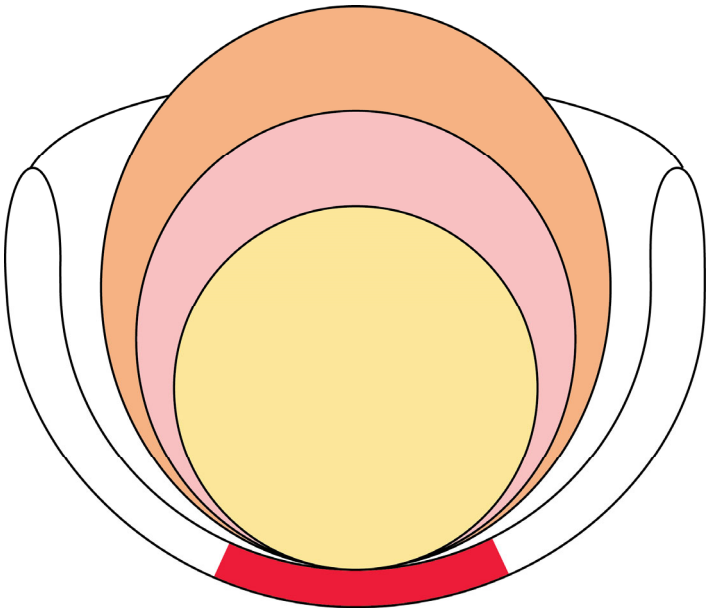
THE SOCIETY FOR PELVIC RESEARCH

FOURTH ANNUAL MEETING



MEETING PROGRAM

November 16-17, 2019
Charleston, SC, USA

#PelvRes19

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The **International Continence Society** ICS

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AUA Urology Research Conferences Advisory Board (URCAB) American Urological Association

Special Thanks to:

Ms. Elizabeth Foss CTC, MCC, MBA, President of My Travel Elf, Inc./MTE Vacations, Naples, FL. As was the case with SPRs 2016 –18, Beth has once again played a pivotal role as an advisor and in organizing the venue, food and beverages, audiovisual, etc., and proofing the vendor contracts involved with this event. She has once again generously donated her time and expertise.

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The 2019 SPR Abstract Review Committee

Vivian Cristofaro, PhD
Kelvin P. Davies, PhD
Carol A. Podlasek, PhD
Michael R. Ruggieri, Sr, PhD
Matthew O. Fraser, PhD

The 2019 SPR Trainee Award Committee

Mary F. Barbe, PhD (Chair)
Maryrose P. Sullivan, PhD
All those faculty that participated in judging

The 2019 SPR Onsite Meeting Preparation / Execution Volunteers

Sylvia O. Saudicani, PhD
Michael R. Odom, PhD Candidate
Zhonghua Aileen Ouyang, PhD Candidate
Vivian Cristofaro, PhD

All those who attended and participated in the Fourth Annual Meeting

Our Mission Statement

To promote the highest standards of basic and translational science research directed toward understanding benign pelvic visceral and musculoskeletal function and dysfunction through education, interaction, and advocacy.

Our Vision Statement

The Society for Pelvic Research will be the premier professional organization for career basic and translational scientists and engineers interested in benign urogenital, distal gut and pelvic floor research.

It will promote multidisciplinary interaction, intellectual cross-fertilization, networking for collaboration and career development through the regular dissemination of information via online resources, annual meetings and workshops, and published guidelines and standards for basic and translational science research.

Our History

The beginnings of the SPR trace back to the 2006 at a scientific meeting reception. Over refreshments, Matt Fraser and Mike DiSanto discussed starting a society that would serve the needs of the career basic/translational researchers in the field of Pelvic Medicine. It took until December of 2013 to take that initial thought and do something about it. An email went out to the original group and discussions and plans began. Additional Board Members were selected and invited to join in order to gain their expertise and a multidisciplinary balance.

The Society for Pelvic Research was born.

The Society For Pelvic Research is a North Carolina Non-Profit Corporation that filed on May 12, 2015. The company's filing status is listed as Current-Active and its File Number is 1444909.

Tax exempt status under Internal Revenue Code (IRC) Section 501(c)(3) was granted effective May 12, 2015. Donors can deduct contributions under IRC Section 170. The Society may accept tax deductible bequests, devises, transfers or gifts under Sections 2055, 2106 0r 2522.

The Society for Pelvic Research Public Charity Status is 509(a)(2).

This year's meeting represents the cumulative efforts of our board, volunteer members, advisors, partners and funders over the past year. We are already looking forward to next year's meeting.

Our Board of Directors

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Associate Professor, Department of Surgery, Duke University and Durham VA Medical Centers

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Associate Professor, Departments of Urology and Neuroscience, Albert Einstein College of Medicine

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Michael R. Odom, PhD Candidate
Graduate Student, Department of Physiology, Brody School of Medicine, East Caroline University

Members at Large
Kelvin P. Davies, PhD
Professor, Departments of Urology and Physiology & Biophysics, Albert Einstein College of Medicine

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Michael R. Ruggieri, Sr, PhD
Professor, Department of Anatomy and Cell Biology, Temple University School of Medicine

Onsight Annual Meeting Trainee Awards Committee Chair
Mary F. Barbe, PhD
Professor, Department of Anatomy and Cell Biology, Temple University School of Medicine

Marketing Strategist and **Webmaster**
China Chien, B.S.

Program Summary

November 15, 2019

6:00 PM **Trainee Affairs Committee Workshop** - **Matthew O. Fraser, PhD**

8:00 PM **Trainee Social Event**

November 16, 2019

7:00 AM **Breakfast / Trainee Breakfast with the Experts**

8:00 AM **Welcome, Opening Remarks, Mission of the SPR** - Matthew O. Fraser, PhD

8:10 AM **Session 1: Physiology I**

Moderators: Ekta Tiwari, PhD, Post-Doctoral Fellow and Matthew O. Fraser, PhD
Key Note Speaker - **Fievos L. Christofi, PhD AGAF**
Q&A
Oral Presentations - Abstracts S1A1-S1A6
Q&A

10:15 AM **Break**

10:30 AM **Session 2: Models and Methods**

Moderators: Shelby N. Harper, Medical Student and Maryrose P. Sullivan, PhD
State of the Art Speaker - **Lance Zirpel, PhD**
Q&A
Oral Presentations - Abstracts S2A7-S2A12
Q&A

12:35 PM **Lunch**

1:35 PM **Session 3: Pharmacology I**

Moderators: Michael R. Odom, PhD Candidate and Johanna L. Hannan, PhD
State of the Art Speaker - **Trinity Bivalacqua, MD PhD**
Q&A
Oral Presentations - Abstracts S3A13-S3A17
Q&A

3:30 PM **Break**

3:45 PM **Session 4: NIH and Societal Funding/Support**

Moderators: Lindsey K. Burleson, Medical Student and Georgi V. Petkov, PhD
NIH/NICHD Guest Speaker - **Donna Mazloomdoost, MD FACOG**
AUA Guest Speaker - **Carolyn J. M. Best, PhD**
ANMS Guest Speaker - **Fievos L. Christofi, PhD**
SMSNA and ISSWSH Speaker - **Johanna L. Hannan, PhD**
Q&A

5:30 PM **Reception**

7:30 PM **Adjourn for the Day**

November 17, 2019

7:00 AM **Breakfast**

8:00 AM **Welcome to Day 2** - Maryrose P. Sullivan, PhD

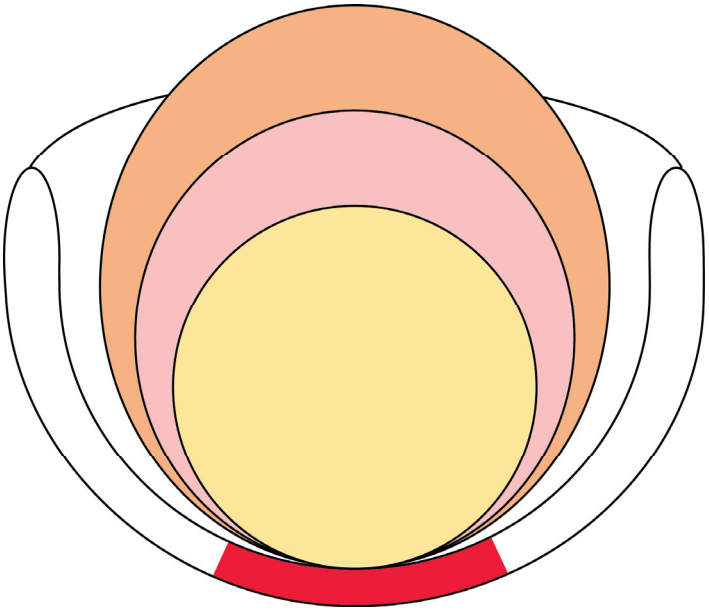
8:05 AM **Session 5: Pharmacology II**

Moderators: Jiajie Jessica Xu, DVM Resident and Sylvia O. Suadicani, PhD
Key Note Speaker– **R. Clinton Webb, PhD**
Q&A
Oral Presentations - Abstracts S5A18-S5A23
Q&A

Program Summary

December 17, 2019

10:10 AM	**Break**
10:25 AM	**Session 6: Physiology II**
	Moderators: Zhonghua Aileen Ouyang, PhD Candidate and Kelvin P. Davies, PhD
	Special Guest Speaker – **Pamela A. Moalli, MD PhD**
	Q&A
	Oral Presentations - Abstracts S6A24-S6A28
	Q&A
12:20 PM	**Lunch**
1:25 PM	**Session 7: Physiology III**
	Moderators: Cody L. Dunton, PhD Candidate and Carol A. Podlasek, PhD
	Oral Presentations - Abstracts S7A26-S7A30
	Q&A
2:25 PM	**Break**
2:40 PM	**Trainee Awards Presentations** - Mary F. Barbe, PhD and Michael E. DiSanto, PhD
2:50 PM	**Closing Remarks** - Matthew O. Fraser, PhD
3:00 PM	**Meeting Adjourns**



#PelvRes19

Program in Detail

November 15, 2019

Trainee Affairs Committee Workshop

6:00 PM 1:00 **Workshop Leader - Matthew O. Fraser, PhD**
On Mentoring and Being Mentored

8:00 PM 2:00 **Trainee Social Event:** Aqua Terrace Roof-Top Bar

November 16, 2019

7:00 AM 1:00 **Breakfast/Trainee Breakfast with the Experts**
Experts - Carolyn J. M. Best, PhD, Trinity Bivalacqua, MD PhD, Fievos L. Christofi, PhD AGAF, Pamela A. Moalli, MD PhD, R. Clinton Webb, PhD, Lance Zirpel, PhD

8:00 AM 0:10 **Welcome, Opening Remarks, Mission of the SPR**
SPR President: Matthew O. Fraser, PhD

Session 1: Physiology I

Moderators: Ekta Tiwari, PhD, Post-Doctoral Fellow and Matthew Fraser, PhD

8:10 AM 0:40 **Key Note Speaker - Fievos Christofi, PhD AGAF**
Targeting Enteric Glia in GI Diseases and Motility Disorders – Recent Progress on Post-operative Ileus

8:50 AM 0:10 **Q&A**

Oral Presentations - Abstracts S1A1-S1A6

9:00 AM 0:10 **S1A1** Fernanda B.M. Priviero, PhD, Post-Doctoral Fellow - *Toll Like Receptor-9 activation impairs cavernosal reactivity in obese mice*

9:10 AM 0:10 **S1A2** Marcia Urban-Maldonado, BS - *Role of Pannexin 1 channels in stress-induced pelvic pain and urinary symptoms in mice*

9:20 AM 0:10 **S1A3** Cody L. Dunton, PhD Candidate - *Differential effects of pressure and stretch on urothelial cell function In vitro*

9:30 AM 0:10 **S1A4** Wenbin Yang, PhD - *Aoah suppresses PPARγ-mediated gut dysbiosis*

9:40 AM 0:10 **S1A5** Francis M. Hughes Jr, PhD - *Chronic bladder outlet obstruction causes NLRP3-dependent inflammation in the hippocampus and depression in rats: a possible mechanism underlying psychosocial disorders associated with LUTS*

9:50 AM 0:10 **S1A6** Violeta N. Mutafova-Yambolieva, MD PhD - *Is NT5E/CD73 involved in adenosine production in suburothelium/lamina propria during filling of the murine bladder?*

10:00 AM 0:15 **Q&A**

10:15 AM 0:15 **Break**

Session 2: Models and Methods

Moderators: Shelby N. Harper, Medical Student and Maryrose P. Sullivan, PhD

10:30 AM 0:40 ~~**State of the Art Speaker - Lance Zirpel, PhD**~~
~~*Neuromodulation for Urological and Bowel Dysfunction: Research-Driven Directions*~~

11:10 AM 0:10 **Q&A**

Program in Detail

November 16, 2019

Session 2: Models and Methods (Continued)

Moderators: Shelby N. Harper, Medical Student and Maryrose P. Sullivan, PhD

Oral Presentations - Abstracts S2A7-S2A12

11:20 AM 0:10 **S2A7** Jiajie Jessica Xu, DVM Resident - *Effects of sedative and anesthetic agents on urodynamic and anesthetic parameters in male cats*

11:30 AM 0:10 **S2A8** Lindsey K. Burleson, Medical Student - *Validation of an animal model of pelvic radiation induced female sexual and urinary dysfunction*

11:40 AM 0:10 **S2A9** Jonathan M. Beckel, PhD - *Increasing the pH of Urothelial Lysosomes Induces Bladder Hyperactivity and Inflammation*

11:50 AM 0:10 **S2A10** Ekta Tiwari, PhD, Post-Doctoral Fellow - *Nerve transfer for restoration of lower motor neuron-lesioned bladder and urethra function: establishment of a canine model and interim pilot study results*

12:00 PM 0:10 **S2A11** Anna S. Nagle, PhD - *Regional and directional variation in bladder wall micromotion instigated by electrostimulation as measured by transabdominal Anatomical Motion Mode (AMM) ultrasound*

12:10 PM 0:10 **S2A12** Zhonghua Aileen Ouyang, PhD Candidate - *Real time closed loop control of bladder function with dorsal root ganglia sensory feedback and sacral root electrical stimulation*

12:20 PM 0:15 **Q&A**

12:35 PM 1:00 **Lunch**

Session 3: Pharmacology I

Moderators: Michael R. Odom, PhD Candidate and Johanna L. Hannan, PhD

1:35 PM 0:40 **State of the Art Speaker: Trinity Bivalacqua, MD PhD**

Surgical and Biomaterial Advances for Regeneration of the Urogenital Tract

2:15 PM 0:10 **Q&A**

Oral Presentations: Abstracts S3A13-S3A17

2:25 PM 0:10 **S3A13** Alan S. Braverman, PhD - *Nicotinic receptors on nerve terminals induce acetylcholine release in canine bladder*

2:35 PM 0:10 **S3A14** Brothely Malique Jones, Graduate Student - *Histamine does not directly contract urinary bladder smooth muscle*

2:45 PM 0:10 **S3A15** Shawn Choe, MS- *Optimization of Sonic hedgehog delivery to the penis from self-assembling nanofiber hydrogels to preserve penile morphology after cavernous nerve injury*

2:55 PM 0:10 **S3A16** Andrew Draganski, PhD - *Curcumin-Loaded Nanoparticles Protect Erectile Function in a Rat Model of Type-2-Diabetes*

3:05 PM 0:10 **S3A17** Shaojing Ye, PhD - *Intravesical macrophage migration inhibitory factor (MIF) and activation of intravesical MIF receptors mediate PAR4-induced bladder pain*

Program in Detail

November 16, 2019

Session 3: Pharmacology I (Continued)

Moderators: Michael R. Odom, PhD Candidate and Johanna L. Hannan, PhD

3:15 PM 0:15 **Q&A**

3:30 PM 0:45 **Break**

Session 4: Special Topic – NIH and Societal Funding/Support

Moderators: Lindsey K. Burleson, Medical Student and Georgi V. Petkov, PhD

3:45 PM 0:30 **NIH/NICHD Guest Speaker** - **Donna Mazloomdoost, MD FACOG**
4:15 PM 0:20 **AUA Guest Speaker** - **Carolyn J. M. Best, PhD**
4:35 PM 0:20 **ANMS Guest Speaker** - **Fievos L. Christofi, PhD**
4:55 PM 0:20 **SMSNA and ISSWSH Speaker** - **Johanna L. Hannan, PhD**
5:15 PM 0:15 **Q&A**

5:30 PM 2:00 **Reception**

7:30 PM **Adjourn for the Day**

November 17, 2019

7:00 AM 1:00 **Breakfast**
8:00 AM 0:05 **Welcome to Day 2**
SPR Treasurer: Maryrose P. Sullivan, PhD

Session 5: Pharmacology II

Moderators: Jiajie Jessica Xu, DVM Resident and Sylvia O. Suadicani, PhD

8:05 AM 0:40 **Key Note Speaker: R. Clinton Webb, PhD**
Dysregulated innate immune responses contribute to erectile dysfunction
8:45 AM 0:10 **Q&A**
Oral Presentations: Abstracts S5A18-S5A23
8:55 AM 0:10 **S5A18** Nora M. Haney, MD, Resident - *Impact of RhoGDI Gene Transfection of Bladder Smooth Muscle Contractility in a Validated Ex-vivo Murine Model*
9:05 AM 0:10 **S5A19** Britney N. Hudson, Graduate Student - *Utilizing Enzymes to Induce Hypoxia for an in vitro Bladder Outlet Obstruction Model*
9:15 AM 0:10 **S5A20** Vanessa Dela Justina, Graduate Student - *NLRP-3 Inflammasomes inhibition improves bladder reactivity in dbdb-/- mice*
9:25 AM 0:10 **S5A21** John Malysz, PhD - *Subtype-specific Kv7/KCNQ channel activators reduce excitability and contractility in human detrusor smooth muscle*
9:35 AM 0:10 **S5A22** Nagat Frara, PhD, Post-Doctoral Fellow - *The nicotinic receptor agonist Epibatidine induces contraction more frequently in sub-mucosal than sub-serosal canine bladder smooth muscle strips*

Program in Detail

November 17, 2019

Session 5: Pharmacology II (Continued)

Moderators: Jiajie Jessica Xu, DVM Resident and Sylvia O. Suadicani, PhD

9:45 AM 0:10 **S5A23** Shelby N. Harper, Medical Student - *Calcium Pyrophosphate and Monosodium Urate Activate the NLRP3 Inflammasome within Bladder Urothelium via Reactive Oxygen Species and Thioredoxin Interacting Protein*

9:55 AM 0:15 **Q&A**

10:10 AM 0:15 **Break**

Session 6: Physiology II

Moderators: Zhonghua Aileen Ouyang, PhD Candidate and Kelvin P. Davies, PhD

10:25 AM 0:40 **State of the Art Speaker: Pamela A. Moalli, MD PhD**
Urogynecologic meshes: a lesson in the development of novel devices

11:05 AM 0:10 **Q&A**

Oral Presentations: Abstracts S6A24-S6A28

11:15 AM 0:10 **S6A24** Michael R. Odom, PhD Candidate - *Castration Mediated Schwann Cell Dedifferentiation Leads to Slower Nerve Conduction, Decreased Neuritogenesis, and Nitrergic Neuron Loss*

11:25 AM 0:10 **S6A25** Mary F. Barbe, PhD - *Functional and histological changes in the dog urinary bladder after different decentralization and reinnervation strategies*

11:35 AM 0:10 **S6A26** Yolanda Cruz, PhD - *Gonadal hormones and anesthetics influence threshold of transcutaneous electrical stimulation to induce external urethral sphincter reflex activity in female rats*

11:45 AM 0:10 **S6A27** Bradley A. Potts, MD, Resident - *Barrington's Reflexes Revisited: Proximal Urethral Electrostimulation Causes Remarkable Excitatory Bladder Response in Spinal Cord Intact Rats*

11:55 PM 0:10 **S6A28** Geneva E. Cruz, BS - *Bladder reinnervation by somatic nerve transfer to pelvic nerve vesical branches does not reinnervate the urethra*

