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TITLE: Functional and Molecular Mechanisms Underlying Detrusor-Sphincter Dyssynergia (DSD) in SCI

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14. ABSTRACT

In patients with spinal cord injury (SCI), neurogenic lower urinary tract dysfunction due to detrusor overactivity (DO) during urine storage and detrusor-sphincter dyssynergia (DSD) during voiding is a great risk factor of urological problems in people with spinal cord injury (SCI). The overall aim of this proposal is therefore to seek to identify the functional and molecular mechanisms of voiding dysfunction and DSD, as well as the involvement of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), following SCI. In the third year of the project, the major achievements included; (1) time-dependent progression of lower urinary tract dysfunction such as DSD in association with BDNF upregulation in the bladder in SCI mice, especially focusing on the mice up to 6 weeks after SCI and (3) effects of early-started BDNF inhibition on lower urinary tract dysfunction such as DO and DSD/inefficient voiding in the SCI model. These results indicated that SCI induces time dependent bladder and urethral sphincter dysfunctions similar to SCI humans, which are associated with the changes in molecular expressions in the bladder (e.g., BDNF) and bladder afferent pathways (e.g., ASIC channels).

15. SUBJECT TERMS
Spinal cord injury, urinary bladder, urethral sphincter, detrusor overactivity, detrusor sphincter dyssynergia, bladder afferent neurons, brain-derived neurotrophic factor (BDNF)

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1. **INTRODUCTION:** In patients with spinal cord injury (SCI), neurogenic lower urinary tract dysfunction associated with detrusor overactivity (DO) during urine storage and detrusor-sphincter dyssynergia (DSD) (i.e., concomitant contractions of detrusor and external urethral sphincter [EUS]) during voiding is a great risk factor of urological problems such as urinary incontinence, retention, urinary tract infection, upper urinary tract deterioration and autonomic dysreflexia. This proposal will seek to identify the functional and molecular mechanisms of voiding dysfunction and DSD, as well as the involvement of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in a mouse model of SCI.
2. **KEYWORDS:** Spinal cord injury, urinary bladder, urethral sphincter, detrusor overactivity, detrusor sphincter dyssynergia, bladder afferent neurons, brain-derived neurotrophic factor (BDNF)
3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - As described in the SOW, the major goals of the third year of the project were to evaluate bladder and external urethral sphincter (EUS) functions as well as molecular and growth factor expression in the bladder and afferent neurons in spinal intact and SCI mice at 4 weeks (Major tasks #5 & 6) and 6 weeks (a part of Major tasks #7 & 8) after spinal cord transection.
  - During the third year of the project, we successfully accomplished the characterization of bladder and urethral dysfunction in 4-week SCI mice and, near completely, in 6-week SCI mice, as planned in the SOW although the last portion of experiments using 6-week SCI mice was delayed because of the COVID-19 pandemic that considerably slowed the research activity for almost 5 months since March, 2020.

Specific Aim 1: To investigate the time-dependent changes in voiding dysfunction induced by DSD and molecular expression of mechanosensitive channels in bladder afferent neurons using a mouse model of SCI.	**Timeline**	**Site 1**
Major Task 5: Evaluating bladder and EUS function by cystometry and EUS-EMG recordings in age-matched spinal intact and SCI mice at 4 weeks after injury.	Months	
Subtasks 1 & 2: Evaluate the changes in bladder and EUS activity using similar methods described in Major Task 2.	20-**28**	Yoshimura Tyagi

