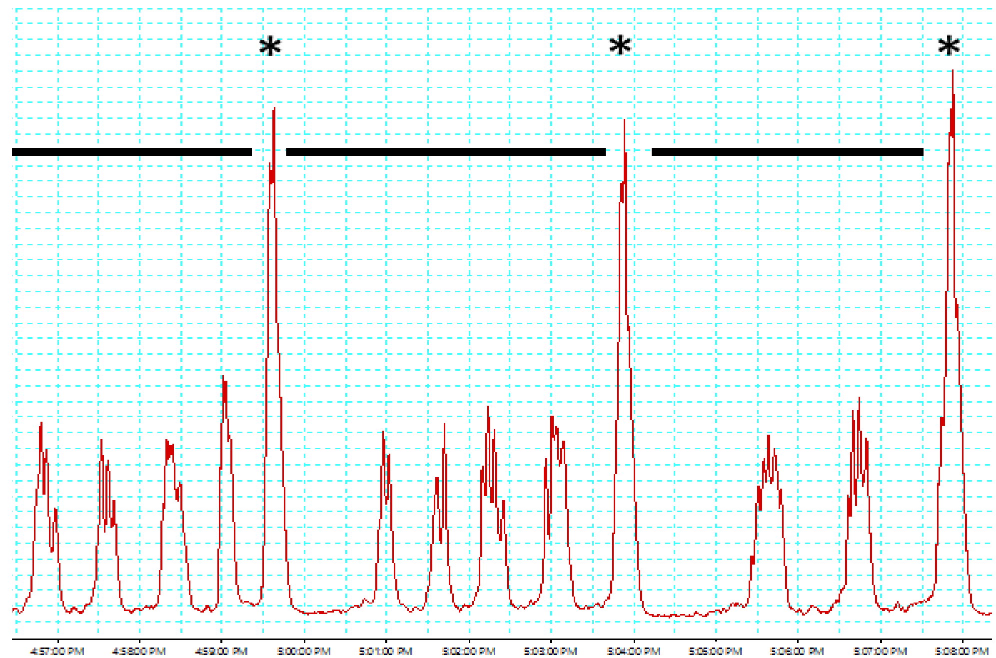


Transvesical Approach

Transurethral Approach

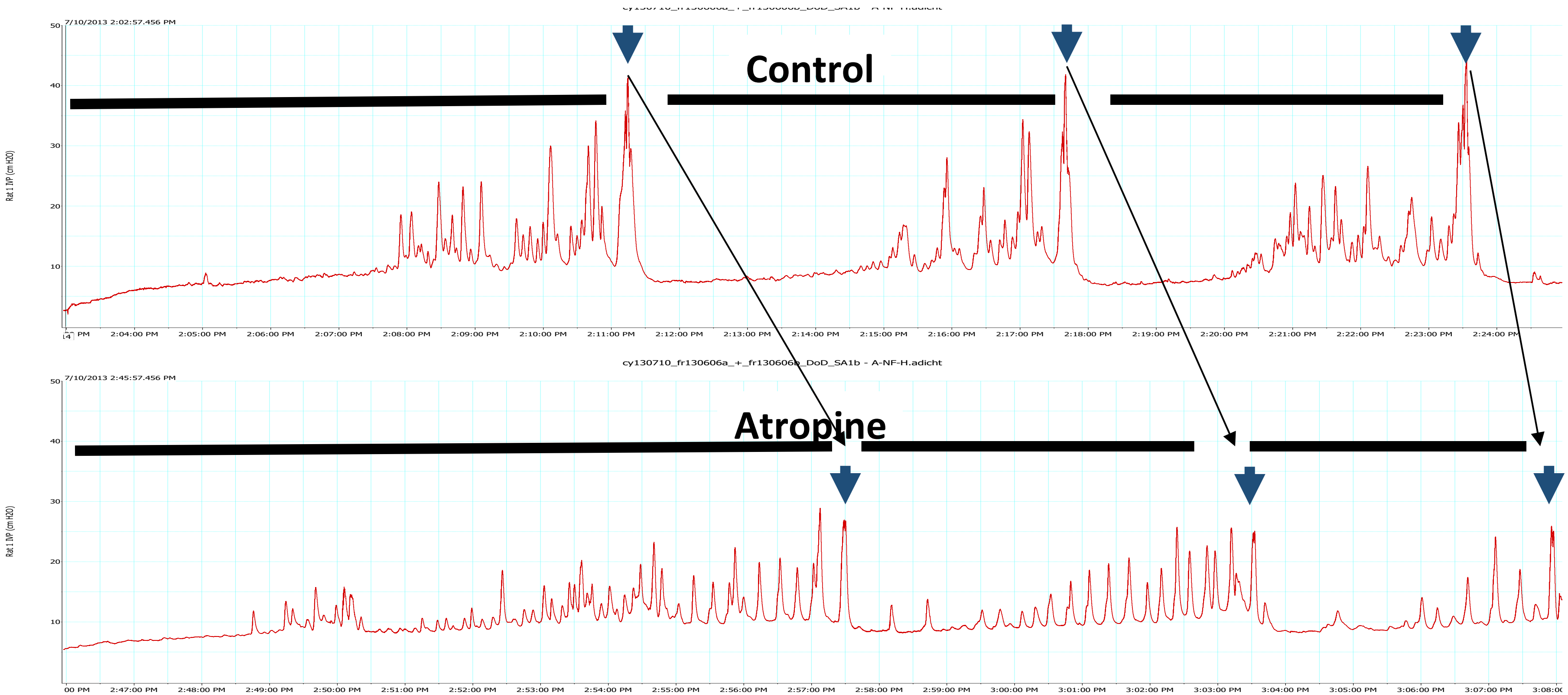
What is Bladder Capacity

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**

Response to Drugs

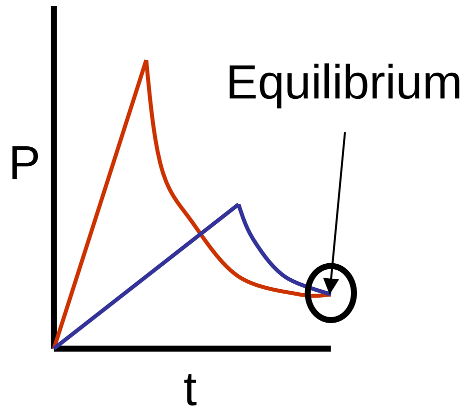


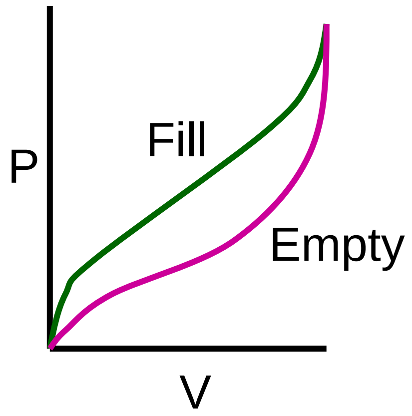