12:05 PM 0:15 **Q&A**

12:20 PM 1:00 **Lunch**

Session 7: Physiology III

Moderators: Cody L. Dunton, PhD Candidate and Carol A. Podlasek, PhD

Oral Presentations: Abstracts S7A29-S7A33

1:20 PM 0:10 **S7A29** John Sobieski, BA - *Comparison of the actomyosin ATPase inhibitor calponin and the sphingosine-1-phosphate cell differentiation pathway regulatory enzymes in vaginal wall smooth muscle of women with and without pelvic organ prolapse*

1:30 PM 0:10 **S7A30** Michael R. Odom, PhD Candidate - *Ex Vivo Akt Inhibition Reverses Castration Induced Penile and Pudendal Artery Endothelial Dysfunction*

Program in Detail

November 17, 2019

Session 7: Physiology III (Continued)

Moderators: Cody L. Dunton, PhD Candidate and Carol A. Podlasek, PhD

1:40 PM	0:10	**S7A31** Vivian Cristofaro, PhD - *Effect of α-synuclein mutation on bladder function*
1:50 PM	0:10	**S7A32** Elizabeth Kalmanek, BS - *Caspase signaling in ED patients and animal models*
2:00 PM	0:10	**S7A33** Laura G. White, BS - *Acute Ozone Exposure Increases Bladder Pro-Inflammatory Cytokines and Mitochondrial Respiration in Female Mice*
2:10 PM	0:15	**Q&A**
2:25 PM	0:15	**Break**
2:40 PM	0:10	**Trainee Awards Presentations:** Mary F. Barbe, PhD and Michael E. DiSanto, PhD
2:50PM	0:10	**Closing Remarks:** Matthew O. Fraser, PhD
3:00 PM		**Meeting Adjourns**

Best Trainee Presentation Awards

Sponsored by The International Continence Society

First Prize Winner - Michael R. Odom, PhD Candidate

S7A30 - "*Ex Vivo Akt Inhibition Reverses Castration Induced Penile and Pudendal Artery Endothelial Dysfunction*"

Second Prize Winner - Shelby N. Harper, Medical Student

S5A23 - "*Calcium Pyrophosphate and Monosodium Urate Activate the NLRP3 Inflammasome within Bladder Urothelium via Reactive Oxygen Species and Thioredoxin Interacting Protein*"

Third Prize Winner - Lindsey K. Burleson, Medical Student

S2A8—"*Validation of an animal model of pelvic radiation induced female sexual and urinary dysfunction*"

The Top Ranked Abstracts by the Abstract Review Committee

S2A8 - **Lindsey K. Burleson, Medical Student** *et al*.
"Validation of an animal model of pelvic radiation induced female sexual and urinary dysfunction "

S3A15 - **Shawn Choe MS, Medical Student** *et al*.
"Optimization of Sonic hedgehog delivery to the penis from self-assembling nanofiber hydrogels to preserve penile morphology after cavernous nerve injury"

S3A17 - **Shaojing Ye PhD** *et al*.
"Intravesical macrophage migration inhibitory factor (MIF) and activation of Intravesical MIF receptors mediate PAR4-induced bladder pain"

S6A25 - **Mary F. Barbe PhD** *et al*.
"Functional and histological changes in the dog urinary bladder after different decentralization and reinnervation strategies"

S6A24 - **Michael R. Odom, PhD Candidate** *et al*.
"Castration Mediated Schwann Cell Dedifferentiation Leads to Slowe Nerve Conduction, Decreased Neuritogenesis, and Nitrergic Neuron Loss"

Keynote Speaker

Dr. Fievos L. Christofi, PhD AGAF

Department of Anesthesiology
Ohio State University, Columbus, Ohio, USA

Dr. Fievos L. Christofi, PhD AGAF, is a Professor and Vice Chair of Research in Basic, Translational and Clinical Research in the Department of Anesthesiology at The Ohio State University. He has served as Chair of the Appointment, Promotion and Tenure Committee in his department and the College of Medicine. At the national level, he is a Councilor of the American Neurogastroenterology and Motility Society (ANMS), member of the advisory panel for the ANMS Institute, Chair of the ANMS Grants Program Review Committee, and Member of the International Scientific Planning Committee for the Federation of Neurogastroenterology and Motility (FNM) Societies. In the past he was appointed as Gastrointestinal Section Editor for Current Opinion in Pharmacology (COPHAR) and is the new Editor for Basic Science Research for Neurogastroenterology and Motility, the journal for the Society. Dr. Christofi has served on numerous NIH study sections, including serving as permanent member of the Clinical, Integrative and Molecular Gastroenterology NIH Study Section. He has been a mentor to students, postdocs, residents, fellows, and faculty trainees in research supported by scholarships, fellowships (F32), Mentored Clinician Scientist Awards (K08), NIH Loan Repayment Grants for physician scientists (NIH LRP), grants from the Pediatric Society of Anesthesiologists (PSA) and NIH pilot grants (CTSA).

A major focus of research efforts in the department of Anesthesiology is on 'neurobiology of disease' in the gut, spinal cord and brain in the context of inflammation – these efforts include studies on post-operative neurological complications such as postoperative ileus and postoperative gastrointestinal dysfunction (POGD), ischemic spinal cord injury, cognitive dysfunction and implementation of enhanced recovery protocols after surgery. Dr. Christofi's research program that has been funded by the National Institutes of Diabetes, Digestive & Kidney Diseases (NIDDK) for the past 25 years, has focused on purinergic signaling in the 'little brain' in the gut (i.e. the enteric nervous system) in health and disease – Purinergic receptors are potential therapeutic targets in GI diseases and motility disorders including postoperative ileus and POGD. Their recent efforts are directed at the study of enteric glia, "γλοια" ("glue of the nervous system") in GI diseases and motility disorders 'in mice and man'. Off-course, glia do much more than just provide structural support. Their specific target of investigation is glial pathogenic mechanisms of postoperative ileus, an acute functional bowel disorder with disruption of motility and constipation induced by surgical trauma and gut manipulation.

Despite the implementation of enhanced recovery protocols after surgery, postoperative ileus remains a huge clinical problem, and extended hospitalization costs can run into the billions in the U.S. Key pathogenic mechanisms include study of (1) 'Reactive glia' in the context of inflammation in enteric neuropathy, disruption of motility and postoperative ileus. (2) Mechanosensation in enteric glia in health and disease. (3) Purinergic signaling pathways. (4) Endothelin signaling pathways and (5) Interleukin pathways. (6) Development of novel innovative human in vitro models to study the 'human little brain' in the gut, enteric glial networks, or motility reflexes is required to translate findings to humans in GI Diseases and Disorders. They developed the 'First in Man' Routine Patch Clamp Recordings in Isolated Neural Networks of Human Myenteric Ganglia – 'A Novel Model of The Human Little Brain' for further studies in normal and diseased states. Their group is in the process of developing a gut mucosal biopsy 'neural network' model from patients with functional gastrointestinal disorders or inflammatory bowel diseases to study enteric neuropathies and channelopathies using patch clamp. Translatability of findings from animals to humans is a key aspect of their studies – after all, 'human is not always a good model for the mouse'.

State of the Art Speakers

Dr. Lance Zirpel, PhD

Pelvic Health & Gastric Therapies, Restorative Therapies Group
Medtronic, Minneapolis, MN

Dr. Lance Zirpel, PhD, is Chief Scientist for the Pelvic Health & Gastric Therapies at Medtronic. He manages the Research portfolio focused on understanding and improving current sacral and tibial neuromodulation therapies, while also defining future therapies through preclinical and early clinical feasibility studies.

Dr. Zirpel received his PhD in Physiology and Biophysics from the University of Washington School of Medicine, conducted post-doctoral work at the University of Utah School of Medicine, and established an NIH-funded research program as a faculty member in the Department of Neuroscience at the University of Minnesota Medical School before joining Medtronic in 2012.

Dr. Trinity Bivalacqua, MD PhD

Departments of Urology and Oncology
Johns Hopkins Hospital and The James Buchanan Brady Urological Institute
Baltimore, MD

Dr. Trinity Bivalacqua, MD MPH, is the R. Christian B. Evenson Professor of Urology and Oncology at the Johns Hopkins Hospital and is Director of Urologic Oncology at The James Buchanan Brady Urological Institute. He received his undergraduate degree, medical and PhD (pharmacology) graduate degrees from Tulane University and completed a urology residency at Johns Hopkins. He completed a Urology Care Foundation post-doctoral fellowship.

He is actively involved in resident and medical student education. He has a clinical subspecialty interest in prostate and bladder cancer as well as sexual dysfunction. He has a basic translational research lab focusing on urothelial carcinoma and regenerative medicine which is supported by peer-reviewed grant funding. He is a previous recipient of the AUA Rising Star in Urology Research Award and Gold Cystoscopy Award.

Special Guest Speakers

Dr. Donna Mazloomdoost, MD FACOG

Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
National Institutes of Health, Bethesda, MD

Dr. Donna Mazloomdoost, MD FACOG, joined NICHD in 2017 as a medical officer and project scientist/ director of the NICHD Pelvic Floor Disorders Network. She completed her medical training at Emory University School of Medicine and pursued an obstetrics/gynecology (OBGYN) residency at the University of Cincinnati. After her residency graduation, Dr. Mazloomdoost joined the faculty at Georgetown University Hospital in Washington, D.C., where she was an assistant professor and served as the assistant clerkship director for the medical students. During this time, she was recognized several times for her teaching. She left Georgetown after 6 years in 2013 to pursue a fellowship in female pelvic medicine and reconstructive surgery at the Tri-Health/Good Samaritan Hospital in Cincinnati, OH and completed training in 2016.

Her research has focused on pelvic floor disorders, postoperative pain control, health-seeking behaviors, and pelvic anatomy, and she has written multiple publications in peer-reviewed journals. Her experience with research networks began as a fellow when she served on the Fellows Pelvic Research Network (FPRN) Steering Committee for 2 years, publishing her own multicenter trial while advising others on theirs.

She also sees and operates on patients with pelvic floor disorders in the DC/Maryland/Virginia area.

Dr. Carolyn J.M. Best, PhD

AUA Office of Research
American Urologic Association, Linthicum, MD

Dr. Carolyn J.M. Best, PhD, is Director of Research for the American Urological Association (AUA). The AUA promotes the highest standards of urological clinical care through education, research, and the formulation of health care policy. Dr. Best is responsible for all programs, events, and resources of the AUA's Office of Research, which supports urologic research through funding, education, and advocacy. This support includes a large diversity of programs that facilitate research for the spectrum of urologic cancers and benign diseases and conditions. This also includes collaborative relationships with federal and non-federal funding agencies, patient advocacy groups, AUA sections and subspecialty societies, and other urologic research stakeholders.

Dr. Best also served at the Program Director of the Department of Defense (DoD) Prostate Cancer Research Program (PCRP) of the Congressionally Directed Medical Research Programs (CDMRP) for eight years, during which she received the Department of the Army Commander's Award for Civilian Service and the Prostate Cancer Foundation's Award for Exemplary Public Service and Outstanding Leadership.

Dr. Best obtained her PhD in experimental pathology from the University of Maryland, Baltimore, and subsequently trained at the National Cancer Institute (NCI) in the Laboratory of Pathology, Laboratory of Biosystems and Cancer, and the Molecular Therapeutics Program. During her training at the NCI, Dr. Best was the inaugural recipient of the Sallie Rosen Kaplan Fellowship for Women Scientists in Cancer Research, among other awards.

Keynote Speaker

Dr. R. Clinton Webb, PhD

Department of Physiology
Medical College of Georgia, Augusta University, Augusta, GA

Dr. R. Clinton Webb, PhD, graduated from the Southern Illinois University, in 1971, and received his Ph.D. in Anatomy from the University of Iowa in 1976. After postdoctoral training at the University of Michigan and the Universitaire Instelling Antwerpen, he joined the faculty of the Department of Physiology at the University of Michigan in 1979, reaching full Professor in 1989. In 1999, he joined the faculty at the Medical College of Georgia serving as Professor and Chairperson of the Department of Physiology. In 2018, he stepped down from the chairperson position. He is now Professor of Physiology and holds the Herbert S. Kupperman Chair in Cardiovascular Disease.

Webb's research interests focus on the physiology of the cardiovascular system with particular emphasis on hypertension and sexual dysfunction. He has published over 350 peer-reviewed papers and 100 book chapters and reviews and is currently funded by a National Institutes of Health (NIH) Program Project Grant.

In addition to being the Editor-in-Chief Elect of Vascular Pharmacology, he is also an Associate Editor of the American Journal of Hypertension and Guest Editor of Hypertension. He is a member of numerous editorial boards, including those of Journal of Cardiovascular Pharmacology and American Journal of Physiology.

Webb is an active member of several societies, including the American Heart Association, American Physiological Society and the American Society for Pharmacology and Experimental Therapeutics.

Dr. Webb has served on numerous peer-review committees for the National Institutes of Health and the American Heart Association.

State of the Art Speaker

Dr. Pamela A. Moalli, MD PhD

Departments of Obstetrics, Gynecology & Reproductive Sciences and Bioengineering, the McGowan Institute of Regenerative Medicine, and the Clinical & Translational Research Institute
University of Pittsburgh, Pittsburgh, PA

Dr. Pamela A. Moalli, MD PhD, is Professor of Obstetrics, Gynecology and Reproductive Sciences (OB/Gyn/RS) with a joint appointment in the Department of Bioengineering. She received a Bachelor in Sciences degree with distinction from Brown University in 1985. After taking a year off to train and compete with the United States Rowing Team, she participated in the NIH sponsored Medical Scientist Training Program at Northwestern University from 1986-1994 earning a PhD in molecular and cellular biology and a medical degree. She completed her residency in Obstetrics and Gynecology at Magee-Womens Hospital from 1994 to 1998. From 1998 to 2000, she completed a fellowship in Urogynecology and Reconstructive Pelvic Surgery.

Dr. Moalli is director of the Division of Urogynecology and Reconstructive Pelvic Surgery and director of the fellowship in Female Pelvic Medicine and Reconstructive Surgery. She directs one of the only laboratories in the country using a bench to bedside approach to develop novel solutions for the diagnosis and treatment of pelvic floor disorders. She has established herself as a national and international leader on biomaterials used to repair pelvic organ prolapse. She employs state of the art technology including computational models to provide insight into mechanisms by which commonly performed surgeries fail so as to improve patient outcomes. Finally, she studies mechanisms of maternal birth injury as a pathway to prevention.

She has played a critical role as both a clinician and a basic scientist in the Urinary Incontinence Treatment and Pelvic Floor Disorders Networks. She has become a nationally and internationally recognized leader in all of her research areas serving as chair, panel expert, and reviewer for multiple organizations. Dr. Moalli has a portfolio of research funding from federal, industry and private foundations. She has been funded by the NIH since entering medical school in 1986. She has served as chair of the Pelvic Floor Disorders Registry since 2016, which includes 522 transvaginal mesh trials. Her experiences have afforded her the expertise to mentor medical and graduate students, and clinical and research fellows to empower them with the appropriate tools that will allow them to succeed. Currently, she has authored over 100 publications, 8 chapters and multiple review papers.

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S1A1

Title: Toll Like Receptor-9 activation impairs cavernosal reactivity in obese mice

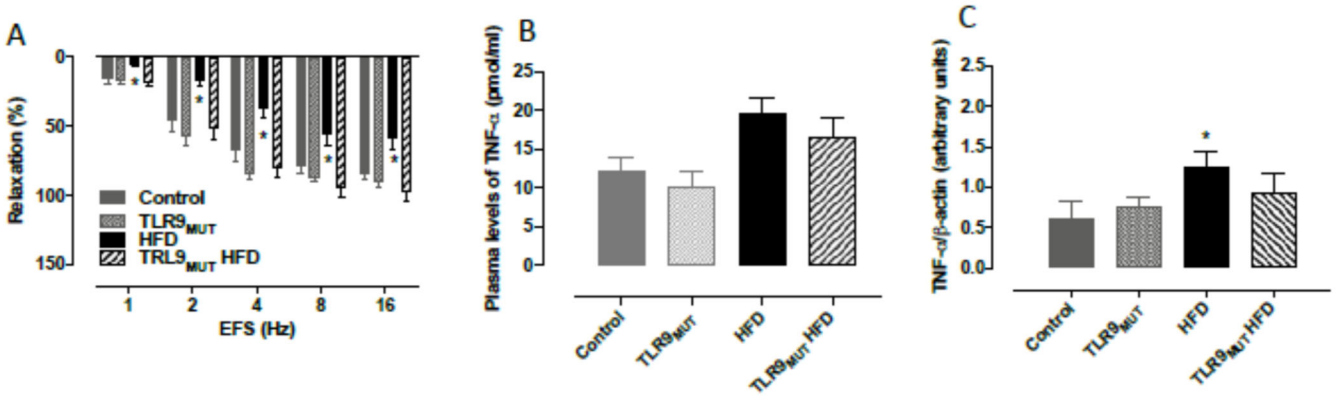
Authors: Fernanda Priviero[1], Fabiano Calmasini[1,2], Vanessa Dela Justina[1], Edson Antunes[2], R. Clinton Webb[1]

Affiliations: [1]Department of Physiology, Augusta University, Augusta, GA, USA
[2]Department of Pharmacology, State University of Campinas, Campinas, SP, Brazil

Introduction/Objectives: Obesity is considered a low grade of chronic inflammation and erectile dysfunction (ED) has been shown to be related with inflammatory markers. Chronic infusion of TNF-α caused ED in mice while TNF-α knockout mice exhibited improvement in the relaxation of the corpus cavernosum. Since obesity triggers an inflammatory process, we aimed to investigate the hypothesis that in obesity, Toll-like receptor 9 (TLR9) activation leads to increased TNF-α levels and an impairment in the cavernosal reactivity.

Methods: Four-week old male C57BL6 (WT) and TLR9 mutant mice ($TLR9_{MUT}$) were fed a standard chow or high fat diet (HFD) for 12 weeks. Blood glucose and epididymal fat were analyzed. The corpus cavernosum was then mounted on a strip myograph for functional evaluation. Contractile and relaxing responses of the corpus cavernosum were evaluated by electrical field stimulation (EFS) and concentration response curves to phenylephrine and acetylcholine. Protein expression of TLR9, MyD88, COX-2, TNF-α, IL-1β, eNOS, nNOS and Rho-kinase α, F4/80 and CD11b were measured by western blot. Plasma levels of TNF-α were measured by ELISA.

Results: After 12 weeks of HFD, compared to standard chow, both WT and $TLR9_{MUT}$ mice exhibited increased body weight, epididymal fat and fasting glucose with a slightly smaller magnitude in $TLR9_{MUT}$ mice. EFS-induced relaxation of the corpus cavernosum was decreased in WT HFD but not in $TLR9_{MUT}$ HFD (Fig A). Reactivity of the corpus cavernosum presented no changes to acetylcholine-induced relaxation while a trend to increased contraction was seen in WT HFD only. In the corpus cavernosum, protein expressions of TLR9, MyD88, COX-2, IL-1β and eNOS were similar in all groups studied. However, nNOS and Rho-kinase α protein expressions were, respectively, decreased and increased in WT HFD (but not in $TLR9_{MUT}$ HFD) compared to control. Although levels of TNF- α presented only a trend to increase in the circulation of mice fed HFD (Fig B), the cavernosal expression of TNF- α was increased in HFD mice but not in $TLR9_{MUT}$ fed HFD Fig C).



Conclusions: These data are suggestive that in obesity, activation of TLR9 in macrophages leads to increased TNF- α and impaired cavernosal relaxation, which may contribute to ED by modulating the expression of nNOS and Rho-kinase.

Funding Source(s): NIH HL-134604; FAPESP 2016/20592-8

S1A2

Title: Role of Pannexin 1 channels in stress-induced pelvic pain and urinary symptoms in mice.