Milestone #7: Characterization of the time-dependent progression of storage and voiding dysfunction in 4-weeks SCI mice	20-28	Yoshimura Tyagi
Major Task 6: Evaluating changes in molecular and BDNF expression of bladder afferent pathways in SCI mice <u>at 4 weeks</u>		
Subtasks 1 to 3: Analyze the changes in mRNA and protein levels using similar methods described in Major Tasks 3 & 4.	20-28	Yoshimura Tyagi
Milestone #8: Characterization of alterations in mechanosensitive channels in bladder afferent pathways and BDNF after SCI, which are correlated with the SCI-induced functional changes in bladder and EUS activity (Major Task 2).	20-28	Yoshimura Tyagi
Major Task 7: Evaluating bladder and EUS function by cystometry and EUS-EMG recordings in age-matched spinal intact and SCI mice <u>at 6 weeks after injury.</u>		
Subtasks 1 & 2: Evaluate the changes in bladder and EUS activity using similar methods described in Major Task 2.	29-36	Yoshimura Tyagi
Milestone #9: Characterization of the time-dependent progression of storage and voiding dysfunction in 6-weeks SCI mice	29-36	Yoshimura Tyagi
Major Task 8: Evaluating changes in molecular and BDNF expression of bladder afferent pathways in SCI mice <u>at 6 weeks</u>		
Subtasks 1 to 3: Analyze the changes in mRNA and protein levels using similar methods described in Major Tasks 3 & 4.	29-36	Yoshimura Tyagi
Milestone #10: Characterization of alterations in mechanosensitive channels in bladder afferent pathways and BDNF after SCI, which are correlated with the SCI-induced functional changes in bladder and EUS activity (Major Task 7).	29-36	Yoshimura Tyagi

- **What was accomplished under these goals?**

1) Major activities

1. We performed experiments using mice with 4-weeks and 6-weeks SCI to characterize time-dependent changes in lower urinary tract dysfunction following SCI by using experimental techniques such as cystometry, urethral sphincter electromyography

(EMG) and molecular assays (Major Task 5-6 for 4-weeks SCI mice and Major Tasks 7-8 for 6-weeks SCI mice).

2. We evaluated the role of BDNF overexpression in changes in bladder and urethral sphincter function, and molecular expression in the bladder and afferent neurons in 2-6-weeks SCI mice (Major Tasks 5-8).

2) Specific objectives

1. To evaluate the time-course effects of SCI on bladder and urethral sphincter activity during voiding using simultaneous recordings of cystometry and external urethral sphincter (EUS)-EMG in an awake condition
2. To evaluate the time-course effects of SCI on molecular expression (TRP, ASIC & Piezo channels) in L6-S1 dorsal root ganglia (DRG) that contain bladder and EUS afferent neurons.
3. To evaluate the effects of BDNF inhibition induced by administration of anti-BDNF antibodies on lower urinary tract dysfunction and molecular changes in SCI mice.
4. To evaluate the effects of chemogenetic inhibition of Aδ-fiber bladder afferent pathways on lower urinary tract dysfunction in SCI mice using virus vector mediated gene delivery of mutant inhibitory ion channels with chemogenetic channel activation by ivermectin, an FDA-approved antiparasitic drug.

3) Significant results or key outcomes, including major findings, developments, or conclusions;

1. <u>The time-course effects of SCI on lower urinary tract dysfunction</u>

   Because we reported the progressive changes in bladder overactivity during the storage phase from 2 weeks to 4 weeks after SCI in the first- and second-year progress reports, our third-year study investigated the time-course changes in bladder and urethral sphincter function in SCI mice up to 6 months after spinal cord transection.

   [Methods] SCI was produced by complete transection of the Th8/9 spinal cord in female C57BL/6N mice. After spinal transection, their bladder was manually squeezed to eliminate the urine once daily until the evaluation. We inserted a PE-50 tube into the bladder from the bladder dome as a cystostomy catheter as well as EMG wires to the bilateral EUS, and evaluated the bladder function at different time-points

(2 weeks, 4 weeks and 6 weeks) after the injury using simultaneous recordings of cystometry and EUS-EMG under an awake condition.