- FBC decreases with atropine
- TBC increases !!! Decreased FBC due to decreased voiding efficiency.

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

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!





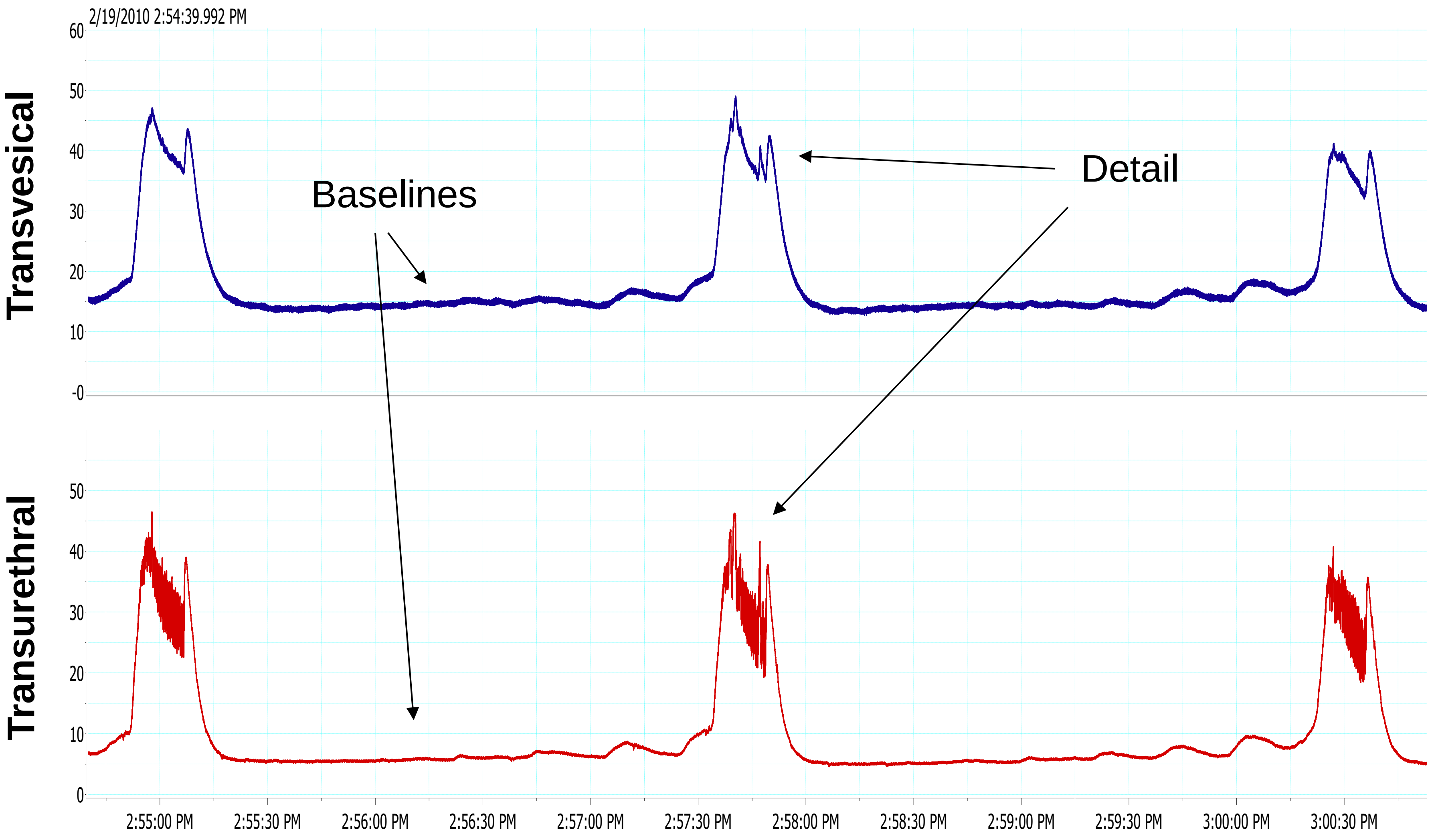
Flow rate affects the compliance measurements!