Authors: Marcia Urban-Maldonado, Melissa E. Laudano and Sylvia O. Suadicani*

Affiliations: Department of Urology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY, USA.

Introduction/Objectives: Pannexin 1 (Panx1) channels are key components of the urothelial mechanosensory, transduction and sensory system. They are mechanosensitive and provide a direct conduit for urothelial ATP release in response to bladder distension. They also interact with purinergic P2X7 receptors to form a functional complex that once activated, triggers a mechanism of self-regenerating ATP release that can amplify and modulate downstream transmission of sensory information within the bladder and from the bladder to the CNS. Little is known, however, of the extent to which Panx1 channels are involved in bladder pathology. The goal of this study is to evaluate the involvement of Panx1 channels in the emergence of pelvic pain and urinary symptoms observed in the context of chronic stress.

Methods: Wildtype (WT) and Panx1-null mice (Panx1tm1a(KOMP)Wtsi strain; 3-5 months old), and the environmental stress model of 96 hrs exposure to continuous illumination (CI) were used in this study. Pelvic tactile sensitivity and bladder function were evaluated at baseline (pre-CI) and at the day after ending the 96 hrs of CI stress using the Von Frey test and the void stain on paper (VSOP) method, respectively. ATP levels in spontaneously voided urine were quantified pre- and post-CI using the luciferin-luciferase assay. At conclusion of the functional evaluations, CI stressed and age-matched controls (maintained under conventional 12 hour/day illumination) were euthanized, the bladder harvested and urothelial Panx1 mRNA levels were quantified by real time PCR. An additional group of WT mice was submitted to awake continuous cystometry at baseline and then at the day after the 96hr of CI to assess stress-induced changes in urodynamic parameters and effect of intravesical treatment with the Panx1 channel blocker mefloquine (300nM). Data is expressed as mean±SEM and statistical difference determined by paired or unpaired Student's t-test.

Results: In WT mice, pelvic tactile threshold decreased 18-fold (pre-CI: 0.22 ± 0.06 vs post-CI: 0.012 ± 0.007; N = 4 $p<0.01$) and micturition frequency increased 2-fold (pre-CI: 0.56 ± 0.04 vs post-CI: 1.11 ± 0.12; N = 4 $p<0.01$) after 96 hrs exposure to CI stress. These signs of emergence of pelvic pain and urinary frequency in stressed WT mice were accompanied by (1) a 2.5-fold increase in Panx1 expression in the bladder urothelium when compared to non-stressed WT mice (2.53 ± 0.29 stressed/non-stressed controls; N=4; $p<0.001$), and (2) a 2-fold increase in urothelial ATP release when compared to baseline levels (1.8 ± 0.24 post-CI/pre-CI; N = 4; $p=0.007$). Notably, emergence of pelvic pain and urinary frequency was abrogated in Panx1-null mice submitted to CI stress and urothelial ATP release was not different from control pre-CI levels. In addition, urodynamic assessment of WT mice after 96 hrs CI stress indicated marked bladder hyperactivity that was normalized by intravesical treatment with the Panx1 channel blocker mefloquine.

Conclusions: Panx1 channels are essential players in mechanisms of stress-induced pelvic pain and urinary frequency, likely by amplifying urothelial ATP release and signaling that can ultimately change the bladder sensory and motor responses, and cause bladder sensitization.

Funding Source(s): None

S1A3

Title: Differential effects of pressure and stretch on urothelial cell function *In vitro*.

Authors: Cody Dunton[1], J. Todd Purves[2,3], Francis M. Hughes[2], Jiro Nagatomi[1]

Affiliations: [1]Department of Bioengineering, Clemson University, Clemson, SC, USA, [2]Departments of Surgery and [3]Pediatrics, Duke University Medical Center, Durham, NC, USA

Introduction/Objectives: Partial bladder outlet obstruction (pBOO) is frequently accompanied by inflammation leading to fibrosis of the bladder wall and reduced tissue compliance[2]. Our previous study demonstrated that exposure of bladder urothelial cells to elevated hydrostatic pressures (40 cmH_2O for 1 min) induced extracellular ATP release, which activated the NLRP3 inflammasome, via purinergic signaling[6]. Since bladder cells *in vivo*, are exposed to cycles of storage and voiding pressures, as well as large strain during storage, in the present study, we examined the mechanisms of pressure- and strain-induced responses in the urothelium.

Methods: A custom apparatus[7] was used to expose rat urothelial cell line MYP3 cells to simulated pressure cycles of either unobstructed, early stage (ES) pBOO, or late stage (LS) pBOO for 72 hours. Following exposure, the soluble collagen content in the supernantant was determined using a Sircol assay, intracellular caspase-1 activity was determined using an established method[8], and RT-PCR was conducted to examine profibrotic gene expression. Using another custom setup , MYP3 cells were exposed to mechanical stimuli with the following configurations: 125% stretch applied at a constant rate over 5 minutes and then held constant for 5 minutes, 40 cmH_2O for 1 minute, or 125% stretch applied in the same manner followed by 40 cmH_2O for 1 minute, to simulate pathologic bladder distension and voiding respectively. After exposure to mechanical stimuli, the extracellular ATP concentration was measured using a commercially available kit (Life Technologies) and intracellular caspase-1 activity was measured using an established method[8]. In addition, urothelial cells were isolated from 2 and 6 week pBOO rat bladders and RT-PCR studies were conducted to examine profibrotic gene expression.

Results: MYP3 cells exposed to all simulated pBOO pressure cycling for 72 hours demonstrated increased mRNA expression for prolyl 4-hydroxylase (P4H) and collagen type 1 (COL1) expression, as well as elevated caspase-1 activity compared to cells exposed to atmospheric pressure. Similarly, urothelial cells isolated from pBOO rats demonstrated increased expression of COL1 and P4H, as well as lysyl-oxidase (LOX), after 6 weeks. Exposure of MYP3 cells to pressure and distension independently or in tandem resulted in a 1.80-fold, 1.73-fold and 1.65-fold increase in extracellular ATP levels and a 1.48-fold, 1.44-fold, and 1.46-fold increase in intracellular caspase-1 activity, respectively, compared to the control.

Conclusions: In the present study, we hypothesized that changes in pressure cycling associated with different stages of pBOO result in progression of associated disorders. Increased voiding and storage pressure associated with late stage pBOO resulted in increased COL1 and P4H indicating fibrosis, which is consistent with elevated expression of COL1, P4H, and LOX found in 6 week pBOO rats. Exposure of urothelial cells to pathologic levels of pressure and strain in tandem did not produce an additive response in extracellular ATP concentration or caspase-1 activity suggesting that pressure and strain may be acting via similar mechanosensory pathways. Further research, however, is needed to improve understanding of mechanically induced bladder pathologies such as pBOO.

Funding Source(s): NIH (R01DK103534, P20GM103444), NSF (1264579)

S1A4

Title: Aoah suppresses PPARγ-mediated gut dysbiosis

Authors:, Wenbin Yang[1], Lizath M Aguiniga[1], Ryan E. Yaggie[1], John M. Rosen[1], Colleen B. Bushell[4], Michael E. Welge[4], Bryan A. White[3], Anthony J. Schaeffer[1], and David J. Klumpp[1,2]

Affiliations: Departments of Urology[1] and Microbiology-Immunology[2], Feinberg School of Medicine, Northwestern University, Chicago, IL and the Carl R. Woese Institute for Genomic Biology[3] and Applied Research Institute[4] at University of Illinois at Urbana-Champaign, IL

Introduction/Objectives: Corticotropin-releasing factor (CRF) is a critical mediator of stress responses and diverse biologic processes including mood and GI function. In a genetic screen for loci modulating pelvic pain severity, we identified *Aoah*, the locus encoding acyloxyacyl hydrolase, as a novel genetic suppressor of *Crf*. Because CRF is a modulator of GI function, we examined the influence of *Aoah* on gut microbiota.

Methods: Microbiota were analyzed by 16S rDNA sequence analyses. Conditional knockout mice were generated by mating Crf-CRE mice with floxed AhR and PPARγ mice. Epithelial barrier function was assessed by quantifying trans-epithelial electrical resistance in an Üssing chamber. Visceral pain in response to bladder distension was quantified by abdominal EMG.

Results: AOAH-deficient mice exhibited visceral pain and altered fecal microbiota, relative to wild type mice, and visceral pain was resolved by co-housing or oral gavage of wild type fecal stool. Dissection revealed an enlarged cecum in AOAH-deficient mice, a phenotype of germ-free rodents, and AOAH-deficient mice exhibited reduced cecal barrier function consistent with a “leaky gut” phenotype. CRF can alter GI permeability, and we observed that conditional knockout of *Crf* transcriptional regulators AhR and PPARγ rescued the leaky gut phenotype of AOAH-deficient mice. Moreover, conditional knockout of PPARγ restored normal microbiota to AOAH-deficient mice.

Conclusions: These data suggest that PPARγ is a driver of dysbiosis and leaky gut downstream of AOAH and presents a novel therapeutic target for CRF-dependent gut dysbiosis.

Funding Source(s): R01 DK103769

S1A5

Title: Chronic bladder outlet obstruction causes NLRP3-dependent inflammation in the hippocampus and depression in rats: a possible mechanism underlying psychosocial disorders associated with LUTS

Authors: Francis M. Hughes Jr.[1,2], Nathan A. Hirshman[1], Huixia Jin[1], Shelby N. Harper[1], J. Todd Purves[1,2,3]

Affiliations: [1]Department of Surgery, Division of Urology, Duke University Medical Center, Durham, NC. [2]Department of Bioengineering, Clemson University, Clemson, SC. [3]Department of Pediatrics, Duke University Medical Center, Durham, NC, USA

Introduction/Objectives: Numerous reports anecdotally link LUTS (and the underlying diseases such as bladder outlet obstruction) with depression, yet causality and mechanisms are unknown. Breakthroughs in other organ systems, particularly in the gastrointestinal system, have demonstrated that peripheral insults may trigger inflammation in the central nervous system (CNS). This inflammation is dependent on activation of the NLRP3 inflammasome and can cause psychosocial disorders, particularly depression. Recently, we have shown that the cyclophosphamide-induced model of acute cystitis can trigger inflammation in the hippocampus and the appearance of depressive symptoms in rats. However, this model has some severe limitations including: possible direct central effects of cyclophosphamide and its metabolites in the CNS, its acute nature (<24 h), and the lack of a clinical correlate since cyclophosphamide-induced hemorrhagic cystitis is relatively rare. Thus, we have pivoted our focus on the bladder-brain axis to bladder outlet obstruction, a more bladder-centric, clinically-relevant model.

Methods: Female rats were divided into 4 groups: control, sham, BOO or BOO + gly (glyburide; an NLRP3 inhibitor). BOO was created by inserting a 1 mm transurethral catheter, tying a suture around the urethra, and removing the catheter. Glyburide (NLRP3 inhibitor) was provided by subcutaneous pellet (50 mg, 21 day release) replaced every 21 days. Rats were analyzed 12 weeks post-op (chronic BOO). Inflammation in the hippocampus was assessed by Evan's blue extravasation. Depression symptoms were assessed by the open field and sucrose preference tests, two standard assays of depression symptoms.

Results: There was a significant increase in Evans blue extravasation into the hippocampus in BOO rats which was completely blocked by the NLRP3 inhibitor glyburide. In addition, there was a significant decrease in exploratory behavior in the open field behavioral assay and a decrease in sucrose preference in the BOO rats, both of which are signs of depression. Like inflammation, these symptoms were diminished to control values by glyburide.

Conclusions: BOO, a bladder-localized event, stimulates NLRP3-fdependent inflammation in the hippocampus of rat after 12 weeks and this inflammation causes depression. This study provides the first-ever causative explanation of the previously anecdotal link between BOO and depression.

Funding Source(s): NIDDK: R01DK103534 (PI - Purves)

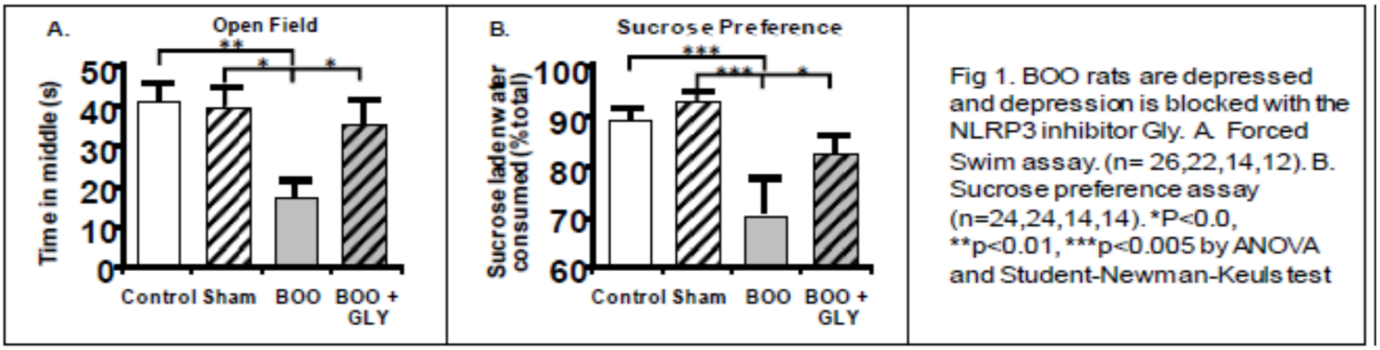


Fig 1. BOO rats are depressed and depression is blocked with the NLRP3 inhibitor Gly. A. Forced Swim assay. (n= 26,22,14,12). B. Sucrose preference assay (n=24,24,14,14). *P<0.0, **p<0.01, ***p<0.005 by ANOVA and Student-Newman-Keuls test

S1A6

Title: Is NT5E/CD73 involved in adenosine production in suburothelium/lamina propria during filling of the murine bladder?

Authors: Violeta N. Mutafova-Yambolieva*, Benjamin Kwok, Priya Kukadia

Affiliations: Department of Physiology and Cell Biology, University of Nevada Reno School of Medicine, Reno, NV 89557, USA

Introduction/Objectives: Adenosine (ADO) is a biologically active nucleoside that participates in the normal functions of key organ systems in the body, including the lower urinary tract. In the bladder, ADO inhibits contraction of the detrusor smooth muscle, reduces release of ATP from the urothelium, and stimulates umbrella cell exocytosis. ADO is generated from AMP by ecto-5'-nucleotidase (NT5E), also known as CD73. The primary extracellular precursors of AMP are ATP, nicotinamide adenine dinucleotide (NAD), and ADP-ribose. We have recently reported that ADO and its precursors are present in suburothelium (SubU)/lamina propria (LP) of the murine bladder during filling. Surprisingly, previous immunohistochemistry studies have suggested that NT5E/CD73 is absent in the murine bladder mucosa. How then is ADO produced in the SubU/LP of the filling bladder? The present study was designed to address key aspects of this question.

Methods: We recently developed a novel *ex vivo* murine bladder preparation with the detrusor smooth muscle removed that allows direct access to the SubU/LP of the filling bladder. We applied the highly-fluorescent analog of AMP, 1*N*6-etheno-AMP (εAMP, 2 μM) to the SubU/LP surface of the preparation and monitored the decrease of εAMP substrate and the appearance of εADO product by HPLC-FLD methodologies. εAMP degradation was compared in preparations of wildtype (C57BL/6J) and *Nt5e-/-* mice (10-12 weeks of age). The preparations were placed in 3-ml chambers and bathed in oxygenated Krebs bicarbonate solution (KBS, pH 7.4, 37oC). Aliquots of the bath solution were collected before adding εAMP and at 2-10-minute intervals after starting bladder filling with KBS at 15 μl/min in the presence of εAMP. We evaluated the possible presence of a releasable AMPase by adding εAMP to aliquots of the bathing solution that were collected during bladder filling and examining whether εAMP was degraded in this solution, in the absence of bladder tissue.

Results: Approximately 40% of εAMP was degraded in SubU/LP of unfilled and filled bladder preparations ($P>0.05$), suggesting that ADO is produced from AMP in SubU/LP in a mechano-insensitive manner. In aliquots of bath solution incubated with unfilled or filled bladders, about 10% of εAMP was degraded to εADO, suggesting that a small portion of the εAMP degradation is mediated by releasable AMPase. In preparations with completely inactivated *Nt5e*, about 20% of εAMP was degraded to εADO, suggesting that at least half of the AMPase activity is associated with CD73. Finally, no releasable AMPase activity was observed in *NT5E-/-* preparations ($P>0.05$, substrate in KBS vs. substrate in aliquot of bath solution).

Conclusions: Adenosine is produced from AMP in the SubU/LP at both low and high intravesical volumes and pressures. The degradation of AMP to ADO in SubU/LP is mediated by at least three enzymes: 1) Membrane-bound CD73, 2) Unknown membrane-bound activity that is different from CD73, and 3) Releasable (soluble) enzyme likely associated with releasable cytosolic NT5E. The availability of ADO in SubU/LP could be altered by inhibitors or activators of AMPases to improve bladder function.

Funding Source: NIH grant DK 41315

S2A7

Title: Effects of sedative and anesthetic agents on urodynamic and anesthetic parameters in male cats

Authors: Jiajie J. Xu*[1,2], Zuha Yousuf[2,3], Zhonghua Ouyang[2,3], Tara Martin[1], Patrick A. Lester[1], Tim M. Bruns[2,3]

Affiliations: [1]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, [2]Biointerfaces Institute, University of Michigan, Ann Arbor, MI, [3]Biomedical Engineering Department, University of Michigan, Ann Arbor, MI

Introduction/Objectives: Urodynamic studies in animals, particularly cats, are essential to understanding the pathophysiology of bladder disorders. However, sedative and anesthetic agents used for these studies can influence bladder function. We compared the effects of select agents (dexmedetomidine [dex], alfaxalone [alfax], propofol, isoflurane [iso], and alpha-chloralose [chloralose]) on urodynamic and anesthetic parameters. We hypothesized that the tested agents would have varied effects on anesthetic and urodynamic parameters.

Methods: Adult male cats (n=5) were sedated at least three times per agent with dex (0.04 mg/kg intramuscular [IM] bolus; reversed with matched volume of atipamazole after session), alfax (5 mg/kg IM bolus followed by 0.08 – 0.13 mg/kg/min intravenous [IV] constant rate infusion [CRI]), and propofol (2 mg/kg IV bolus followed by 0.15 – 0.2 mg/kg/min IV CRI) on separate days, and sedated one time with iso (inhaled 0-2.5 %), then transitioned to chloralose (70 mg/kg IV induction, 20 mg/kg IV maintenance) during a terminal procedure. At least two cystometrograms (2 ml/min, 41°C saline) were conducted in each sedated session. Urodynamic parameters (peak pressure, bladder capacity, bladder compliance, non-voiding contractions, pressure curve slopes) and anesthetic parameters (change in heart rate [ΔHR], average heart rate [HR], reflexes, induction/recovery times) were evaluated.

Results: Many urodynamic and anesthetic parameters differed significantly between agents. Peak pressure was greatest with propofol (117 ± 10 cm H20) and lowest with iso (84 ± 13 cm H20). Bladder capacity was highest with chloralose (60 ± 9 ml) and lowest with dex (43 ± 9 ml). The number of non-voiding contractions per trial was greatest with chloralose. Propofol, chloralose, and dex had the highest slopes during the beginning (start to 100s before peak), middle (100-50s before peak), and final (50s to peak) portions of the pressure curve respectively. Cats progressed to a deeper plane of anesthesia (lower HR, smaller ΔHR, decreased reflexes) under dex (HR = 117 ± 6; ΔHR = 19 ± 21 bpm), compared to propofol (HR = 174 ± 6 bpm; ΔHR = 85 ± 22 bpm,) and alfax (HR = 211 ± 6 bpm; ΔHR = 77 ± 21 bpm). Time to induction was shortest with propofol, and time to recovery was shortest with dex combined with atipamazole reversal.

Conclusions: In this study, we compared the effects of select sedative and anesthetic agents on urodynamic and anesthetic parameters in cats, and found agent-specific differences. These findings will help interpret the confounding effects of anesthetic choice.

Funding sources: The Cohen Comparative Medicine Research Award

S2A8

Title: Validation of an animal model of pelvic radiation induced female sexual and urinary dysfunction

Authors: Lindsey K. Burleson[1]*, Shelby A. Powers[1], Michael R. Odom[1], Dillon A. Ellis[2], Jae W. Jung[2], Bridget F. Koontz[3], Johanna L. Hannan[1]

Affiliations: [1]Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA; [2]Department of Physics, East Carolina University, Greenville, NC, USA; [3]Department of Radiation Oncology, Duke University School of Medicine, Durham, NC, USA

Introduction/Objectives: Women with cervical and endometrial cancers treated with radiation therapy (RT) can experience injury to surrounding normal tissues leading to genitourinary dysfunction. While radiation therapy is indicated in 53% of cervical cancer treatment regimens, little is known on the short or long-term effects of pelvic RT induced injury. This study characterizes the impact of pelvic RT on sexual and urinary function in female rats.

Methods: Female Sprague-Dawley rats (10 weeks) received a single dose (0 or 20 Gy) of x-ray radiation to the cervix (Sham: n=5, RT: n=16). At 4 or 9 weeks post-RT, sexual function was measured in vivo by pelvic nerve stimulated increases in vaginal blood flow (VBF) via laser Doppler. VBF was normalized to mean arterial pressure (MAP) measured by carotid cannulation. Ex vivo tissue bath experiments assessed vaginal adrenergic, cholinergic and electrical field stimulated (EFS) contractions. Bladder and urethral internal and external sphincters were also assessed for cholinergic, caffeine and EFS neurogenic contractility.

Results: Following RT, maximal VBF/MAP and the area under the curve (AUC) was markedly lower 4 weeks post-RT ($p<0.05$). Interestingly by 9 weeks, VBF and AUC had normalized. Cholinergic-mediated vaginal contraction was reduced at 4 weeks post-RT but recovered at 9 weeks post-RT ($p<0.05$). After 9-weeks RT, adrenergic-mediated vaginal contraction remained elevated ($p<0.05$). Neurogenic EFS vaginal contractions were unchanged with RT. Cholinergic bladder contractions to carbachol were unchanged with RT but EFS-mediated contractions were reduced in both RT groups ($p<0.05$). Internal urethral sphincter cholinergic contractility was unchanged with RT. In contrast, external urethral sphincter contractions to both caffeine and EFS were elevated at both 4 and 9 weeks post-RT ($p<0.05$).

Conclusions: Our model of pelvic RT demonstrates impaired sexual arousal and increased vaginal contractility. Acute decrease in VBF paired with chronic increase in vaginal contractility may contribute to vaginal dryness and dyspareunia experienced post-RT. Similar to previous RT studies, the bladder developed decreased neurogenic contractions. This model will allow us to understand the pathophysiology of RT-induced sexual and urinary dysfunction and discover radioprotective therapies.

Funding Source(s): BSOM Summer Scholars Research Award, International Society for the Study of Women's Sexual Health

S2A9

Title: Increasing the pH of Urothelial Lysosomes Induces Bladder Hyperactivity and Inflammation

Authors: Jonathan M. Beckel, Chinonso Obidike, Raider Rodriguez, Stephanie L. Daugherty, William C. de Groat

Affiliations: Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA USA

Introduction/Objectives: It is known that the prevalence of bladder pathology increases with age, however the cellular mechanisms responsible remain unclear. One possibility may be defects in the endolysosomal pathway of the urothelium, which exhibits age-related changes in lysosomal degradation concurrent with increases in lysosomal pH. Thus, increased lysosomal pH in urothelial cells may play a role in bladder pathology. This project aimed to determine if direct modulation of lysosomal pH in the urothelium through intravesical administration of the lysosomotropic weak base chloroquine (CHQ) is sufficient to cause bladder hyperactivity and inflammation similar to that previously reported in aged rats.

Methods: Female Sprague-Dawley rats (~200-250g, n=10) were anesthetized using isoflurane and CHQ (100μM in sterile saline, 0.5ml) was instilled in the bladder through a transurethral catheter for 1 hour. The animals were then allowed to recover overnight for use in cystometry (n=5) or plasma extravasation experiments (n=5) the next day. For cystometry, CHQ treated rats or non-treated controls (n=5) were anesthetized with urethane and catheterized through the bladder dome. Open cystometry was then performed by perfusing Krebs solution into the bladder at a rate of 0.08ml/min. For plasma extravasation, CHQ treated rats or non-treated controls (n=5) were anesthetized using urethane, and Evans Blue (50mg/kg) was injected through a jugular vein catheter. Fifteen minutes after dye injection, the rats were sacrificed by decapitation, exsanguinated and the bladder removed. After weighing, the bladder was placed in 3 ml formamide for 72 hours. The dye present in the formamide solution was quantified by measuring optical density and then the concentration was estimated using a standard curve. Final results are expressed as μg of dye per g bladder tissue. ATP and IL-1β release were determined with the luciferin-luciferase assay and ELISA, respectively, in cultured human cells.

Results: CHQ did not alter bladder activity during the first 2 hours after intravesical administration, but on the next day it significantly decreased intercontraction interval (36.6% decrease, $p<0.0001$) and micturition threshold pressure (19.07% decrease, $p<0.01$). Plasma extravasation of Evans Blue dye also increased significantly (5.3 fold increase, $P<0.001$) in animals treated with CHQ for 1 day. Stimulation of cultured urothelial cells with CHQ (100μM) increased extracellular concentrations of ATP (33.2% after 30 min, $p<0.001$) and IL-1β (75.9% after 2hrs, $p<0.001$).

Conclusions: Our results demonstrate that intravesical instillation of CHQ can induce bladder inflammation and hyperactivity in the rat, possibly through the release of ATP and IL-1B from the urothelium suggesting that intravesical chloroquine treatment may be a suitable model for studying age-related bladder pathology.

Funding Source(s): NIH (DK106115, DK114492, DK117884)

S2A10

Title: Nerve transfer for restoration of lower motor neuron-lesioned bladder and urethra function: establishment of a canine model and interim pilot study results

Authors: Ekta Tiwari,[1]* Danielle M. Salvadeo,[2] Alan S. Braverman,[2] Nagat A. Frara,[2] Lucas Hobson,[2] Geneva Cruz,[2] Justin M. Brown,[3] Michael Mazzei,[4] Michel A. Pontari,[5] Amanda R. White,[2] Mary F. Barbe,[2] and Michael R. Ruggieri Sr.,[1,2,6]

Affiliations: [1]Department of Computer and Electrical Engineering, College of Engineering Temple University, 1947 North 12th Street, Philadelphia, PA 19122, [2]Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, [4]Department of Medicine and [5]Department of Urology, Temple University, 3501 North Broad Street, Philadelphia, PA 19140, [3]Department of Neurosurgery, Neurosurgery Paralysis Center, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114 and [6]The Shriners Hospital of Philadelphia, 3501 North Broad Street, Philadelphia, PA 19140

Introduction/Objectives: Previous patient surveys show that patients with spinal cord or cauda equina injuries prioritize recovery of bladder function. We sought to determine if nerve transfer after long-term decentralization restores bladder and sphincter function in canines.

Methods: Twenty-four female canines underwent transection of sacral roots and hypogastric nerves (S Dec, n=6), or additional transection of L7 dorsal roots (L7d+S Dec; n=7). Twelve months later, three L7d+S Dec animals underwent obturator-to-pelvic nerve and sciatic-to-pudendal nerve transfers (L7d+S Dec+Reinn). Eleven animals served as controls. Squat-and-void behaviors were tracked before and after decentralization, after reinnervation, and following awake bladder filling procedures. Bladders were cystoscopically injected with Fluorogold three weeks before euthanasia. Immediately prior to euthanasia, transferred nerves were stimulated to evaluate motor function. Dorsal root ganglia were assessed for retrogradely labelled neurons.

Results: Transection of only sacral roots failed to reduce squat-and-void postures. L7 dorsal root transection was necessary for significant reduction. Three L7d+S Dec animals showing loss of squat and void postures post-decentralization were chosen for reinnervation and recovered these postures 4-6 months post reinnervation. Each showed obturator nerve stimulation-induced bladder contractions and sciatic nerve stimulation-induced anal sphincter contractions immediately prior to euthanasia. One showed sciatic nerve stimulation-induced external urethral sphincter contractions and voluntarily voided twice following non-anesthetized bladder filling. Reinnervation was confirmed by increased labelled cells in L2, L4-L6 dorsal root ganglia (source of obturator nerve in canines) of L7d+S Dec+Reinn animals, compared to controls.

Conclusions: New neuronal pathways created by nerve transfer can restore bladder sensation and motor function in lower motor neuron-lesioned canines even 12 months after decentralization.

Funding Source(s): NIH 1R01NS070267

S2A11

Title: Regional and directional variation in bladder wall micromotion instigated by electrostimulation as measured by transabdominal Anatomical Motion Mode (AMM) ultrasound

Authors: Anna S. Nagle[1], Zachary E. Cullingsworth[1], Andrea Balthazar[2], Charles R. Blocher[2], Adam P. Klausner[2], and John E. Speich[1]

Affiliations: [1]Department of Mechanical & Nuclear Engineering, Virginia Commonwealth University School of Engineering, Richmond, VA. [2]Department of Surgery, Virginia Commonwealth University School of Medicine, Richmond, VA.

Introduction/Objectives: Non-voiding rhythmic contractions are often associated with feelings of urinary urgency in individuals with Overactive Bladder. In order to better understand and improve non-invasive techniques to measure the micromotions of the bladder wall that cause these rhythmic contractions, an anesthetized pig model was developed. The objective of this study was to determine how the region of and direction through the bladder wall in which micromotion is monitored affect the accuracy of measurement of micromotion rhythm using transabdominal AMM ultrasound.

Methods: An isoflurane anesthetized female pig underwent an ultrasound urodynamic study. With the bladder filled to 500 ml, filling was paused and electrotrodes were placed on the bladder surface to deliver electrostimulation of 5 V in a square wave every 20s (3 cycles/ minute). An ultrasound transducer was positioned above the bladder transabdominally to obtain AMM one-dimensional lines perpendicular to the anterior and posterior bladder wall as well as parallel along the anterior bladder wall (Fig. 1). A custom MATLAB program monitored bladder wall thickness over 85s AMM cine loops and used Fourier analysis to measure peak frequencies of the signals.

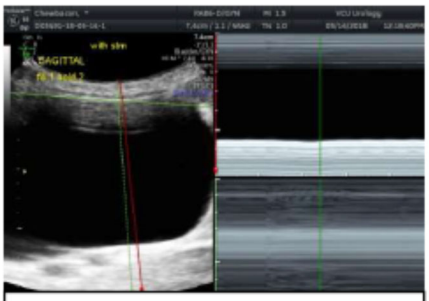


Fig. 1. Right: 2D ultrasound image of the bladder. Left: AMM tracked lines perpendicular (top) and parallel (bottom) to the bladder wall.

Results: The frequency components of changes in bladder wall thickness are presented in the below table for when electrostimulation was on 3 cycles/minute and when it was off (Table 1).

Table 1. The peak frequency closest to 3 cycles/minute is shown. If there was no peak frequency component in the range of interest (1-8 cycles/minute), zero is reported.

Location/Direction	Stimulation (cycles/min)	Frequency (cycles/min)	Amplitude (cm)
Anterior/Parallel	On: 3	2.2	0.18
	Off: 0	0	0
Anterior/Perpendicular	On: 3	2.9	0.08
	Off: 0	0	0
Posterior/Parallel	On: 3	4.4	0.03
	Off: 0	6.14	0.05

Conclusions: Measurement of bladder wall changes in thickness in the anterior portion of the bladder using an AMM tracking line perpendicular to the bladder wall yielded a peak frequency nearly identical to the frequency of electrostimulation.

Funding Source(s): This research was funded by the SUFU-Cogentix Medical OAB grant.

S2A12

Title - Real time closed loop control of bladder function with dorsal root ganglia sensory feedback and sacral root electrical stimulation

Authors - Zhonghua Aileen Ouyang, Zachariah J. Sperry, Elizabeth C. Bottorff, Tim M. Bruns

Affiliations - Biomedical Engineering and Biointerfaces Institute, University of Michigan, Ann Arbor, MI, USA

Introduction/Objectives - Overactive bladder (OAB) affects millions of people worldwide. Patients suffer from a frequent and uncontrollable urge to urinate, which can lead to a poor quality of life. Current sacral neuromodulation therapy uses open-loop electrical stimulation to alleviate symptoms. In this study, we aim to improve therapy by developing a conditional stimulation paradigm using neural recordings from dorsal root ganglia as sensory feedback. Stimulation is only given when an increase in bladder pressure is detected. Patients can potentially benefit from improved stimulation efficacy, a reduced occurrence of neural habituation over time, and a longer battery life.

Methods - Experiments were performed in acute, anesthetized felines, in which the first and second sacral-level dorsal root ganglia (DRG) and roots were exposed bilaterally. A bipolar cuff electrode was placed on the left or right S1 root distal to the DRG for stimulation. One or two Utah arrays were implanted in the opposite S1 and/or S2 DRG. We implemented a Kalman filter-based model to estimate the bladder pressure in real-time using threshold crossings from the DRG recordings. The Medtronic Summit research development kit was used to control stimulation applied by an RC+S neurostimulator connected to the cuff electrode. Closed-loop neuromodulation was performed during continuous cystometry or when the bladder was partially full and non-voiding contractions were present.

Results - S1 stimulation at 5 Hz (200 µs pulse width, 1-2 times motor threshold) decreased the peak amplitude of non-voiding contractions by 63% and increased bladder capacity by 18-23% when applied continuously, in preliminary experiments. We observed that a model-estimated 6 cmH2O pressure increase within 4 seconds was effective at identifying 100% of non-voiding contractions, and used that threshold to initiate conditional stimulation. Non-randomized closed-loop stimulation trials increased bladder capacity by 26% compared to no stimulation in one experiment.

Conclusions - This study demonstrates the utility of decoding bladder pressure from neural activity for closed-loop control. In the future, real-time validation during behavioral studies is necessary prior to clinical translation.

Funding source - Medtronic

S3A13

Title: Nicotinic receptors on nerve terminals induce acetylcholine release in canine bladder

Authors: Alan S. Braverman[1], Nagat Frara[1], Danielle M. Salvadeo[1], Mary F. Barbe[1], Michael R. Ruggieri, Sr.[1,2]

Affiliations: [1]Department of Anatomy and Cell Biology, Temple University School of Medicine, and [2]Shriners Hospitals for Children, Philadelphia, PA, USA

Introduction/Objectives: Carbachol, a mixed muscarinic and nicotinic agonist similar to acetylcholine, is often used for in-vitro bladder contraction with the implicit assumption that it causes contraction by only activating bladder smooth muscle muscarinic receptors. We sought to determine whether nicotinic receptors may also be involved in canine detrusor muscle contractions in- vitro.

Methods: Mucosa denuded female canine bladder muscle strips from sham operated animals were used from a larger study of nerve transfer for bladder reinnervation. Strips were fixed between force transducers and positioners and suspended in Tyrode's solution bubbled with 95% O_2/5% CO_2 at 37 C. After stretching to a length of optimal force production, maximal responses to 120 mM KCl were determined then various agents were added for 20 minutes before inducing contraction with the nicotinic agonists epibatidine and nicotine itself.

Results: Epibatidine induced contractions that were approximately 40% of the maximal response to 120 mM KCl whereas nicotine only induced contractions that were 20% of KCl. The muscarinic receptor antagonist atropine (10 uM) completely blocked 10 uM epibatidine or 1 mM nicotine induced contractions but desensitization of purinergic receptors with 10 uM α,β methylene ATP only blocked these contractions by 40%. Blocking sodium channels with 1 uM tetrodotoxin (TTX) had no statistically significant inhibitory effect on epibatidine or nicotine induced contractions. Desensitizing nicotinic receptors by exposure to nicotine blocked contractile responses to epibatidine and vice versa. The α7 nAChR-selective agonist AR-R17779 had no effect on its own but blocked epibatidine contractions whereas the α7 selective antagonist MLA had no effect at α7 selective concentrations but blocked epibatidine at higher concentrations. The α4β2 selective agonist TC2559 also had no effect alone but blocked epibatidine induced contractions. The skeletal muscle neuromuscular junction nicotinic receptor antagonist atracurium besylate (5 uM) blocked both epibatidine and nicotine induced contractions. Epibatidine contractions were also completely blocked by another skeletal muscle neuromuscular junction nicotinic receptor antagonist tubocurarine (1 uM) and the ganglionic nicotinic antagonists hexamethonium (100 uM) or mecamylamine (10uM), but not by the depolarizing neuromuscular junction blocker succinylcholine (10 uM).

Conclusions: Because of atropine blockade but only minor blockade by α,β methylene ATP desensitization, the nicotinic agonists induce bladder contractions indirectly by releasing predominately acetylcholine from intramural nerve terminals. Because TTX was ineffective, these nicotinic receptors do not need to induce action potentials and thus are likely located near the neuromuscular junction. The nature of these nicotinic receptors appears to be somewhat unusual in that they can be blocked by antagonists thought to be selective for skeletal muscle neuromuscular junction nicotinic receptors (atracurium and tubocurarine) as well as ganglionic nicotinic receptors (hexamethonium and mecamylamine).

Funding Source(s): NINDS R01NS070267

S3A15

Title: Optimization of Sonic hedgehog delivery to the penis from self-assembling nanofiber hydrogels to preserve penile morphology after cavernous nerve injury

Authors: Shawn Choe[1], Elizabeth Kalmanek[1], Christopher Bond[2], Daniel Harrington[3], Samuel Stupp[2], McVary KT[4], Podlasek CA[1]*

Affiliations: [1]University of Illinois at Chicago; [2]Northwestern University, [3]UT Health; [4]Loyola University Stritch School of Medicine.

Introduction/Objectives: Erectile dysfunction (ED) is a significant medical condition, with high impact on patient quality of life. Current treatments are minimally effective in prostatectomy, diabetic and aging patients due to injury to the cavernous nerve (CN); loss of innervation causes extensive smooth muscle (SM) apoptosis, increased collagen and ED. Sonic hedgehog (SHH) is a critical regulator of penile SM. We developed a self-assembling peptide amphiphile (PA) nanofiber hydrogel for extended release of SHH protein to the penis after CN injury, to suppress SM apoptosis. We propose that the marked improvements in penile morphology observed with this technology can be significantly further enhanced with optimization of delivery conditions for SHH PA, which is vital for clinical translation. In this study we optimize the CN injury model, the concentration of SHH protein delivered, the duration of apoptosis suppression, and simultaneous distribution of SHH to the penis and CN, to maximize SM preservation.

Methods: Adult Sprague Dawley rats (n=97) underwent: 1.) CN crush with SHH treatment of the penis by PA for 4 days with two SHH protein concentrations, 2.) Increased duration of SHH treatment after CN injury to 9 days with 2 SHH PA injections, 3.) Simultaneous SHH PA delivery to the penis and CN after CN crush. Sham, CN crush only, and MSA PA treated controls were also performed for each group. Penile morphology was examined by quantifying the apoptotic index, SM, collagen, and the proliferative index (Ki67/DAPI).

Results: Apoptotic index increased 117% 4 days after CN injury. SHH PA suppressed apoptosis 27%. SM was 48% higher with SHH treatment, and doubling the concentration of SHH resulted in higher SM preservation (76%). Increasing the duration of SHH delivery to 9 days with two SHH PA injections continued to suppress apoptosis 22%, and resulted in 100% more SM. Simultaneous SHH PA delivery to the penis and CN was most effective for SM preservation (127%). Proliferative index was increased 50% at 2 days, 38% at 4 days and 31% at 7 days after CN injury. Proliferation occurred in both SM and endothelium. Apoptotic index was not significantly different in corpora cavernosa of mild (117%) and severe CN crush (119%), and with CN resection (125%).