Coolsaet 1985

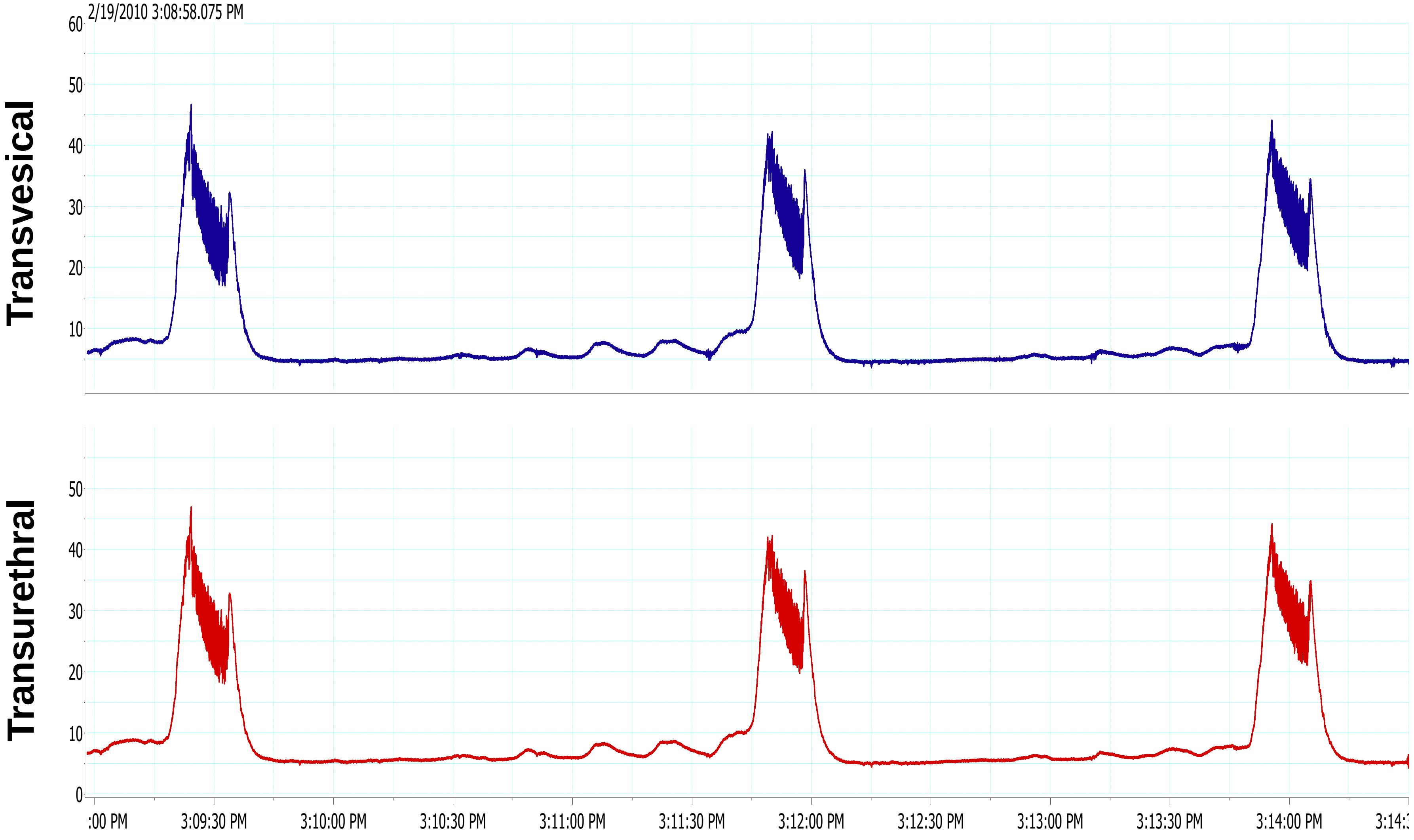
Response of the Bladder to Filling:
Measurement System Considerations

- 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

Transvesical Filling



Transureteral Filling



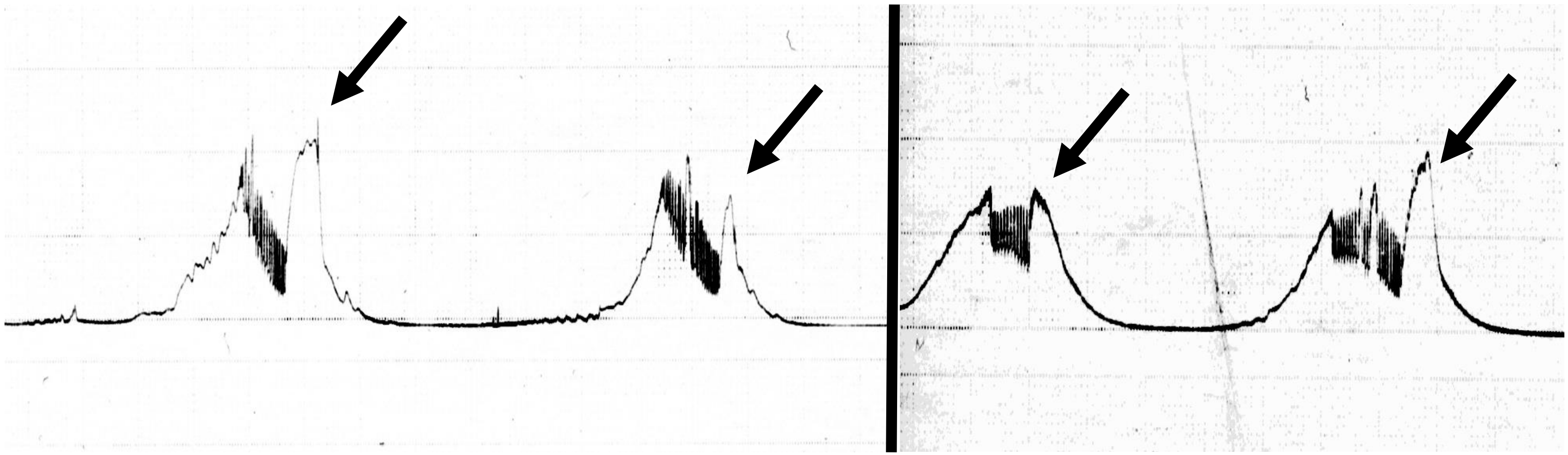
Response of the Bladder to Filling:
Measurement System Considerations

- 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

Transvesical Filling

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

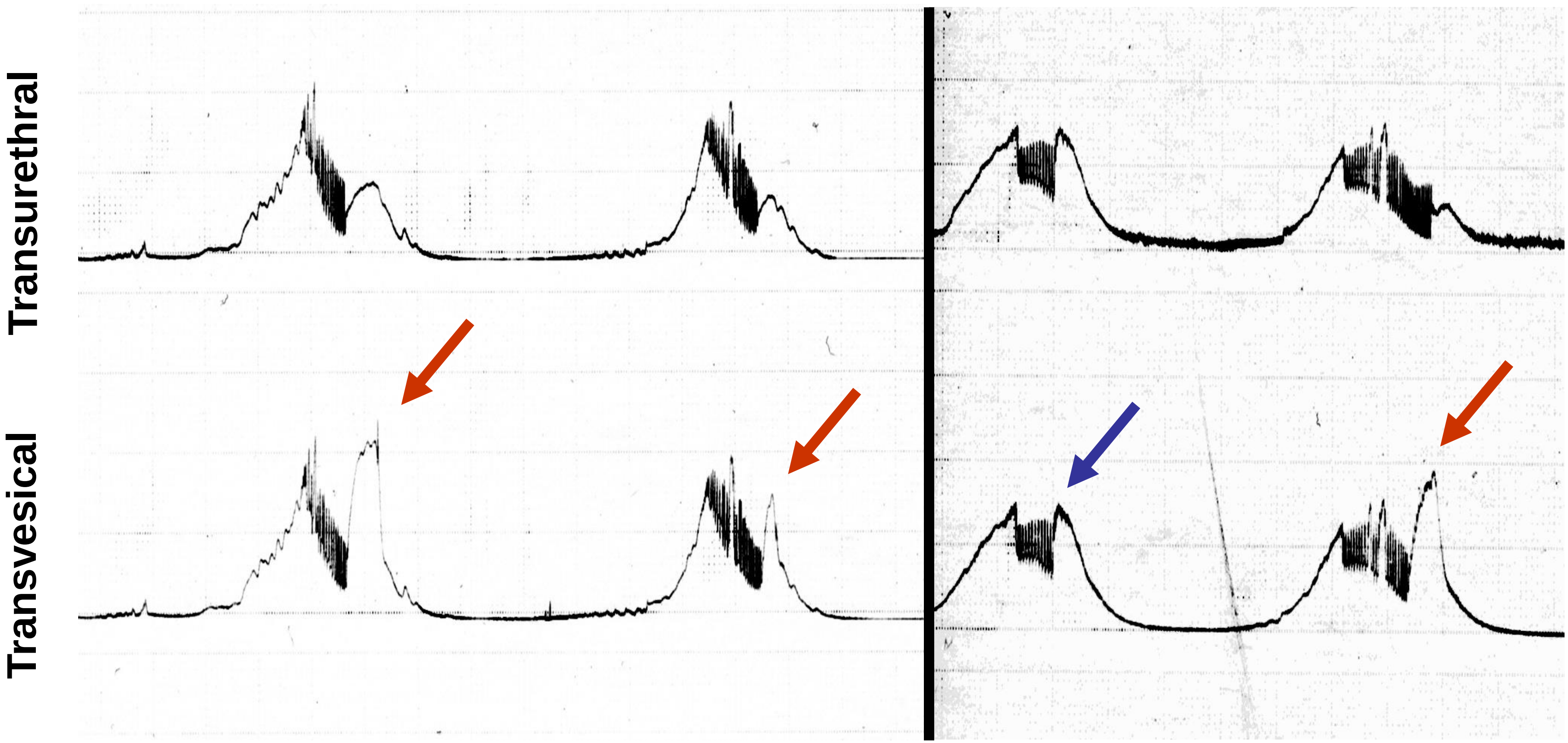
Transvesical



Arrows Point to Apparent Closing Pressures

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



Outline

- **Animal models for basic and translational research**
  - Species differences in LUT anatomy and physiology
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- **Measurement of LUT function**
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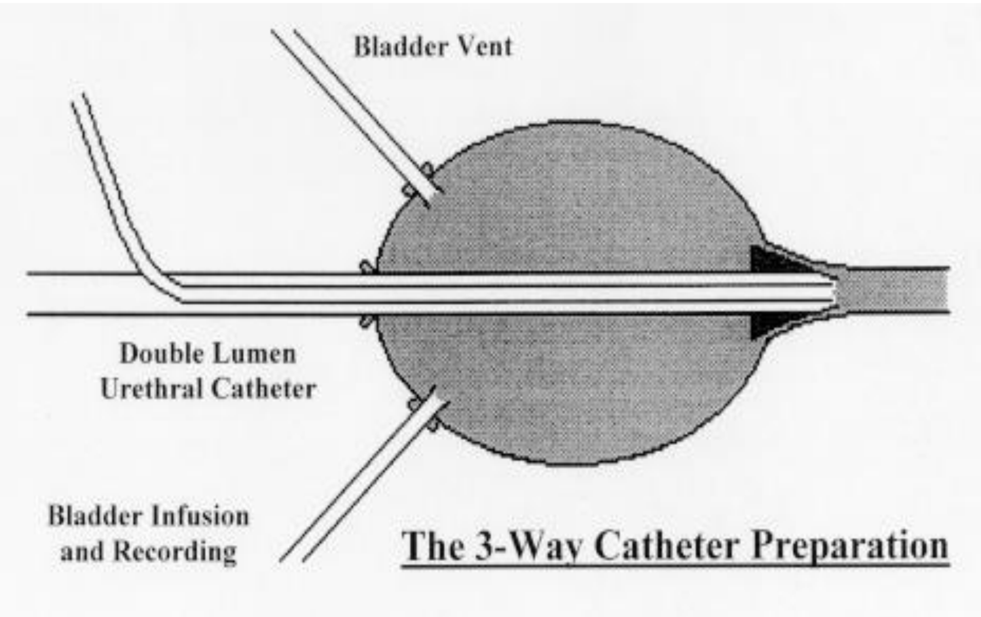
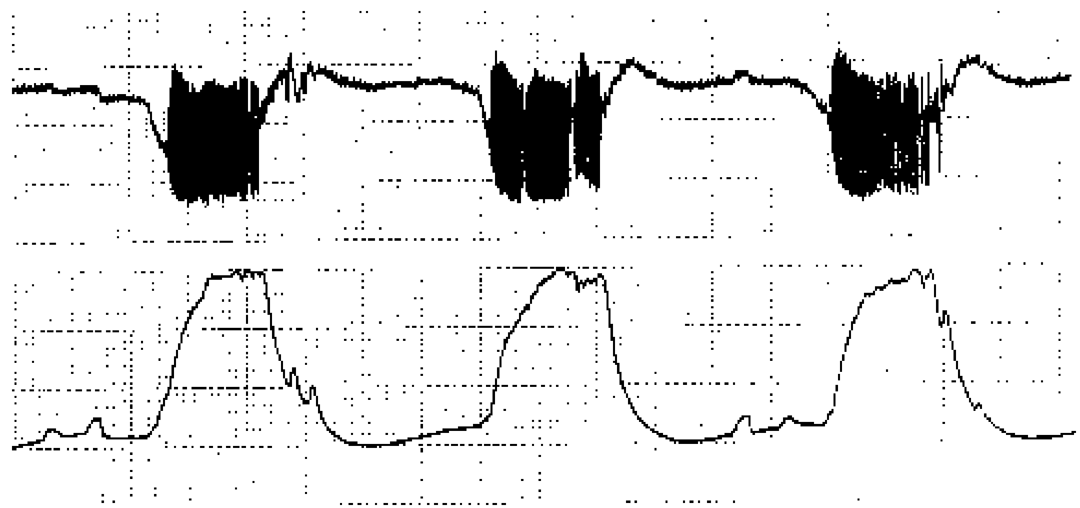
Simultaneous Isolated Bladder and Urethra