Conclusions: Optimization of SHH delivery by PA improved SM preservation 80%. An all or nothing apoptotic response of the penis was observed with loss of innervation. Dampening the intensity of the early apoptotic response is critical to preserving SM and erectile function. Proliferation of SM and endothelium also occur in the corpora cavernosa after CN injury, and this response is increased with SHH PA treatment. Optimization of SHH delivery by PA is indispensable for clinical translation to ED patients, and the PA nanofiber distribution mechanism, may be broadly applicable as an in vivo delivery tool.

Funding Source(s): NIH/NIDDK DK101536

S3A14

Title: Histamine does not directly contract urinary bladder smooth muscle

Authors: Brothely M. Jones, Osvaldo Vega Rodríguez, Nathan R. Tykocki

Affiliation: Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan, U.S.A

Introduction/Objective: Bladder dysfunction is largely associated with lower urinary tract symptoms indicative of overactive bladder (OAB) or underactive bladder (UAB). In bladder diseases such as interstitial cystitis and urinary tract infections, mast cells and mast cell degranulation are implicated as a potential cause for these symptoms. Mast cells degranulate in response to various stimuli and release histamine. Histamine is a well-studied in other tissues, but little is known about the role of histamine and histamine receptors within the bladder. Since other types of smooth muscle cells express H1 (contraction) and H2 (relaxation) histamine receptors, **we tested the hypothesis that histamine cause detrusor muscle to contract.**

Methods: All procedures followed institutional guidelines and were approved by the Institutional Animal Care and Use Committees of Michigan State University. 6-12-week-old C57BL/6 mice were euthanized, and bladders were removed. Dissected bladders were cut into approximately 2 mm wide bladder strips, either with or without the urothelium attached. Tissue was hung in an isolated tissue bath for isometric contractility experiments. Passive tension (1g) was applied, and tissue were exposed to an EC_{50} concentration of the cholinergic agonist carbachol (CCh; 200 nM) at the beginning and end of the experiment to verify tissue viability. Histamine concentration response curves (100 nM – 300 µM) were then performed. Other tissues were first contracted with 200 nM CCh before addition of 300 µM histamine to test for histamine-mediated relaxation. Analyses were performed using GraphPad Prism software. Comparisons were made using twoway ANOVA with Bonferroni's post hoc analysis, or paired t-test.

Results: Histamine had no effect on CCh-induced contractions in any tissue. While histamine caused minimal contraction to bladder strips with urothelium, no contractile response occurred in the absence of the urothelium. Although bladder strips with urothelium show a small contraction, it was not statistically significant ($P > 0.05$, $N = 4\text{-}5$).

Conclusions: Histamine does not directly cause detrusor contraction in C57BL/6 mice, however further investigation is needed to determine if histamine causes release of a contractile substance from the urothelium.

Funding Sources: NIH K01 DK103840 and NIH R01-DK119615

S3A16

Title: Curcumin-Loaded Nanoparticles Protect Erectile Function in a Rat Model of Type-2-Diabetes.

Authors: Andrew Draganski[1], Moses T. Tar[2], Guilllermo Villegas[2], Joel Friedman[1] and Kelvin P. Davies[1,2,] *.

Affiliations: [1]Department of Physiology and Biophysics, [2]Department of Urology, Albert Einstein College of Medicine, Bronx, New York; NY10461.

Introduction/Objectives: Curcmin, a naturally occurring anti-inflammatory compound, has shown promise in preclinical studies to treat erectile dysfunction (ED) associated with type-1-diabetes (T1D). However, poor bioavailability following oral administration limits its efficacy. The present study evaluated the potential of topical application of curcumin loaded nanoparticles (curc-np) to prevent ED in a rat model of type-2-diabetes (T2D).

Methods: Curc-np (4mg curcumin) or blank nanoparticles (blank-np) were applied every 2-days for 2-weeks to the shaved abdomen of 20-weeks old Zucker Diabetic Fatty male rats (ZDF) (*N*=5 per group). Lean ZDF controls were treated with blank-np (*N*=5). Penetration of nanoparticles and curcumin release were confirmed by two-photon fluorescence microscopy and histology. Erectile function was determined by measuring intracorporal blood pressure normalized to systemic blood pressure (ICP/BP) following cavernous nerve stimulation. Corporal tissue was excised and RT-qPCR used to determine expression of the following markers: nuclear factor-κB (NF-κB), NF-κB-activating protein (NKAP), nuclear factor erythroid 2-related factor-2 (Nrf2), Kelch-like ECH-associated protein-1 (Keap1), heme oxygenase-1 (HO-1), variable coding sequence-A1 (Vcsa1), phosphodiesterase-5 (PDE5), endothelial and neuronal nitric oxide synthase (eNOS, nNOS), Ras homolog gene family member A (RhoA), and Rho-associated coiledcoil containing protein kinases-1 and -2 (ROCK1, ROCK2).

Results: Nanoparticles penetrated the abdominal epidermis and persisted in hair follicles for 24-hours. Curc-np treated animals exhibited higher average ICP/BP than animals treated with blank-np at all levels of stimulation and was statistically significant ($p<0.05$) at 0.75mA. In corporal tissue, NKAP expression decreased 60% and HO-1 expression increased 60% in curc-np compared to blank-np treated animals. ICP/BP values inversely correlated with NKAP and directly correlated with HO-1 expression levels.

Conclusions: Topical application of curc-np to a rat model of T2D can systemically deliver curcumin, protect erectile function and modulate corporal expression of inflammatory markers. These studies demonstrate the potential for topical application of curc-np as a treatment for ED in T2D patients. The T2D animal model of ED represents a more prevalent disease than the more commonly studied T1D model. Although there is improved erectile response in curc-np treated animals, only at the lower levels of stimulation (0.75mA) was this significantly compared to the blank-np treated animals suggesting more studies are needed to optimize protocols and evaluate toxicity.

S3A17

Title: Intravesical macrophage migration inhibitory factor (MIF) and activation of intravesical MIF receptors mediate PAR4-induced bladder pain

Authors: Shaojing Ye[1,2], Fei Ma[1,2], Katherine L Meyer-Siegler[3], Lin Leng[4], Richard Bucala[4], Pedro L Vera[1,2]

Affiliations: [1]Lexington VA Health Care System, R&D Service, Lexington, KY; [2]University of Kentucky, Physiology, Lexington, KY; [3]St Petersburg Community College, St Petersburgh, FL.; [4]Yale University, Internal Medicine, New Haven, CT

Introduction/Objectives: Activation of intravesical protease activated receptor-4 (PAR4) releases urothelial MIF to mediate bladder pain. We hypothesized that MIF mediates bladder pain by interacting with intravesical MIF receptors. Therefore, we tested whether intravesical antagonism of MIF or MIF receptors (CXCR4, CD74 or CXCR2) reduces or prevents bladder pain.

Methods: Intravesical PAR4 (100 µM; 1hr) induced bladder pain in female C57 BL/6 mice under isoflurane anesthesia. Bladder pain (lower abdominal von Frey (VF) 50% threshold) was measured before (baseline) and 24 hr post-PAR4 infusion. Scrambled PAR4 peptide (Scramb) was used as control. Intravesical pre-treatment (15 min prior to PAR4) with PBS, isotype control, neutralizing anti-MIF, anti-CD74 monoclonal antibodies and AMD-3100 (CXCR4 inhibitor) tested the effect of antagonists on PAR4- induced bladder pain. Voided volume and frequency were also measured at 24 hr. At end of experiment, bladders were collected for histology. ANOVA with post-hoc tests were used.

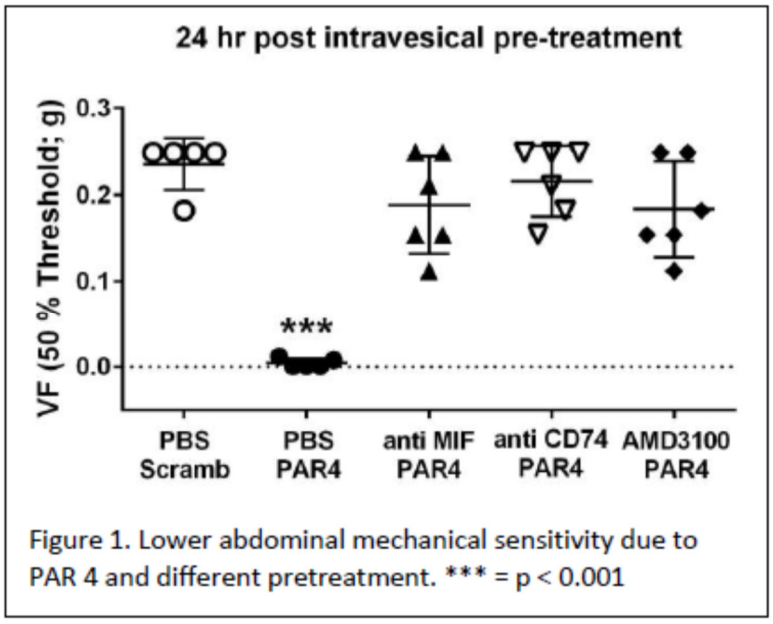


Figure 1. Lower abdominal mechanical sensitivity due to PAR 4 and different pretreatment. *** = $p < 0.001$

Results: Intravesical scrambled peptide did not produce bladder pain. PAR4 induced bladder pain that was not blocked by PBS (control) pretreatment but was blocked by intravesical pre-treatment with anti-MIF, anti- CD74 antibodies or CXCR4 antagonist (Figure 1). No significant effects were noted on micturition volume or frequency (Table 1).

Table 1. Effect of intravesical antagonists and treatment on micturition parameters (Mean± SEM)

Intraves. Antagonist	PBS (control)	PBS	IgG1 (control)	Anti MIF	Anti CD74	AMD3100
Intraves. Treatment	Scramb. (control)	PAR4	PAR4	PAR4	PAR4	PAR4
Volume (µl)	167 (± 21.4)	148 (±13.4)	224 (± 36)	164 (± 18)	118 (±18)	169 (±26)
Frequency (voids/3 hr)	3.8 (± 0.9)	3.2 (± 0.7)	3 (± 0.7)	3.7 (± 0.3)	5.5 (± 0.5)	3.5 (± 0.3)

Conclusions: Intravesical MIF and/or activation of MIF receptors mediate bladder pain. Future experiments will examine additional MIF receptors (CXCR2). In addition, we will examine how intravesical antagonism of MIF or its receptors contributes to release of urothelial high mobility group box 1 to mediate bladder pain.

Funding Source(s): DK121695 (PLV); AR049610 (RB)

S5A18

Title: Impact of RhoGDI Gene Transfection of Bladder Smooth Muscle Contractility in a Validated Ex-vivo Murine Model

Authors: Gregory Joice[1], Nora M Haney[1]*, Nikolai Sopko[1], James M Bell[1], Justin D La Favor[1], Takahiro Yoshida[1], Gonzalo Torga[1], Kelly T Harris[1], Xiaopu Liu[1], Matthew Kiedrowski[2], Marc Penn[2], Trinity Bivalacqua[1]

Affiliations: [1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]Northeast Ohio Medical University, Rootstown, OH

Introduction: Plasmid-based gene therapy is an intriguing option for treating malignant and benign bladder pathologies. The RhoA pathway is involved in bladder smooth muscle contraction, cancer invasion, and metastasis. Rho GDP-dissociation inhibitor (RhoGDI) is an inhibitor of the RhoA pathway keeping it bound in the cytosol in its inactive form. We previously validated an ex-vivo bladder gene transfer model to facilitate assessment of gene targets for treating bladder pathology.
Methods: Bladders were harvested from female Lewis Rats (~250g) and sectioned longitudinally into 4 equal strips. Bladder strips were transfected with a previously developed human RhoGDI containing plasmid with a CMV promoter using two techniques: passive and microinjection transfection. For passive transfection, FuGENE (Promega, USA) was used at a ratio of 6:1 as a transfection reagent and bladders were cultured for 72 hours. For microinjection, naked plasmid was injected directly into the detrusor muscle of rat bladder strips prior to being cultured for 72 hours. Transfected bladder tissues were analyzed for downstream effectors of the RhoA pathway via qPCR. Transfection localization within the bladder was determined with immunofluorescence (IF) using a GFP plasmid on a similar DNA backbone. Finally, functional effects of RhoGDI transfection were assessed with detrusor response to KCl and carbachol on bladder strip myography.

Results: Human RhoGDI was detected in transfected ex-vivo cultured bladder segments in both passive and microinjection experiments. In both groups, qPCR analysis demonstrated rodent RhoA and RhoGDI levels were not impacted but ROCK1 and ROCK2 mRNA were significantly decreased by 15.0% ($p=0.035$) and 22.4% ($p=0.010$) after FuGENE transfection and 20.5% ($p=0.024$) and 21.4% ($p=0.015$) after microinjections. IF revealed GFP transfection in urothelial cells in both groups but microinjections lead to improved (6.8 vs. 2.2 cells/hpf, $p<0.01$) transfection and more positive cells deeper within the bladder wall. Ex-vivo cultured bladder strips successfully contracted to KCl (mean 0.88+/-0.48 mN/mg tissue) and carbachol (1.82+/-0.97 mN/mg tissue) stimulation. After microinjection, RhoGDI caused a significant reduction in maximum KCl mediated constriction (0.418 vs. 0.778 mN/mg tissue, $p=0.024$) that was not observed after passive transfection (0.775 vs. 1.098 mN/mg, $p = 0.44$).

Conclusion: Our human RhoGDI plasmid interacts with and appears to downregulate the rat RhoA pathway after both passive and microinjection transfection. Microinjection appears to allow for increased transfusion in the deeper muscle layers providing a slight functional benefit.

Funding Source(s): None

S5A19

Title: Utilizing Enzymes to Induce Hypoxia for an in vitro Bladder Outlet Obstruction Model

Authors: Britney N Hudson[1], J. Todd Purves[2,3], Francis M. Hughes[2], and Jiro Nagatomi[1]

Affiliations: [1]Clemson University, Clemson, SC USA, [2]Departments of Surgery and [3]Pediatrics, Duke University Medical Center, Durham, NC, USA

Introduction: Partial bladder outlet obstructions (PBOO) impairs bladder function [1] and we previously demonstrated that PBOO triggered NLRP3 inflammasome activation and increased collagen synthesis [2]. In addition, studies have shown that PBOO causes blood flow disruption, resulting in tissue hypoxia [3]. Thus, we hypothesized that hypoxia plays a role in the pathology of PBOO bladder such as inflammasome activation and fibrosis. The present *in vitro* study used enzyme glucose oxidase (GOX) in culture to expose rat bladder urothelial cell line to transient hypoxic condition. Following exposure to hypoxia, cell viability, nitric oxide levels, ATP release and caspase-1 activity were examined.

Methods: Hypoxic media was prepared with various GOX concentrations. Since this reaction results in the production of hydrogen peroxide and gluconic acid, we also included the enzyme catalase (CAT, 120 U/mL) and HEPES (25 mM) in the culture media to counteract the cytotoxicity of hydrogen peroxide, and to buffer pH, respectively. Oxygen tension of media were measured using Biostat B Benchtop Oxygen Probe at various timepoints. MYP3 cells were exposed to hypoxic conditions (O2%, 7-11% or below 5%) via GOX (0.078 µg/ml or 0.15 µg/ml, respectively) for up to 2 hours. Cell viability was assessed using Vybrant MTT cell viability assay. Extracellular ATP release in supernatant media was quantified using a commercially available assay kit. Nitric oxide release was measured through nitrite concentration in supernatant media via the Griess reagent assay [4]. Intracellular caspase-1 activity levels were measured following an established method [1]. Data were statistically analyzed using two-way analysis of variance (ANOVA) and a *post hoc* Tukey test. P-values less than 0.05 were considered statistically significant.

Results: Oxygen tension at GOX concentrations of 0.078 or 0.15 µg/mL were stable after a 1.5 hour incubation and for additional 2 hours during which MYP3 cells were cultured. In the presence of catalase, MYP3 cell viability under GOX induced hypoxia was maintained at 95%. However, in the absence of CAT, cell viability was significantly reduced to 36%. Under hypoxic conditions at 7~11 % O2, MYP3 cells demonstrated a trend of higher nitrite concentration than the normoxic conditions at 21% O2. For hypoxic conditions under 5%, nitrite concentration was significantly higher than normoxic conditions. However, intracellular caspase-1 activity in MYP3 cells was similar under both hypoxic and normoxic conditions at the 2 hr timepoint.

Conclusion: The results of the present study provide evidence that GOX was able to maintain low oxygen tension to simulate pathological hypoxic conditions of pBOO, which was demonstrated by an increased NO release by MYP3 cells. Although by-product hydrogen peroxide negatively impacted cell viability, addition of CAT provided a remedy. Using this enzyme system for future studies can help investigate the role of hypoxia in various pBOO related pathology of urothelial cells.

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Funding Source(s): NIH (R01DK103534, P20GM103444), NSF (1264579)

S5A20

Title: NLRP-3 Inflammasomes inhibition improves bladder reactivity in dbdb-/- mice

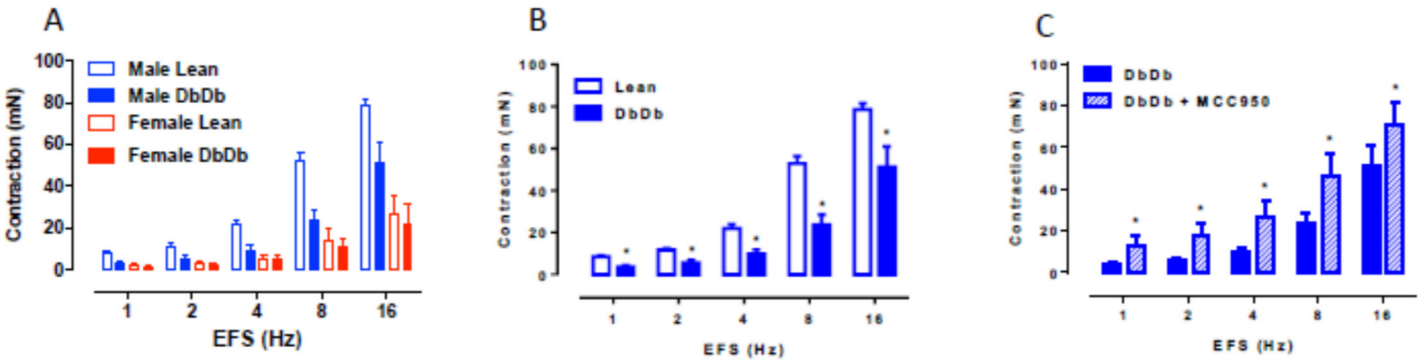
Authors: Vanessa Dela Justina, Fabiano Calmasini, Nina Onuoha, R. Clinton Webb, Fernanda Priviero

Affiliation: Department of Physiology, Augusta University, Augusta, GA, USA

Introduction/Objectives: NLRP3 inflammasomes activation has been demonstrated in both type 1 and type 2 diabetes mellitus (T1DM and T2DM) and it has been correlated with dysfunctional organs. Activation of NLRP3 inflammasomes contributes to bladder dysfunction in models of cystitis, bladder outlet obstruction and hyperactive bladder in T1DM. However, it is still unknown whether inflammasomes contribute to the development of diabetic bladder dysfunction in T2DM. Thus, we aimed to evaluate the hypothesis that NLRP3 inflammasomes activation contributes to hypoactive bladder in the late phase of diabetic bladder dysfunction in T2DM.

Methods: Bladder were excised from male and female wild type (WT) and dbdb-/- mice at 14 – 16-week old, cut in strips and mounted in a muscle strip myograph system. Contraction of the bladder was evaluated by a single concentration of KCl (120 mM), concentration-response curves to carbachol (1 nM – 30 µM) and frequency-response curves to electrical field stimulation (EFS: 1-16 Hz). Carbachol and EFS were repeated in the presence of the NLRP3 inhibitor MCC950 (1 µM).