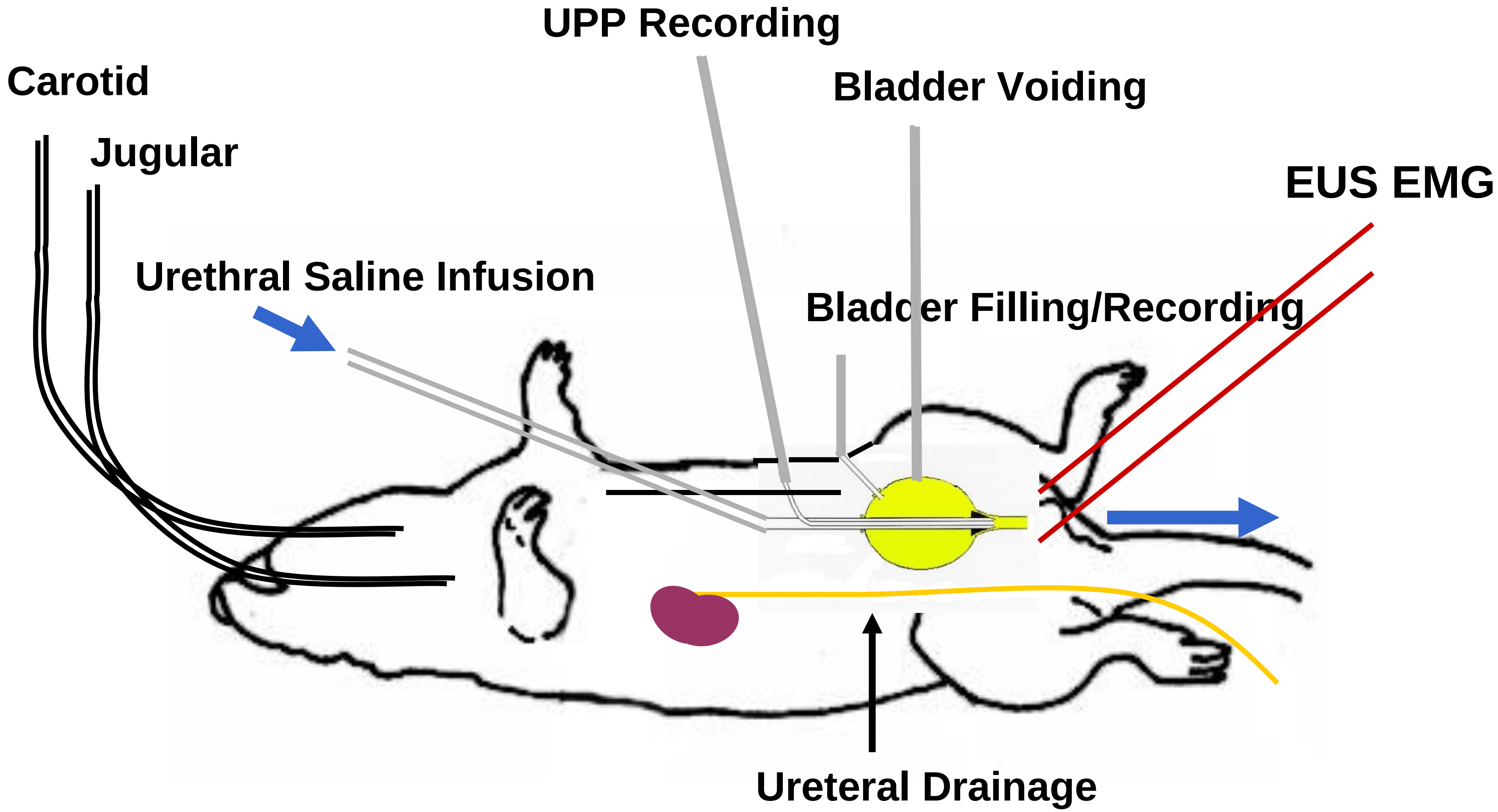
- Fraser MO, Flood HD, de Groat WC, 1995, *Journal of Urology*, 153: 461A.
- Jung SY, Fraser MO, et al., 1999, *Journal of Urology*, 162: 204.
- Kakizaki H, Fraser MO, de Groat WC, 1997, *American Journal of Physiology*, 272: R1647.