Results: A higher magnitude of contraction was observed in the bladder of males compared to females for all contractile agents tested (Fig A shows EFS-induced contractions in bladder of males and females). The contractile responses to KCl were similar between strains (Female WT: 42 ± 7 mN vs dbdb-/-: 40 ± 4 mN; Male WT: 54 ± 6.5 mN vs dbdb-/-: 51 ± 17 mN). In males, the potency of carbachol was significantly reduced in the bladder of dbdb-/-mice (pEC_{50}: 5.53 ± 0.09) compared to WT (pEC_{50}: 6.11 ± 0.07) and it was not restored by the NLRP3 inhibitor MCC950 (pEC_{50}: 5.66 ± 0.08). On the other hand, EFS-induced contraction was significantly lower for all frequencies in the bladder of male dbdb-/- mice (at 16 Hz: 51 ± 10 mN) compared to WT (at 16 Hz: 77 ± 3 mN; Figure B) and it was restored in the presence of MCC950 (at 16 Hz: 71 ± 11 mN; Figure C). In female mice, there was no difference in the contraction of the bladder of dbdb-/- mice when compared to WT and addition of MCC950 did not altered the responses neither in WT nor in dbdb-/-.



Conclusions: In conclusion, our data suggest that NLRP3 inflammasomes contribute to the development of hypoactive detrusor response in the late phase of diabetic bladder dysfunction in males.

Funding Source(s): NIH HL-134604.

S5A21

Title: Subtype-specific Kv7/KCNQ channel activators reduce excitability and contractility in human detrusor smooth muscle

Authors: John Malysz[1], Sarah E. Maxwell[1], Eric S. Rovner[3], Robert Wake[2], and Georgi V. Petkov[1,2]

Affiliations: [1]College of Pharmacy, University Tennessee Health Science Center, Memphis, TN; [2]College of Medicine, University Tennessee Health Science Center, Memphis, TN; [3]Medical University of South Carolina, Charleston, SC.

Introduction/Objectives: The voltage-gated Kv7 channel family — comprised of 5 members (Kv7.1-7.5) encoded by 5 respective KCNQ genes (KCNQ1-5) — regulates various body functions. Recent findings on animal models (guinea pig and rat) from our group and others have identified Kv7 channels as emerging regulators of detrusor smooth muscle (DSM) function[1-8]. The current data revealed detections of mRNA and proteins for Kv7 channel subtypes in DSM tissues and single-cells, and their pharmacological modulation affecting *in vitro* and *in vivo* urinary bladder functions[1-8]. The roles of Kv7 channels in human DSM, however, are yet to be fully established.

Methods: Human DSM specimens were obtained from adult patients, lacking clinical symptoms of OAB/DO, undergoing open-bladder surgeries. Mucosa-free DSM tissues and enzymatically freshly-isolated DSM cells were prepared and investigated by molecular (RT-PCR, qRT-PCR, immunocytochemistry, *in situ* Proximity Ligation Assay/PLA, or Western blot) and functional studies (isolated DSM strip contractility or single DSM cell Amphotericin-B perforated patch-clamp electrophysiology). The following Kv7 channel activators were studied: retigabine (RET: panselective, Kv7.2-5>>Kv7.1), ICA-069673 (ICA: Kv7.2/Kv7.3>Kv7.3/Kv7.5>>Kv7.1), ML213 (ML: Kv7.2~Kv7.4>>Kv7.3~Kv7.5~Kv7.1), and the pan-selective inhibitor XE-991 (XE, Kv7.1-7.5).

Results: Human DSM cells and tissues expressed mRNAs and proteins for Kv7 channel subtypes. Most enriched in single DSM cells were mRNAs for Kv7.4 and Kv7.5 channels, while only minimal detection for Kv7.2 channels was observed. *In situ* PLA on DSM cells revealed close proximity expressions of Kv7.4 and Kv7.5 consistent with these subtypes forming heteromers. RET, ICA, ML at 10 or 30 μM effectively inhibited spontaneous, 20 mM-KCl induced and/or electrical field stimulated DSM phasic contractions. In contrast, XE (30 μM) enhanced DSM contractility. In the current clamp mode (I=0), all three Kv7 channel activators (10-30 μM) induced hyperpolarization, which was reversed upon subsequent addition of XE (10 μM). XE alone also caused depolarization. RET at 10 μM increased Kv7 currents (ramps) measured in the voltage-clamp mode.

Conclusions: Human DSM cells and tissue strips express Kv7 channel subtypes, most predominantly Kv7.4 and Kv7.5 channels likely expressed as heteromers. Pharmacological activation of Kv7 channels with pan- and subtype-specific Kv7 channel activators leads to attenuation of human single DSM cell excitability and tissue strip contractility. These results identify Kv7 channels as key regulators of human DSM function and illuminate the need for their further exploration as potential targets for urinary bladder diseases.

Funding Source(s): NIH R01 DK106964.

References: [1]Afeli *et al.* (2013) *PLoS One*; [2]Provence *et al.* (2015) *JPET*; [3]Provence *et al.* (2018) *JPET;* [4]Andersson *et al.* (2013) *Br J Pharmacol*; [5]Svalo *et al.* (2012) *Basic Clin Pharmacol Toxicol*; [6]Rode *et al.* (2010) *Eur J Pharmacol*; [7]Wang *et al.* (2014) *Int J Urol*; [8]Streng *et al. (2004) J Urol*

S5A22

Title - The nicotinic receptor agonist Epibatidine induces contraction more frequently in sub-mucosal than sub-serosal canine bladder smooth muscle strips.

Authors – Nagat Frara[1]*, Alan S. Braverman[1], Lucas Hobson[1], Dania Giaddui[1], Geneva Cruz[1], Mary F. Barbe[1] and Michael R. Ruggieri Sr.[1,2]

Affiliations - [1]Department of Anatomy and Cell Biology, Lewis Katz School of Medicine and [2]The Shriners Hospital of Philadelphia, Philadelphia, PA

Introduction/Objectives - The expression of nicotinic acetylcholine receptors (nAChRs) in the urinary bladder of different mammalian species and their role in regulating bladder function are still under investigation. In this study, we explored the contractile responsiveness of smooth muscle strips dissected from different areas of the normal canine bladder.

Methods - The whole bladder was removed from sham operated animals used for other studies, rinsed with Tyrode's solution and stored in HTK organ preservation media on ice overnight. Bladder smooth muscle strips were dissected from the dorsal and ventral aspects of the bladder base (below the ureteral orifices), the mid bladder and the bladder dome. The mucosa and submucosa was removed by sharp dissection and for each of the 6 areas (dorsal and ventral bladder neck, middle and dome) and smooth muscle strips were dissected along visible fascicle lines from tissue adjacent to the mucosa and separate strips from tissue adjacent to the serosa. Strips were fixed between force transducers and positioners and suspended in Tyrode's solution bubbled with 95% O2/5% CO2 at 37 C. After stretching to a length of optimal force production, maximal responses to electric field stimulation (EFS) and 120 mM KCl were determined. After washing and re-equilibration, the responses to 10µM of the nicotinic agonist epibatidine was determined followed by responses to EFS and 30µM of the muscarinic receptor agonist bethanechol in the continued presence of epibatidine.

Results – Data was obtained from 18-52 strips obtained from 2-5 animals for each bladder region. The table at right shows the percentage of strips that produced at least 0.2 grams of tension in response to 10µM epibatidine was greater for each of the regions for the strips obtained adjacent to the mucosa than those dissected immediately beneath the serosal surface. All strips from all regions produced robust contractions to EFS and bethanechol in the continued presence of epibatidine, including those that responded to epibatidine with less than 0.2 grams of tension.

	dorsal aspect		ventral aspect	
	mucosa	serosa	mucosa	serosa
dome	77.8	55.6	77.8	55.6
middle	81.8	59.1	86.5	48.1
neck	72.2	66.7	72.2	38.9

Conclusions – Much of the previous literature on contractile responses of bladder muscle strips incorporates the implicit assumption that strips from the dorsal versus ventral aspects and across the thickness of the bladder respond similarly to pharmacological agents. This is the first finding, to our knowledge, that muscle strip taken from close to the bladder lumen show a difference in contractile response than strips taken closer to the outer surface of the bladder. Because epibatidine contractions are virtually abolished by muscarinic receptor blockade with atropine, nicotinic receptor stimulation causes contraction indirectly by inducing acetylcholine release from bladder nerve endings. Apparently, the density of these nerve endings is greater in the bladder smooth muscle that is closer to the mucosal surface. This finding may have important implications in the understanding of the physiology and biophysics of bladder emptying.

Funding Source(s) - NIH 1R01NS070267

S5A23

Title: Calcium Pyrophosphate and Monosodium Urate Activate the NLRP3 Inflammasome within Bladder Urothelium via Reactive Oxygen Species and Thioredoxin Interacting Protein

Authors: Shelby N. Harper[1]*, Patrick D. Leidig[1], Francis M. Hughes Jr.[1], Huixia Jin1, J. Todd Purves[1,2]

Affiliations: [1]Division of Urology, Department of Surgery, Duke University Medical Center, Durham, NC, USA; [2]Department of Pediatrics, Duke University Medical Center, Durham, NC, USA

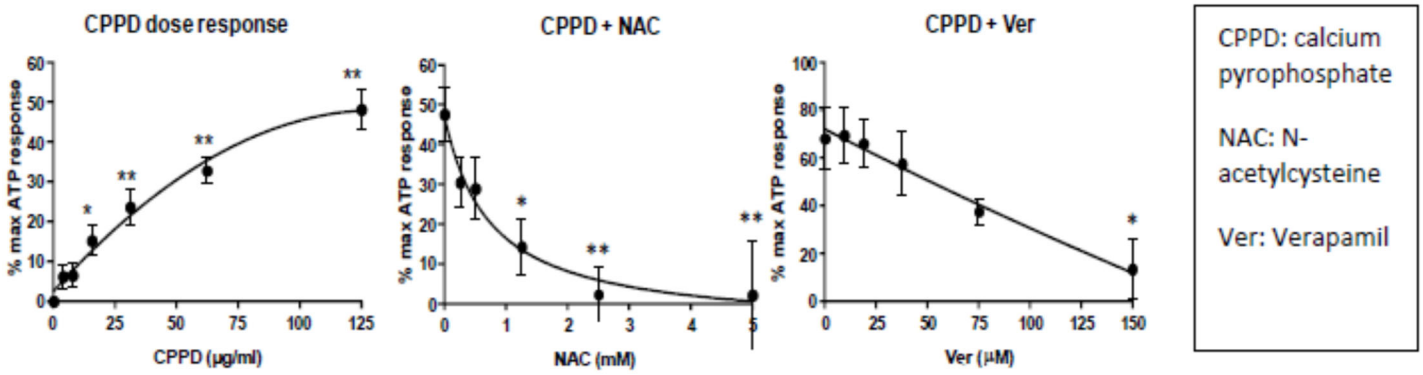
Introduction/Objectives: Urinary stones provoke inflammation within the urinary tract and this can result in pain, fibrosis, and scarring of the tissue. In this study, we investigated the mechanism by which stone-forming components activate the NLRP3 inflammasome within urothelium to promote pro-inflammatory mediator production. Specifically, we aimed to describe the contributions of reactive oxygen species (ROS) and thioredoxin interacting protein (TXNIP), an important structural component of the inflammasome, to this activation.

Methods: Urothelial cells were harvested and incubated overnight. For agonist studies, cells were treated with varying concentrations of calcium pyrophosphate and monosodium urate. For inhibitor studies, cells were treated with either N-acetylcysteine (1 hour) or Verapamil (4 hours) prior to incubation with either calcium pyrophosphate (62.5 ug/mL) or monosodium urate (1.25 ug/mL) for 24 hours. Untreated controls were incubated with ATP (1.25 mM) for 1 hour to maximally stimulate NLRP3 inflammasome activity (measured as caspase-1 cleavage of the fluorogenic substrate Ac-YVAD-AFC). Results are reported as a percentage of maximum ATP response.

Results: Calcium pyrophosphate and monosodium urate activate capase-1, the functional component of NLRP3, in urothelial cells in a dose-dependent manner, reaching ~50% and ~25% of the ATP response, respectively. Pre-treatment with the ROS scavenger N-acetylcysteine reduces this activation in a dose-dependent manner. Additionally, activation was suppressed through treatment with Verapamil, a previously described downregulator of TXNIP expression.

Conclusions: The stone components calcium pyrophosphate and monosodium urate activate NLRP3 in a ROS and TXNIP-dependent manner in urothelium. These findings demonstrate the importance of ROS and TXNIP and suggest that targeting either may be a way to decrease inflammation in the urinary tract that results from stone formation.

Funding Source(s): Funding was provided by NIH (R01DK103534); the 2019 Urology Care Foundation Summer Medical Student Fellowship from the American Urological Association and by the Poindexter Fellowship from Duke University School of Medicine.



S6A24

Title: Castration Mediated Schwann Cell Dedifferentiation Leads to Slower Nerve Conduction, Decreased Neuritogenesis, and Nitrergic Neuron Loss

Authors: Michael R Odom*, Elena S Pak, Stefan Clemens, Johanna L Hannan.

Affiliations: Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA

Introduction/Objectives: Androgen deprivation therapy (ADT) as a prostate cancer treatment leads to erectile dysfunction (ED). ADT induced ED is perpetuated by damage to pelvic nerves and a loss of nitrergic nerves required to initiate erections. Schwann cells (SC) release neurotrophic factors to promote pelvic nerve growth and survival. It is unknown how testosterone deficiency impacts SC function. This study determines how testosterone deficiency influences SC phenotype and trophic support in neuronal regeneration. We hypothesize testosterone deficiency will decrease myelinated SC populations, delay nerve conduction and reduce nitrergic neurons.

Methods: Male Sprague Dawley rats (12 wks) were divided into 2 groups: control (CON) and surgical castration (CAST). All experiments were performed six weeks following castration. Dorsal penile nerves (DPN; 15-16 mm) were excised, placed in a sylgard dish containing oxygenated buffer and conduction velocity of myelinated fast fibers was assessed (n=8 per group). The major pelvic ganglia (MPG) were collected and dissociated into SC or neurons. Dissociated neurons were grown on laminin coated coverslips. To determine whether SCs could provide trophic support, we co-cultured neurons on top of confluent SCs. The co-culture groups we assessed were CON SC + CON MPG, CON SC + CAST MPG, CAST SC + CON MPG (n=4 per group, cultured in triplicates). After 72 hours, all cultures were fixed and stained with beta tubulin class III to assess neurite length, neurite branching, nitrergic (neuronal nitric oxide synthase; nNOS), and sympathetic neurons (tyrosine hydroxylase; TH). Dissociated SC were characterized via qPCR for markers of all SC (Sox10), myelinated SC (Krox20), and dedifferentiated SC (glial fibrillary acidic protein; GFAP).

Results: CAST slows DPN myelinated fast fiber conduction velocity (CON: 16.9±0.7 m/s, CAST: 13.6±0.8 m/s; $p<0.05$). In dissociated cultured MPG neurons, castration reduces both neurite length (CON: 1057±19.9 µm, CAST: 874±18.9 µm, $p<0.05$) and number of branches (CON: 5.3±0.1, CAST: 3.9±0.1, $p<0.05$). Interestingly, CAST MPG neurons co-cultured with CON SC restore neurite length and branching to same level as CON MPG neuron co-cultured with CON SC; however, CAST SC do not impact CON MPG neurite length (CON SC + CON MPG: 1473±57.8 µm, CON SC + CAST MPG: 1587±120.1 µm, CAST SC + CON MPG: 1522±29.7 µm). CAST MPG neurons co-cultured with CON SC still have less nitrergic neurons than CON SC + CON MPG and CON MPG neurons co-cultured with CAST SC also have less nitrergic neurons (CON SC + CON MPG: 38.1±4.6, CON SC + CAST MPG: 23.8±3.6, CAST SC + CON MPG: 19.8±2.1; $p<0.05$). CAST does not impact the number of TH neurons in single or co-cultured conditions. CAST reduces gene expression of Sox10 (-44%), myelinated SC (Krox20; -86%), and dedifferentiated SC (GFAP; -34%).

Conclusions: CAST markedly impairs penile nerve conduction, reduces neurite outgrowth and prevalence of pro-erectile nitrergic neurons. Healthy SC are able to rescue neuritogenesis but not the survival of nitrergic neurons. Additionally, healthy MPG neurons plated on CAST SC have reduced number of nitrergic neurons. We believe CAST SC are undergoing a phenotypic switch from a myelinated neurotrophic form into dedifferentiated SC promoting neuritogenesis and loss of nitrergic neurons. Preserving the myelinated SC phenotype may prevent impaired nerve damage and subsequent ED in patients with testosterone deficiency.

Funding Source(s): BSOM start-up grant, Sexual Medicine Society of North America

S6A25

Title: Functional and histological changes in the dog urinary bladder after different decentralization and reinnervation strategies

Authors: Mary F. Barbe[1]*, Geneva E. Cruz[1], Brian S. McIntyre[2], Emily P. Day[1], Dania Giaddui[1], Courtney L. Testa[1], Alan S. Braverman[1], Ekta Tiwari[1], Justin M. Brown[3], Michael R. Ruggieri, Sr[1,4].

Affiliations: [1]Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, Philadelphia, PA, USA; [2]Drexel University College of Medicine, Philadelphia, PA, USA; [3]Department of Neurosurgery, Massachusetts General Hospital, Boston, MA, USA; [4]Shriners Hospitals for Children, Philadelphia, PA, USA

Introduction/Objectives: To compare the effects of different spinal root decentralization and nerve transfer reinnervation strategies, including time of reinnervation surgery, on the function, innervation and histology of the urinary bladder in a dog model.

Methods: Fifty-five female dogs were divided into 8 groups: 3 different decentralization procedures, 4 decentralized and reinnervation strategies, and a sham control group. The 3 different decentralized procedures were: 1) transection of sacral (S) 1-3 dorsal and ventral roots and an 8 month recovery (n=8, termed S Dec 8 month); 2) S Dec with a 12 month recovery (n=3, termed S Dec 12 month); or 3) a more complete decentralization (transection of hypogastric nerves, dorsal roots of lumbar (L) 7, and dorsal and ventral roots of S1-3) and a 12 month recovery (n=4, termed H+L7d+S Dec 12 month). Twenty-six other dogs underwent decentralization before undergoing one of three bladder reinnervation surgeries during which portions of either the genitofemoral, femoral, or obturator nerve was transferred to the anterior vesicle branch of the pelvic nerve (GFNT, FNT and ObtNT, respectively) either immediately after decentralization or 12 month later. There were 4 surgical reinnervation groups: a) S Dec + Immediate GNT, n=10; b) S Dec + Immediate FNT, n=10; c) S Dec + Immediate ObtNT, n=3; or d) H+L7d+S Dec 12 month ObtNT, n=3. Results were compared to 14 sham control dogs. Maximal detrusor pressure induced by electrical stimulation of T12 to S3 spinal cord segments under isoflurane anesthesia was determined immediately prior to euthanasia and collection of the urinary bladder for histology. One-way ANOVAs were performed, followed by posthoc pairwise comparisons.

Results: All nerve transfer strategies resulted increased detrusor pressure during electrical stimulation of the spinal segments corresponding to the transferred nerves. Restoration of detrusor pressure was greater with obturator nerve transfer than with genitofemoral or femoral nerve transfer. Numbers of intramural ganglia in the bladder wall were lowest after the most complete decentralization (i.e., in the H+L7d+S Dec 12 month and H+L7d+S Dec 12 month ObtNT), as were numbers of neurons per ganglia, compared to sham controls, and trended towards further decreases with increased time of decentralization. In contrast, the mean widths of nerve bundles within the bladder wall were higher in S Dec + Immediate GNT and H+L7d+S Dec 12 month ObtNT animals, than in sham controls, and higher in each rerouted group, compared to sacral decentralized animals. Lastly, percent muscle within the bladder wall did not differ across groups.