Urethral Perfusion Pressure

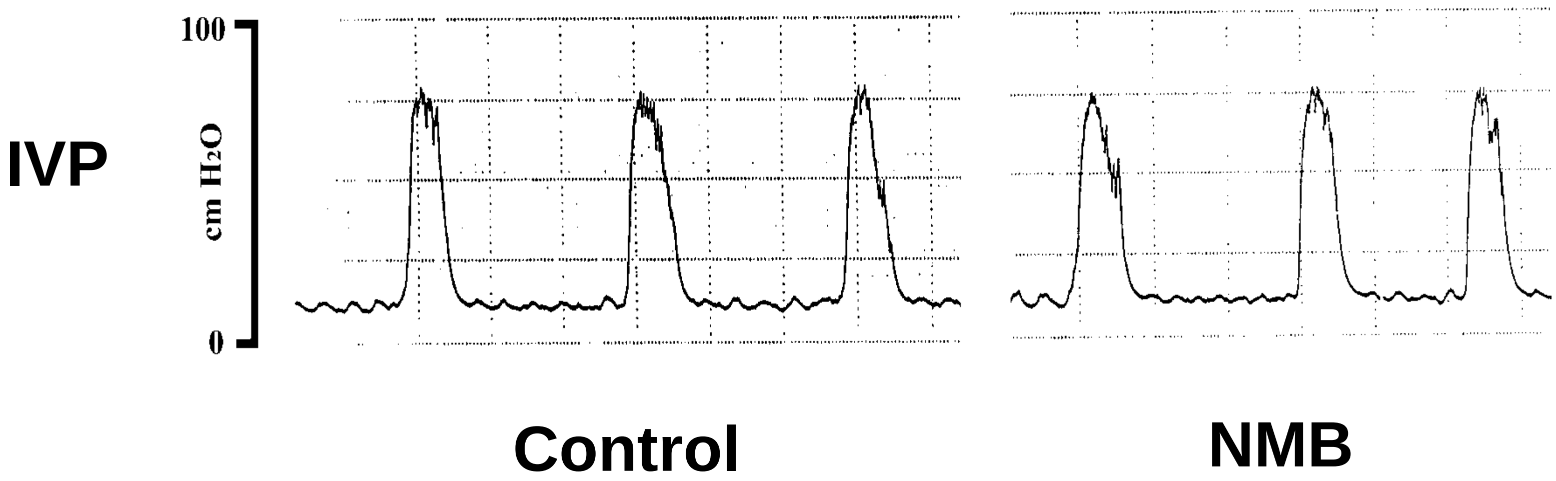
Bladder Pressure



Rat UPP (3-Way System)

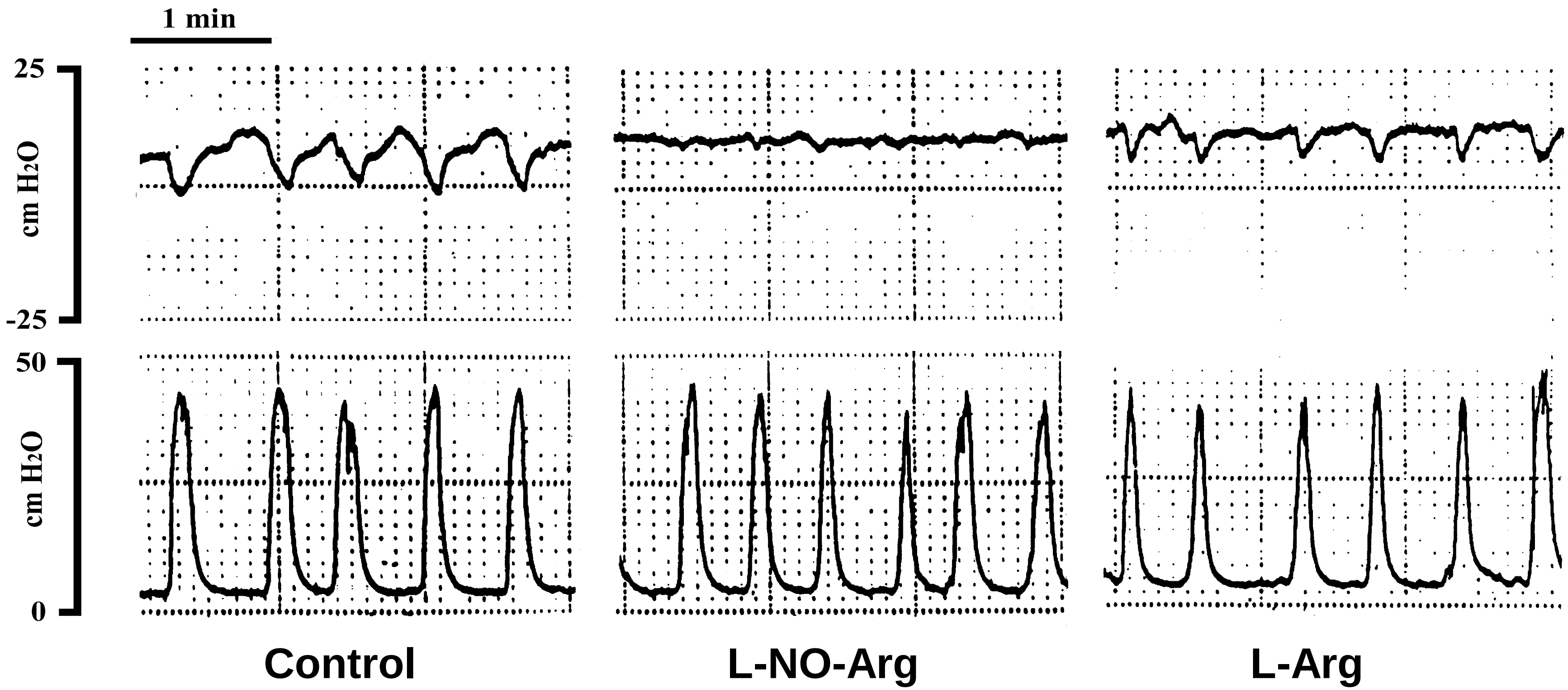


Isovolumetric IVP and UPP



- **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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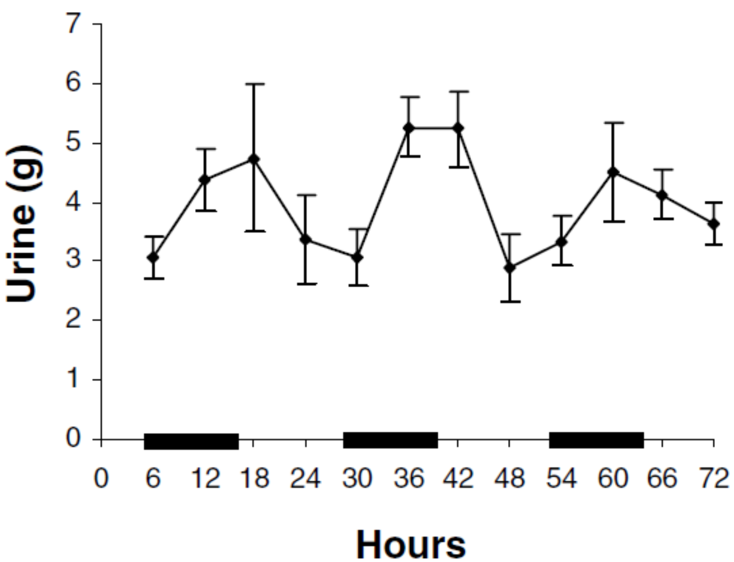
Metabolism Cage / VSOP

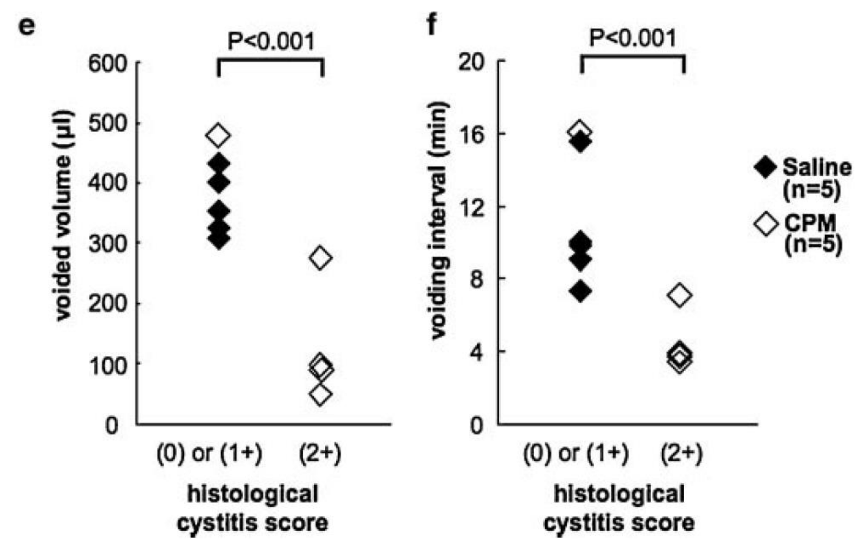
- **Measurement of LUT function**

Metabolism Cage



2 moa SD Rats, 3 days
Eriksson et al. 2004 Exp Physiol 89(4):427-433

Voided Spots on Paper



7 woa ddy mice, 2 hr
Sugino et al. 2008 NUU 27:548-552

Both measure functional bladder capacity, similar to a bladder diary for humans.
VSOP suffers from short sampling window and need to fix time due to diurnal variation.