Conclusions: Despite the loss of intramural ganglia and ganglionic neurons, the percent muscle and function were maintained within the bladder wall of reinnervated animals apparently due to a change in innervation.

Funding Source(s): NIH 1R01NS070267

S6A26

Title: Gonadal hormones and anesthetics influence threshold of transcutaneous electrical stimulation to induce external urethral sphincter reflex activity in female rats

Authors: Ricardo Juárez Mirto[1], José L Palacios Galicia[2], Plácido Zaca-Morán[3], Margarita Juárez[4], Rosa A. Lucio[4], Yolanda Cruz[4]*

Affiliations: [1]Doctorado en Investigaciones Cerebrales, Universidad Veracruzana, México. [2]Centro Universitario del Norte, Universidad de Guadalajara, Colotlán, México. [3]ICUAP, Benemérita Universidad Autónoma de Puebla, Puebla, México. [4]Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, México.

Introduction/Objectives: Electrical stimulation of pudendal nerve facilitates the recovery of the external urethral sphincter (EUS) after its injury. The EUS can also be reflexively activated through mechanical stimulation of the clitoral sheath, suggesting that pudendal motor nerve can be transinaptically stimulated to facilitate regenerative process after nerve injury. Clitoral sheath is innervated by the dorsal nerve of the clitoris (DNC), whose receptive field varies along the estrous cycle. The aim of the present study was to determine if threshold of transcutaneous electrical stimulation (TES) to induce EUS reflex activity is influenced by gonadal hormones variation during estrous cycle and anesthesia in rats.

Methods: Sixteen adult Wistar female rats were used. Rats in diestrus (n=10) or estrus (n=6) were anesthetized with urethane (1.2 g/kg) or Zoletil-Xilazine (Z+X; 40 mg/kg; 7.5 mg/kg, i.p.) and TES of the clitoral sheath was performed. TES threshold was determined by applying electrical current to the clitoral sheath (200-ms square pulses, at .20 Hz) using a pair of electrodes connected to a Grass stimulator. The current started from 100 µA and was progressive increased until EUS electromyographic (EMG) activity was observed. The amplitude of EUS EMG activity was also determined.

Results: TES of the clitoral sheath triggered EUS EMG tonic activity. Threshold for EUS response was significantly greater in diestrus rats (5.9±0.32 mA) compared to estrus rats (0.82±0.01 mA) ($p<0.05$). Conversely, the amplitude of the EUS EMG activity was significantly higher in estrus rats (56.11±13.2 µV) in comparison to diestrus rats (15.22±2 µV) ($p=0.01$). Threshold of Z+X anesthetized rats (9.62±1.47 mA) was significantly higher compared to urethane anesthetized rats (5.9±0.32 mA) ($p=0.01$). In contrast, the amplitude of the EUS EMG activity was significantly higher in urethane anesthetized-rats (15.22±2 µV) in comparison to Z+X anesthetized -rats (8.1±0.9 µV) ($p=0.02$).

Conclusions: It is concluded that both, threshold and amplitude of EUS reflex activity are influenced by gonadal hormones variation through estrous cycle and anesthesia. These factors may affect neuromodulation and should be considered in the clinic.

Funding Source(s): CONACYT: 825130 (RJM); FC2016-2/2319; PADES, DGESU-SEP.

S6A27

Title: Barrington's Reflexes Revisited: Proximal Urethral Electrostimulation Causes Remarkable Excitatory Bladder Response in Spinal Cord Intact Rats

Authors: Bradley A. Potts[1] and Matthew O. Fraser[1,2]*

Affiliations: [1]Division of Urology, Department of Surgery, Duke University Medical Center, Durham, NC, USA; [2]Veteran's Affairs Medical Center, Durham, NC, USA

Introduction/Objectives: Detrusor underactivity (DU) is an important contributor to voiding dysfunction with few reliable treatment options. In the early 1900's, Barrington discovered three excitatory urethra-to-bladder reflexes shown to work via pudendal, hypogastric, or pelvic nerve afferents. We hypothesized that these reflexes may provide a therapeutic target for the treatment of DU. We electrically field-stimulated nerves of the proximal urethras (PUES) of spinal-intact rats before and, in some, after acute suprasacral spinal cord injury to determine if we could elicit these reflexes in normal and acute spinal shock conditions.

Methods: Using urethane-anesthetized female Sprague-Dawley rats (230-290g, n=8), bilateral ureteral diversion and transvesical catheters were placed via laparotomy. The ventral pubis was carefully dissected and excised sharply in order to reveal the proximal and mid-urethra. Dorsally, the rats were prepped with either posterior vertebral dissection or laminectomy to facilitate future spinal cord injury. Following continuous cystometry, electrical stimulation was performed under stable isovolumetric conditions using two 50 micron stainless steel wires secured over and perpendicular to the rostral and caudal proximal urethra. The proximal urethra was stimulated for 30 seconds with a 60 second recovery period at frequencies ranging 5-250hz (10-60V). Following spinal intact stimulation, the SCI was performed using electrocautery to transect the cord at T9-10. At this point, the bladder was filled to pre-SCI total bladder capacity and stimulation was again performed from 5-250hz (50-75V). Extracted data included presence/absence of bladder contraction and evidence of lower extremity motor activity. With a goal of identifying positive bladder responses without simultaneous motor response, data were assigned a positive score of 1 if there was a bladder without motor response, 0 with no response or both bladder and motor response, and -1 with only motor response. Data were analyzed graphically and frequencies with non-negative results were further analyzed with one-way ANOVA.

Results: Overall positive responses were observed in the spinal-intact rats at 20 hz (0-86% success across V) and 50 hz (0-75% success across V). The rate of isolated bladder contraction by PUES at 20 hz was significantly greater at 30 V and 40 V ($P<0.03$ and <0.04, respectively), and, although 40 V produced the highest isolated bladder contraction rate for stimulation at 50 hz, ANOVA did not detect significance. No bladder responses to PUES were seen post-SCI, even in the presence of lower extremity motor activity.

Conclusions: In the spinal-intact rat, PUES at 20 Hz and 30-40 V elicited reliable bladder contractions in the absence of motor responses. Failure to elicit bladder responses post-SCI suggests that urethra-to-bladder reflex arcs require suprasacral involvement. Although PUES was unsuccessful in eliciting reflex bladder contractions under acute spinal shock conditions, we hope to assess bladder response using this approach in other DU rodent models.

Funding Source(s): Discretionary research funds

S6A28

Title: Bladder reinnervation by somatic nerve transfer to pelvic nerve vesical branches does not reinnervate the urethra

Authors: Mary F. Barbe[1], Alan S. Braverman[1], Danielle M. Salvadeo[1], Sandra M. Gomez-Amaya[1], Neil S. Lamarre[1], Justin M. Brown[2], Elise J. De[3], Brian S. McIntyre[4], Emily P. Day[1], Geneva E. Cruz[1]*, Nagat Frara[1], Michael R. Ruggieri, Sr.[1,5]

Affiliations: [1]Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, Philadelphia, PA, USA; [2] Neurosurgery Paralysis Center, Department of Neurosurgery, Massachusetts General Hospital, Boston, MA, USA; [3]Department of Urology, Massachusetts General Hospital, Boston, MA, USA; [4]Drexel University College of Medicine, Philadelphia, PA, USA; [5] Shriners Hospitals for Children, Philadelphia, PA, USA

Introduction/Objectives: We sought to determine whether somatic lumbar nerve transfer to the pelvic nerve's anterior vesical branch following sacral decentralization for detrusor muscle reinnervation also leads to aberrant innervation of the bladder outlet.

Methods: Twenty-six female mongrel hound dogs underwent transection of sacral dorsal and ventral spinal roots. Immediately afterwards, 12 received genitofemoral nerve transfer (GFNT) and 9 received femoral nerve branch transfer (FNT). Five were left sacrally decentralized. Controls included 3 sham-operated and 6 unoperated. Eight months post-surgery, the bladder and urethra were injected with retrograde tracing dyes cystoscopically. Three weeks later, detrusor and urethral pressures were assayed electrophysiologically immediately before euthanasia and characterization of neural reinnervation.

Results: Electrical stimulation of spinal cords or roots did not lead to increased urethral sphincter pressure in nerve transfer animals, compared to decentralized animals, confirming a lack of functional reinnervation of the bladder outlet. In contrast, mean detrusor pressure increased after lumbar cord/root stimulation. In sham/unoperated animals, urethral and bladder dye injections resulted in labelled neurons in sacral level neural structures (dorsal root ganglia (DRG), sympathetic trunk ganglia (STG) and spinal cord ventral horns); labelling absent in decentralized animals. Urethral dye injections did not result in labelling in lumbar or sacral level neural structures in either NT group, while bladder dye injections lead to increased labelled neurons in lumbar level DRG, STG and ventral horns, compared to sacrally decentralized animals.

Conclusions: Pelvic nerve transfer for bladder reinnervation must be accompanied by pudendal nerve transfer to achieve urethral sphincter reinnervation.

Funding Source(s): NIH 1R01NS070267

S7A29

Title: Comparison of the actomyosin ATPase inhibitor calponin and the sphingosine-1-phosphate cell differentiation pathway regulatory enzymes in vaginal wall smooth muscle of women with and without pelvic organ prolapse

Authors: John Sobieski, B.A.[1], Shefali Sharma, M.D.[2], Colin Sperling, B.A.[1], and Michael E DiSanto, Ph.D.[3]

Affiliations: Cooper Medical School of Rowan University[1]; Division of Female Pelvic Medicine and Reconstructive Surgery, Cooper University Hospital[2]; Department of Biomedical Sciences, Cooper Medical School of Rowan University[3]

Introduction/Objectives: Pelvic Organ Prolapse (POP) is the descent of the vaginal walls and uterus leading to herniation of nearby organs into the vagina. POP prevalence as reported by patient symptoms is ~3%, but it has been reported as high as 50% upon physical examination. Many women with POP also present with voiding dysfunction, defecatory dysfunction or dyspareunia. Abnormalities in smooth muscle (SM) in the vagina and endopelvic structures play a role in POP pathogenesis. Calponin is a protein thought to regulate SM contraction by inhibiting both myosin ATPase and bundling of actin filaments via binding to alpha-SM actin. Sphingosine kinase-1 (SPHK1) and sphingomyelin synthase (SMS) regulate the sphingosine-1-phosphate (S1P) pathway of SM contraction and differentiation. The objectives of this study were 1) compare expression of calponin, alpha-SM actin, SPHK1 and SMS in vaginal wall of women with and without POP, 2) determine cellular localization of calponin, alpha-SM actin and SPHK1 in vaginal SM tissue.

Methods: Case-control study comparing vaginal tissue samples from five patients with POP and five without POP. Tissues were approximate 1 x 1 x 1 cm sections of full-thickness anterior vaginal apical tissue. Exclusion criteria: history of pelvic radiation, previous pelvic reconstruction surgery or connective tissue disorders. Tissue samples were pulverized to a powder under liquid nitrogen using a freezer mill and then homogenized in extraction buffer. Western Blot was performed using a no-stain gel line. Fluorescently tagged antibodies were used to determine relative expressions of α-actin, calponin, SPHK1 and SMS. Blots were imaged and quantified using image analysis software with normalization to total lane protein. Independent t-test was performed to compare SM protein expression in POP versus control specimens.

Full-thickness sections were placed in buffered formalin and then embedded in paraffin blocks. 4 µm tissue sections were cut by microtome and placed on slides. One slide was stained with hematoxylin and eosin. Sequential slides were de-paraffinized in xylene, blocked in serum, and incubated with alpha-SM, calponin, SPHK1 or SMS antibodies. Expression and cellular localization of proteins were observed using immunofluorescence probes and microscopy.

Results: Calponin expression was ~35% lower in vagina of POP women ($p = 0.11$) while alpha-SM actin expression was not altered ($p = 0.94$). Calponin to alpha-SM actin ratio was also lower in POP women (1.58 vs 1.94). SPHK1 expression was ~46% lower, but SMS expression was ~30% higher ($p = 0.26$). On IF imaging, calponin more reliably localized to vaginal wall SM as compared to alpha-SM actin, which also stained vascular SM in the full-thickness samples.

Conclusions: Decreased calponin in vaginal SM of women with POP suggests a decreased tonicity and inability of the vagina to prevent POP. Greater localization of calponin to vaginal wall SM further supports this conclusion. Lower expression of SPHK1 and higher expression of SMS predicts a movement of the S1P pathway away from S1P towards more to cellular apoptosis, decreasing vaginal SM contractile function. Although findings only trended towards significance, we will increase our sample size. Future research will leverage these findings to further explore SM regulation and signaling pathways as a target for POP therapeutics.

S7A30

Title: Ex Vivo Akt Inhibition Reverses Castration Induced Penile and Pudendal Artery Endothelial Dysfunction

Authors: Michael R Odom*, Elena S Pak, Johanna L Hannan.

Affiliations: Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA

Introduction/Objectives: Androgen deprivation therapy (ADT) is frequently used to slow prostate cancer progression and reduce tumor size; however, erectile dysfunction (ED) is a common side effect. Following cessation of ADT, testosterone levels may take years to recover. Some men remain testosterone deficient leaving them prone to long-term ED. Testosterone deficiency can induce ED by damaging the vascular endothelium, yet the exact mechanisms remain unknown. For an erection to occur, Akt must phosphorylate the dimer form of endothelial nitric oxide synthase (eNOS) to generate nitric oxide; monomeric eNOS phosphorylation leads to superoxide production. Interestingly, both acetylcholine (ACh) and testosterone produce nitric oxide through Akt dependent mechanisms. This study will determine how testosterone deficiency impacts both Akt and eNOS phosphorylation. We hypothesize testosterone deficiency will reduce Akt mediated eNOS phosphorylation and impair endothelial dependent relaxation.

Methods: Male Sprague Dawley rats (12 wks) were divided into 2 groups: control (CON; n=8) and castration (CAST; n=8). Six weeks following castration, internal pudendal arteries (IPA) and penises were excised and mounted into tissue baths. Testosterone and ACh mediated relaxations were assessed in phenylephrine precontracted tissue. ACh mediated relaxation was also examined in the presence of 10-DEBC, an Akt inhibitor. Separate IPA and penis segments were incubated in testosterone or ACh (30min, in Krebs at 37C) and snap frozen. Western blots of incubated tissues measured phosphorylated Akt (p-Akt) normalized to total Akt, phosphorylated e-NOS (p-eNOS) normalized to total eNOS, and eNOS dimer/monomer protein expression.

Results: CAST decreases testosterone mediated penile relaxation (CON: 55.2%±2.9, CAST: 30.2%±4.1; $p<0.05$) but does not change IPA relaxation to testosterone. CAST reduces ACh relaxation in both the penis (CON: 23.4%±2.6, CAST: 10.9%±1.1; $p<0.05$) and the IPA (CON: 37.5%±2.7, CAST: 21.5%±1.7; $p<0.05$). In testosterone incubated tissues, CAST does not impact Akt or eNOS phosphorylation. To our surprise, in ACh incubated tissues, CAST increases both IPA Akt phosphorylation (CON: 1.00±0.04, CAST: 1.27±0.03; $p<0.05$) and eNOS phosphorylation (CON: 1.00±0.12, CAST: 1.23±0.09; $p<0.05$). In the penis, CAST increases both ACh mediated Akt phosphorylation (CON: 1.00±0.07, CAST: 1.51±0.17; $p<0.05$), and eNOS phosphorylation (CON: 1.00±0.08, CAST: 1.36±0.04; $p<0.05$). Interestingly, CAST markedly decreases penile eNOS dimer/monomer protein expression. In the tissue bath, Akt inhibition restores ACh relaxation in both the IPA (CON: 59.8%±5.7, CAST: 55.9%±3.0) and penis (CON: 26.2%±6.3, CAST: 20.7%±2.7). We are currently measuring superoxide production in CAST IPA and penis following Akt inhibition.

Conclusions: CAST reduces eNOS dimer to monomer ratio, decreasing nitric oxide production and leading to endothelial dysfunction. Though CAST increases both Akt and eNOS phosphorylation, we believe the monomeric form of eNOS is being phosphorylated to generate superoxide rather than nitric oxide. Thus, Akt inhibition may prevent superoxide production and increase endothelial dependent relaxation. Clinical trials are currently evaluating novel Akt inhibitors as prostate cancer treatments. These same Akt inhibitors may also restore erectile function to prostate cancer survivors.

Funding Source(s): BSOM start-up grant, Sexual Medicine Society of North America

S7A31

Title: Effect of α-synuclein mutation on bladder function

Authors: Vivian Cristofaro[1,4]*, Josephine A. Carew[2,4], Sean D. Carey[3], Nicolas Siegelman[1], Raj K. Goyal[2,4], Maryrose P. Sullivan[1,4]

Affiliations: [1]Division of Urology, VA Boston Healthcare System, [2]Department of Medicine, VABHS, [3]Department of Neuroscience, Georgetown University, [4]Harvard Medical School.

Introduction/Objectives: Bladder dysfunction in patients with Parkinson's disease (PD) is generally attributed to central neurodegeneration caused by loss of dopaminergic neurons in the substantia nigra, resulting in disinhibition of bladder reflexes and detrusor overactivity. The pathologic aggregation of α-synuclein (α-syn) in Lewy bodies within these neurons is a distinguishing feature of PD. Although it is known to be a pre-synaptic protein implicated in trafficking and release of synaptic vesicles, the biologic function of α-syn remains poorly understood. While α-syn expression and distribution in the central nervous system has been well described, its localization and function in the bladder have not been defined. Therefore, the purpose of this study was to identify the distribution of α-syn in the bladder and determine the temporal changes in bladder function associated with an α-syn mutation in an animal model of PD.

Methods: Mice lacking the murine α-syn gene but overexpressing human α-syn with a familial mutation (A53T) were used in this study (SNCA-A53T). Mice that overexpress the wild-type human α-syn gene were used as controls (SNCA-WT). Bladders removed from both groups were cut longitudinally, placed in tissue baths and equilibrated for an hour. Contractile responses to electrical field stimulation over a range of frequencies (1-64 Hz) were determined in each group at 14, 28, 42 and 58 weeks of age. Motor function was assessed in each group at each time point by the rotarod test. Data were analyzed by ANOVA. The distribution of endogenous α-syn and its localization with neuronal markers (VAChT, synaptophysin) were examined in normal mice by immunofluorescence/confocal imaging.

Results: At the youngest age tested (14 weeks), the frequency-response curve generated after EFS in the mutant transgenic group (SNCA-A53T) was similar to that of the wild type transgenic group (SNCA-WT). At 28 weeks of age, neurogenic responses were significantly higher in SNCAA53T mice compared to responses in SNCA-WT mice. However, by 42 weeks the frequencyresponse curves in SNCA-A53T were lower than at 28 weeks, while at 58 weeks of age, evoked responses in SNCA-A53T mice were reduced compared to SNCA-WT mice. Although motor function was similar between groups at 14 and 28 weeks, rotarod scores were significantly decreased in SNCA-A53T mice at 42 and 58 weeks. No changes were detected in bladder muscarinic M3 or purinergic P2X1 receptors over time. Immunoreactivity of endogenous α-syn was distributed throughout the bladder wall and co-localized with VAChT in normal mice.

Conclusions: Profound temporal changes in neurogenic contractions detected in an animal model of PD suggest that altered bladder function may occur at an early stage of disease progression prior to the onset of centrally mediated deficits. The localization of α-syn within excitatory nerve fibers is consistent with its potential role in modulating peripheral neurotransmission in the bladder.

Funding Source(s): Department of Veterans Affairs, Biomedical Laboratory R&D, I21 BX003680.

S7A32

Title: Caspase signaling in ED patients and animal models

Authors: Elizabeth Kalmanek[1], Shawn Choe[1], Daniel Harrington[2], Samuel Stupp[3], McVary KT[4], Podlasek CA[1]*

Affiliations: [1]University of Illinois at Chicago, [2]UT Health, [3]Northwestern University, [4]Loyola University Stritch School of Medicine.