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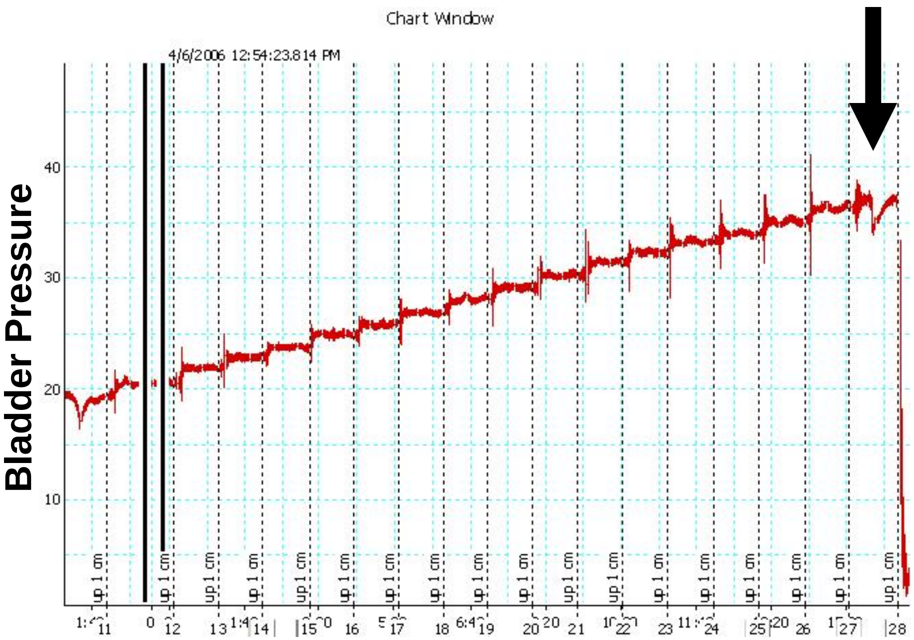
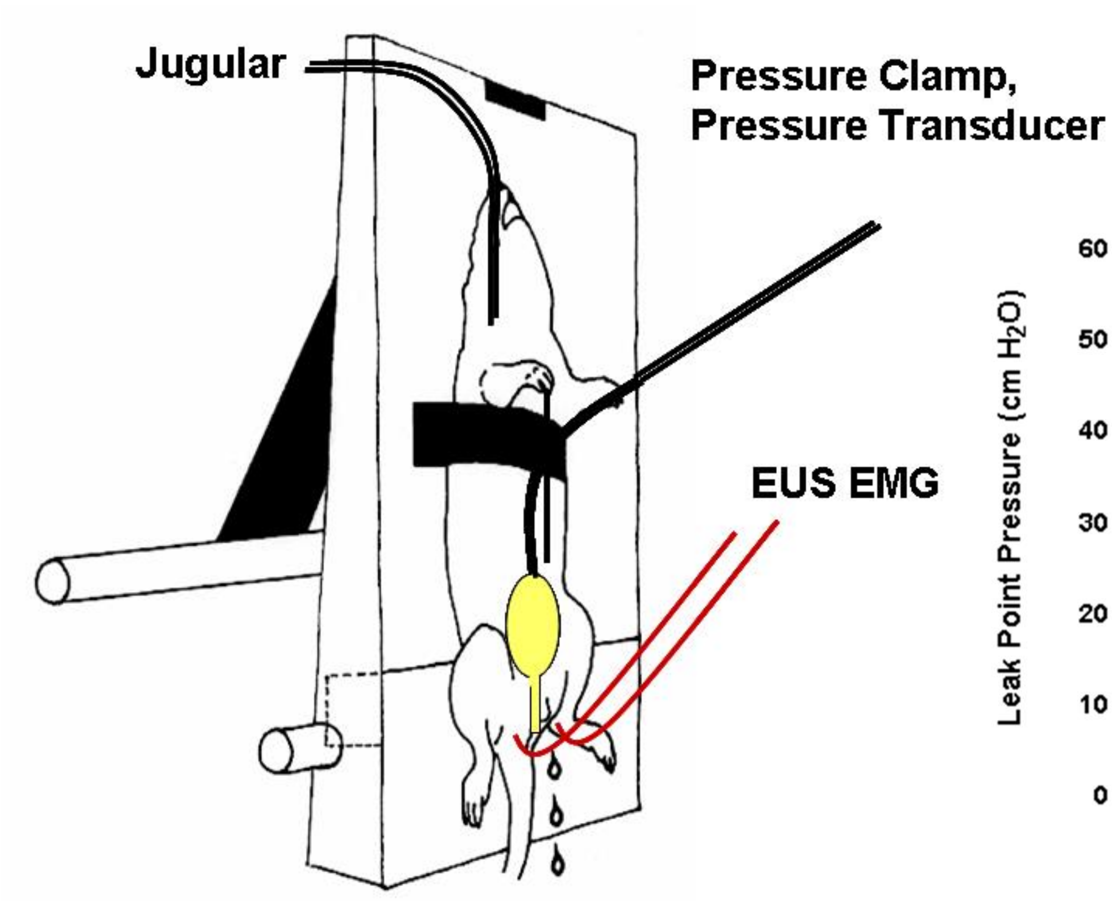
Leak Point Pressure Measurements

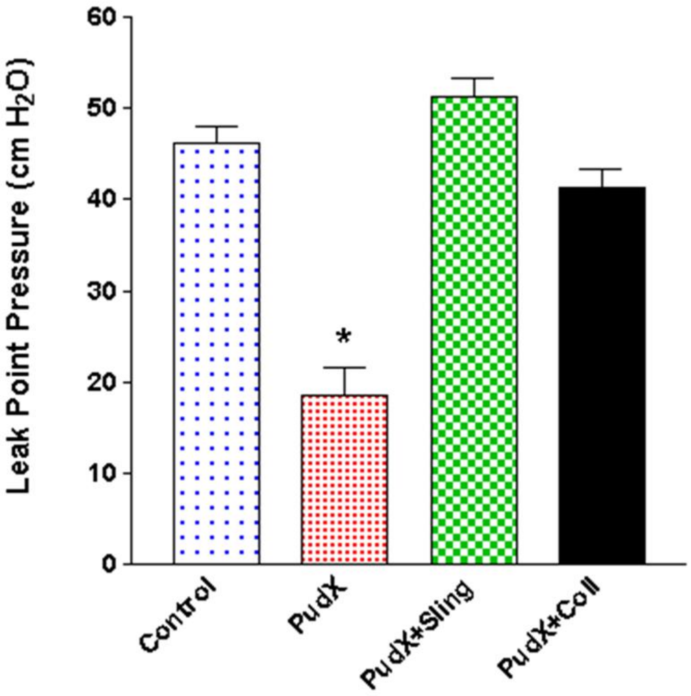
- **Leak Point Pressure Measurement**
  - Developed a method whereby bladder pressure could be increased in an experimental animal without evoking a micturition reflex
    - Does not require rat to cough or sneeze, animal can be supine or vertical
    - Includes entire outlet (from bladder neck through meatus)
    - Incremental increases in pressure until bladder pressure exceeds outlet resistance → Leak!
    - Affected by both striated and smooth muscle surgical and pharmacological manipulations



Intravesical Pressure Clamp Trace

Rat Leak Point Pressure





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Conclusions

- Significant species differences in anatomy and normal physiology, drug metabolism and SAR
- These differences must be addressed, understood and accounted for in order to interpret experimental results properly
- All animal appear to age similarly (health span/life span)
- Species and/or approach differences may yield seemingly disparate results, only by head-to-head comparison can these seeming differences be parsed out
- A variety of techniques are available for measuring LUT function, proper interpretation depends on in depth consideration of LUT physiology and measurement technique interaction with it

End