Introduction/Objectives: Erectile dysfunction (ED) affects ~50% of men aged 40-70 and has a high impact on men's health. Current treatments are ineffective in the difficult to treat prostatectomy (16-82%) and diabetic (56-59%) patients due to injury to the cavernous nerve (CN). With denervation the critical smooth muscle (SM) undergoes apoptosis and the penis becomes fibrotic, thus altering the corpora cavernosal architecture. In order to devise novel ED therapies, prevention of corpora cavernosal remodeling is critical. Apoptosis can take place via the intrinsic (caspase 9) or extrinsic (caspase 8) pathway. We examine the mechanism of how apoptosis occurs in ED patients and in a CN injury rat model, to determine points of apoptosis intervention for therapy development.

Methods: Immunohistochemical analysis and western analysis for caspase 3 cleaved, -8 and -9 (pro and active forms) were performed in corpora cavernosal tissue from Peyronie's, prostatectomy and diabetic ED patients (n=56), and in penis from adult Sprague Dawley rats that underwent CN crush and were sacrificed after 1-9 days (n=16).

Results: Caspase 3 cleaved was observed in corpora cavernosa from Peyronie's patients, and at higher abundance in prostatectomy and diabetic tissues. Apoptosis takes place primarily through the extrinsic (caspase 8) pathway in penis tissue of ED patients. In the CN crushed rat, caspase 3 cleaved was abundant from 1-9 days after injury, and apoptosis takes place primarily via the intrinsic (caspase 9) pathway. Caspase 9 was first observed and most abundant in a layer under the tunica, and after several days was observed in the lining of and between the sinuses of the corpora cavernosal tissue. Caspase 8 staining was observed initially at low abundance in the rat corpora cavernosa, and was not observed at later time points after CN injury (4 and 9 day).

Conclusions: Apoptosis takes place primarily through the extrinsic caspase 8 dependent pathway in ED patients and via the intrinsic caspase 9 dependent pathway in the commonly used CN crush ED model. This is significant when considering points of intervention to suppress the apoptotic response to CN injury for therapy development. The differences in apoptotic signaling between patient tissues and animal models is an important consideration for study design and interpretation, and our therapeutic targets should ideally inhibit both apoptotic mechanisms.

Funding Source(s): NIH/NIDDK DK101536

S7A33

Title: Acute Ozone Exposure Increases Bladder Pro-Inflammatory Cytokines and Mitochondrial Respiration in Female Mice

Authors: Laura G White[1], Zoe S Terwilliger[1], Elena S Pak[1], Myles Hodge[2], Sky W Reece[2], Elizabeth A Browder[2], Kymberly M Gowdy[2], and Johanna L Hannan[1]

Affiliations: [1]Department of Physiology, Brody School of Medicine, East Carolina University; [2]Department of Pharmacology & Toxicology, Brody School of Medicine, East Carolina University

Introduction/Objectives: Ground level ozone (O_3) is a pollutant and irritant that can negatively impact human health. Inhalation of O_3 can increase pro-inflammatory cytokines resulting in vascular inflammation and smooth muscle dysfunction. Increasing evidence suggests that exposure to ambient air pollutants, such as O_3 or particulate matter, can increase bladder inflammation leading to bladder cancer. In the United States, 25-33% of men and women suffer from bladder dysfunction, significantly affecting overall quality of life. The impact of acute O_3 exposure on bladder physiology is unknown. We hypothesize that acute O_3 exposure will increase pro-inflammatory cytokines in the mucosal and smooth muscle layers of the bladder and will increase smooth muscle contractility.

Methods: Female C57BL/6J mice (8-12 weeks old) were exposed to filtered air (FA) or O_3 (1 ppm) for 3 hours. Mice were euthanized at 6 or 24 hours post exposure. Bladders were excised, fixed, stained with H&E and scored for edema. Remaining bladders were separated into the mucosal layer and detrusor. Contractility of the detrusor was determined via a concentration response curve to carbachol or electric field stimulation (EFS). RNA was isolated from the mucosal and detrusor layers and qPCR measured the expression of several pro-inflammatory cytokines. Mitochondrial oxygen consumption of the mucosal layer was measured with OROBOROS oxygraphy-2K (O2K).

Results: O_3 exposure markedly increased edema of the lamina propria 6hrs post-O_3, however, this edema was significantly reduced by the 24hr time point. Detrusor smooth muscle contraction to carbachol was unchanged following acute O_3 exposure. Similarly, following acute inhalation of O_3, there were no changes in EFS-mediated bladder contraction, with or without purinergic and cholinergic antagonists. Acute O_3 exposure increased the gene expression of pro-inflammatory cytokines IL-6 and CXCL1 in bladder mucosa 6hr post-O_3. Following 24hr, all cytokines had normalized to FA levels of gene expression. In contrast, pro-inflammatory cytokine levels were unchanges in the detrusor layer with O_3 exposure. Acute O_3 exposure also increased bladder mucosal mitochondrial respiration in the presence of complex-I activating substrates pyruvate/malate and glutamate 24hr post-O_3 exposure.

Conclusion: Acute O_3 exposure elicited a pro-inflammatory response in the bladder's mucosal layer in female mice. Marked edema within the lamina propria was present 6hr post O_3 exposure. Both cholinergic and EFS-mediated contractions were unaffected by acute O_3 exposure. Increased complex-I mediated mitochondrial respiration was evident in the mucosal layer indicating that acute O_3 may lead to impaired mitochondrial function. Our next steps are: 1) to assess the impact of acute O_3 exposure in male mice to determine if sex-related differences are present and 2) to establish a chronic O_3 exposure model to mimic levels observed in major urban regions.

Funding Source(s): NC State Center for Human Health and the Environment

4th Annual Meeting of the Society for Pelvic Research

November 16-17, 2019

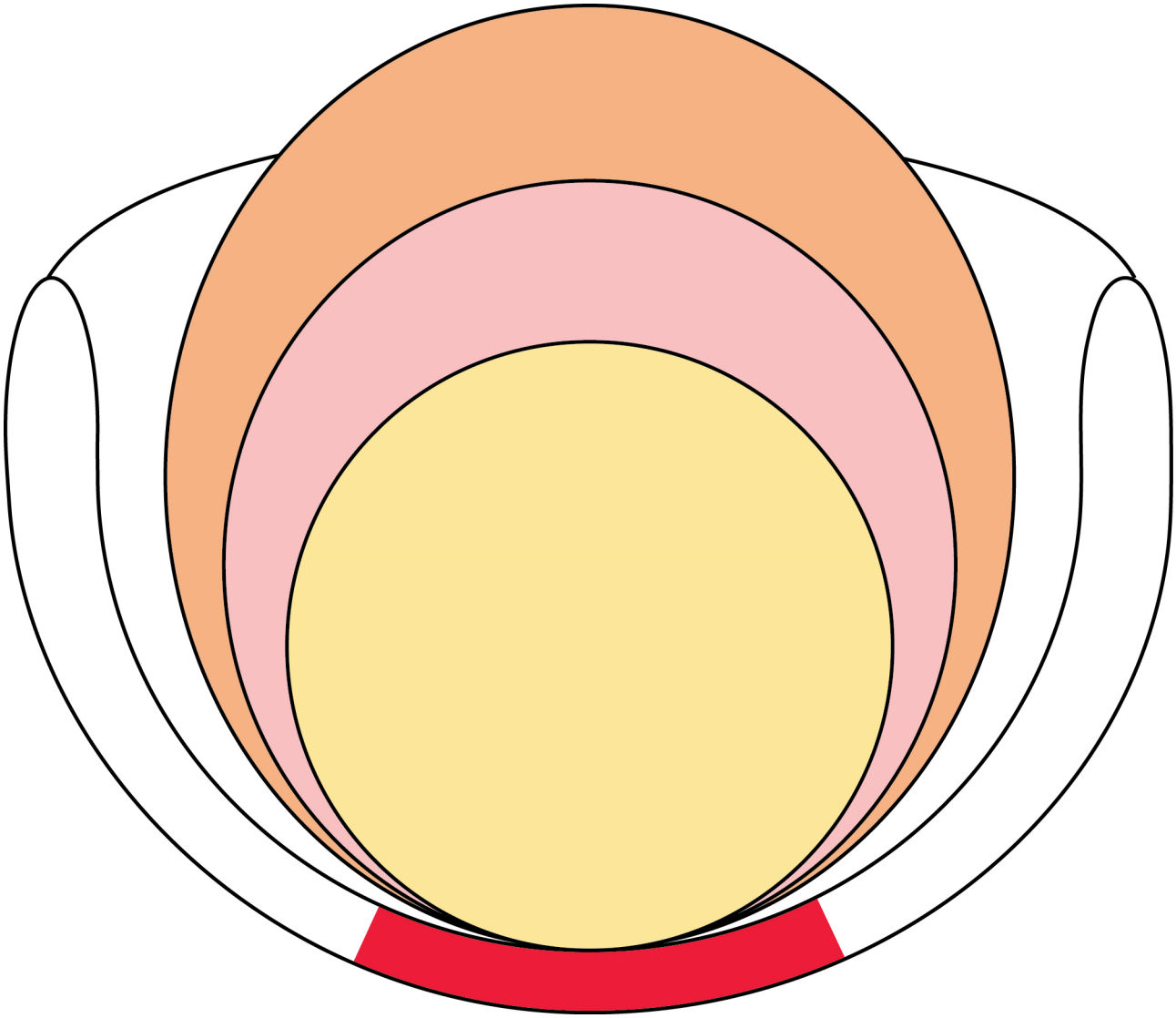
Charleston Marriott, 170 Lockwood Boulevard, Charleston, SC

Start Time	End Time	Duration	Abst #		Presenter
Saturday, November 16, 2019					
7:00 AM	8:00 AM	1:00		BREAKFAST	
8:00 AM	8:10 AM	0:10		Welcome, Opening Remarks, Mission of SPR	Matthew Fraser, PhD
				Session 1: Physiology I - Moderators: Ekta Tiwari, PhD, Post-Doctoral Fellow and Matthew Fraser, PhD	
8:10 AM	8:50 AM	0:40		Keynote Address - Targeting Enteric Glia in GI Diseases and Motility Disorders – Recent Progress on Postoperative Ileus	Fievos Christofi, PhD
8:50 AM	9:00 AM	0:10		Q & A	
9:00 AM	9:10 AM	0:10	S1A1	Toll Like Receptor-9 activation impairs cavernosal reactivity in obese mice	Priviero, F
9:10 AM	9:20 AM	0:10	S1A2	Role of Pannexin 1 channels in stress-induced pelvic pain and urinary symptoms in mice.	Urban-Maldonado, M
9:20 AM	9:30 AM	0:10	S1A3	Differential effects of pressure and stretch on urothelial cell function in vitro.	Dunton, C
9:30 AM	9:40 AM	0:10	S1A4	Aoah suppresses PPARγ-mediated gut dysbiosis	Yang, W
9:40 AM	9:50 AM	0:10	S1A5	Chronic bladder outlet obstruction causes NLRP3-dependent inflammation in the hippocampus and depression in rats: a possible mechanism underlying psychosocial disorders associated with LUTS	Hughes, FM
9:50 AM	10:00 AM	0:10	S1A6	Is NT5E/CD73 involved in adenosine production in suburothelium/lamina propria during filling of the murine bladder?	Mutafova-Yambolieva, VN
10:00 AM	10:15 AM	0:15		Q & A	
10:15 AM	10:30 AM	0:15		BREAK	
				Session 2: Models and Methods - Moderators: Shelby Harper, Medical Student and Maryrose Sullivan, PhD	
10:30 AM	11:10 AM	0:40		State of the Art Lecture - Neuromodulation for Urological and Bowel Dysfunction: Research-Driven Directions	Lance Zirpel, PhD
11:10 AM	11:20 AM	0:10		Q & A	
11:20 AM	11:30 AM	0:10	S2A7	Effects of sedative and anesthetic agents on urodynamic and anesthetic parameters in male cats	Xu, JJ
11:30 AM	11:40 AM	0:10	S2A8	Validation of an animal model of pelvic radiation induced female sexual and urinary dysfunction	Burleson, LK
11:40 AM	11:50 AM	0:10	S2A9	Increasing the pH of Urothelial Lysosomes Induces Bladder Hyperactivity and Inflammation	Beckel, JM
11:50 AM	12:00 PM	0:10	S2A10	Nerve transfer for restoration of lower motor neuron-lesioned bladder and urethra function: establishment of a canine model and interim pilot study results	Tiwari, E
12:00 PM	12:10 PM	0:10	S2A11	Regional and directional variation in bladder wall micromotion instigated by electrostimulation as measured by transabdominal Anatomical Motion Mode (AMM) ultrasound	Nagle, AS
12:10 PM	12:20 PM	0:10	S2A12	Real time closed loop control of bladder function with dorsal root ganglia sensory feedback and sacral root electrical stimulation	Ouyang, ZA
12:20 PM	12:35 PM	0:15		Q & A	
12:35 PM	1:35 PM	1:00		LUNCH	
				Session 3: Pharmacology I - Moderators: Michael Odom, PhD Candidate and Johanna Hannan, PhD	
1:35 PM	2:15 PM	0:40		State of the Art Lecture - Surgical and Biomaterial Advances for Regeneration of the Urogenital Tract	Trinity Bivalacqua, MD PhD
2:15 PM	2:25 PM	0:10		Q & A	
2:25 PM	2:35 PM	0:10	S3A13	Nicotinic receptors on nerve terminals induce acetylcholine release in canine bladder	Braverman, AS
2:35 PM	2:45 PM	0:10	S3A14	Histamine does not directly contract urinary bladder smooth muscle	Jones, BM
2:45 PM	2:55 PM	0:10	S3A15	Optimization of Sonic hedgehog delivery to the penis from self-assembling nanofiber hydrogels to preserve penile morphology after cavernous nerve injury	Choe, S
2:55 PM	3:05 PM	0:10	S3A16	Curcumin-Loaded Nanoparticles Protect Erectile Function in a Rat Model of Type-2-Diabetes	Draganski, A
3:05 PM	3:15 PM	0:10	S3A17	Intravesical macrophage migration inhibitory factor (MIF) and activation of intravesical MIF receptors mediate PAR4-induced bladder pain	Ye, S
3:15 PM	3:30 PM	0:15		Q & A	
3:30 PM	3:45 PM	0:15		BREAK	
				Session 4: NIH and Societal Funding/Support - Moderators: Lindsey Burleson, Medical Student and Georgi Petkov, PhD	
3:45 PM	4:15 PM	0:30		Special Guest Lecture - Opportunities for Funding at NICHD	Donna Mazloomdoost, MD
4:15 PM	4:35 PM	0:20		Special Guest Lecture - Opportunities for Funding/Support at AUA	Carolyn Best, PhD
4:35 PM	4:55 PM	0:20		Special Guest Lecture - Opportunities for Funding/Support at ANMS	Fievos Christofi, PhD
4:55 PM	5:15 PM	0:20		Special Lecture - Opportunities for Funding at SMSNA/ISSWSH	Johanna Hannan, PhD
5:15 PM	5:30 PM	0:15		Q & A	
5:30 PM	7:30 PM	2:00		RECEPTION	
Sunday, November 17, 2019					
7:00 AM	8:00 AM	1:00		BREAKFAST	
8:00 AM	8:05 AM	0:05		Welcome to Day 2 of the 4th Annual SPR Meeting	Maryrose Sullivan, PhD
				Session 5: Pharmacology II - Moderators: Jessica Xu, DVM Resident and Sylvia Suadicani, PhD	
8:05 AM	8:45 AM	0:40		Keynote Address - Dysregulated innate immune responses contribute to erectile dysfunction	R. Clinton Webb, PhD
8:45 AM	8:55 AM	0:10		Q & A	
8:55 AM	9:05 AM	0:10	S5A18	Impact of RhoGDI Gene Transfection of Bladder Smooth Muscle Contractility in a Validated Ex-vivo Murine Model	Haney, NM
9:05 AM	9:15 AM	0:10	S5A19	Utilizing Enzymes to Induce Hypoxia for an in vitro Bladder Outlet Obstruction Model	Hudson, BN
9:15 AM	9:25 AM	0:10	S5A20	NLRP-3 Inflammasomes inhibition improves bladder reactivity in dbdb-/- mice	Dela Justina, V
9:25 AM	9:35 AM	0:10	S5A21	Subtype-specific Kv7/KCNQ channel activators reduce excitability and contractility in human detrusor smooth muscle	Malysz, J
9:35 AM	9:45 AM	0:10	S5A22	The nicotinic receptor agonist Epibatidine induces contraction more frequently in sub-mucosal than sub-serosal canine bladder smooth muscle	Frara, N
9:45 AM	9:55 AM	0:10	S5A23	Calcium Pyrophosphate and Monosodium Urate Activate the NLRP3 Inflammasome within Bladder Urothelium via Reactive Oxygen Species and Thioredoxin Interacting Protein	Harper, SN
9:55 AM	10:10 AM	0:15		Q & A	
10:10 AM	10:25 AM	0:15		BREAK	
				Session 6: Physiology II - Moderators: Aileen Ouyang, PhD Candidate and Kelvin Davies, PhD	
10:25 AM	11:05 AM	0:40		State of the Art Lecture - Urogynecologic meshes: a lesson in the development of novel devices	Pamela Moalli, MD PhD
11:05 AM	11:15 AM	0:10		Q & A	
11:15 AM	11:25 AM	0:10	S6A24	Castration Mediated Schwann Cell Dedifferentiation Leads to Slower Nerve Conduction, Decreased Neuritogenesis, and Nitrergic Neuron Loss	Odom, MR
11:25 AM	11:35 AM	0:10	S6A25	Functional and histological changes in the dog urinary bladder after different decentralization and reinnervation strategies	Barbe, MF
11:35 AM	11:45 AM	0:10	S6A26	Gonadal hormones and anesthetics influence threshold of transcutaneous electrical stimulation to induce external urethral sphincter reflex activity in female rats	Cruz, Y
11:45 AM	11:55 AM	0:10	S6A27	Barrington's Reflexes Revisited: Proximal Urethral Electrostimulation Causes Remarkable Excitatory Bladder Response in Spinal Cord Intact Rats	Potts, BA
11:55 AM	12:05 PM	0:10	S6A28	Bladder reinnervation by somatic nerve transfer to pelvic nerve vesical branches does not reinnervate the urethra	Cruz,GE
12:05 PM	12:20 PM	0:15		Q & A	
12:20 PM	1:20 PM	1:00		LUNCH	
				Session 7: Physiology III - Moderators: Cody Dunton, PhD Candidate and Carol Podlasek, PhD	
1:20 PM	1:30 PM	0:10	S7A29	Comparison of the actomyosin ATPase inhibitor calponin and the sphingosine-1-phosphate cell differentiation pathway regulatory enzymes in vaginal wall smooth muscle of women with and without pelvic organ prolapse	Sobieski, J
1:30 PM	1:40 PM	0:10	S7A30	Ex Vivo Akt Inhibition Reverses Castration Induced Penile and Pudendal Artery Endothelial Dysfunction	Odom, MR
1:40 PM	1:50 PM	0:10	S7A31	Effect of α-synuclein mutation on bladder function	Cristofaro, V
1:50 PM	2:00 PM	0:10	S7A32	Caspase signaling in ED patients and animal models	Kalmanek, E
2:00 PM	2:10 PM	0:10	S7A33	Acute Ozone Exposure Increases Bladder Pro-Inflammatory Cytokines and Mitochondrial Respiration in Female Mice	White, LG
2:10 PM	2:25 PM	0:15		Q & A	
2:25 PM	2:40 PM	0:15		BREAK	
2:40 PM	2:50 PM	0:10		Trainee Awards Presentations	Mary Barbe, PhD & Michael DiSanto, PhD
2:50 PM	3:00 PM	0:10		Closing Remarks	Matthew Fraser, PhD
	3:00 PM			MEETING ADJOURNS	

Final Announcements

SPR 2020 to be held in Charlotte, NC

Please Welcome and Support our New President, Michael R. Ruggieri, Sr, PhD



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