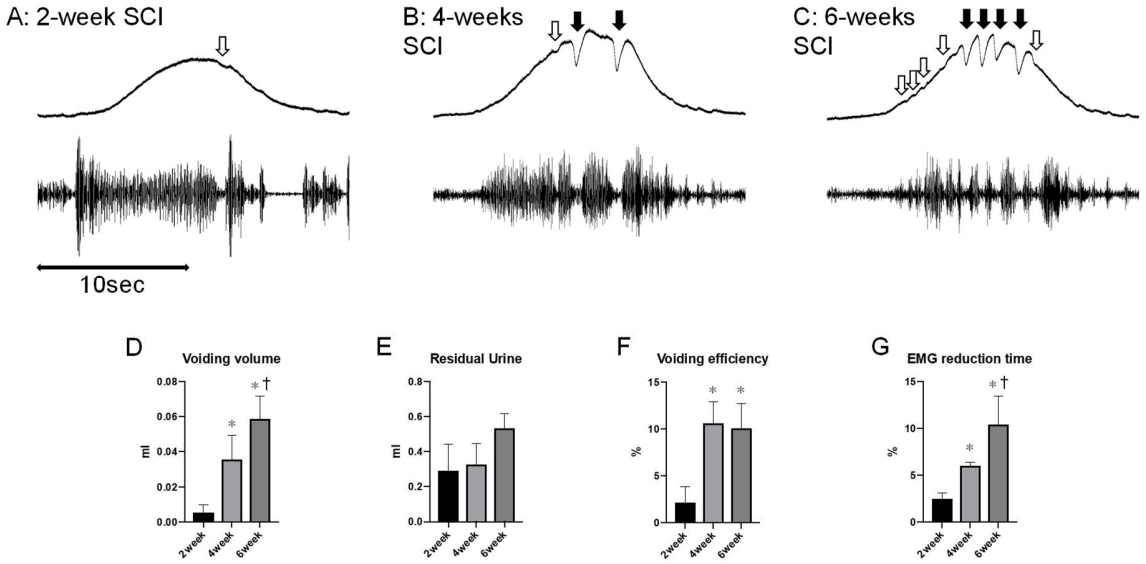

Fig. 1. A-C: Representative cystometry and EUS-EMG tracings of (A) 2 weeks post spinal cord injury (SCI), (B) 4 weeks post SCI, and (C) 6 weeks post SCI during voiding contraction. In the 2week post SCI mice, EUS activity did not show a much reduction. leading to urinary retention (A). At 4weeks post SCI, EUS reductions during voiding bladder contractions were increased, resulting in notch-like bladder pressure reductions as shown by black arrows, which corresponded to actual urination (B). At 6weeks post SCI, the EUS activity during voiding was further reduced and showed an increase of notch-like reductions (C). **D-G:** In comparison of 2-, 4-, and 6weeks post SCI, voiding volume and EMG reduction time during voiding bladder contractions were increased with time-progression. Due to larger bladder capacity, residual urine did not show a difference between the groups. Voiding efficiency showed an improvement at 4weeks compared to 2weeks, but did not show the difference between 4- and 6-weeks post SCI. ∗: $P<0.05$ vs 2week. †: $P<0.05$ vs 4week. N=7-8 mice at each group.

[Results] In SCI mice, non-voiding contractions (NVC) during bladder filling were confirmed at 2 weeks post-SCI and did not increase over time to 6 weeks (data not shown). In EUS-EMG measurements, DSD was observed at 2 weeks, but periodic EMG reductions during bladder contraction, resulting in urination, were not observed in most 2-weeks SCI mice, thereby leading to urinary retention (Fig. 1). At 4 weeks, SCI mice showed increases of EMG activity reduction time with increased voiding efficiency. At 6 weeks, SCI mice exhibited further increases in bladder capacity, residual volume and EMG reduction time compared to 2-weeks and 4-weeks SCI mice without significant changes in voiding efficiency compared to 4 weeks (Fig. 1)

[Conclusions] These results indicate that DO evident as NVC is established in the early phase (2weeks) whereas DSD is completed later at 4weeks with a slight improvement evident as increased EMG reduction time at 6 weeks post SCI.

2. <u>The time-course effects of SCI on afferent marker expression in bladder sensory pathways</u>

We also evaluated the time-course changes of expression of C-fiber afferent nociceptive channels such as TRPV1 and Aδ-fiber afferent mechanosensitive channels such as ASIC1-3 & Piezo2 in L6-S1 dorsal root ganglia (DRG), which contain bladder and EUS afferent neurons, obtained from 2-, 4- and 6-weeks SCI mice as well as spinal intact mice.

[Methods] Using the same mouse model of SCI, we measured the transcript levels of afferent nerve markers such as TRPV1, ASICs (1-3) and Piezo2 in L6-S1 DRG, which contain bladder and EUS afferent neurons, by real-time PCR methods.

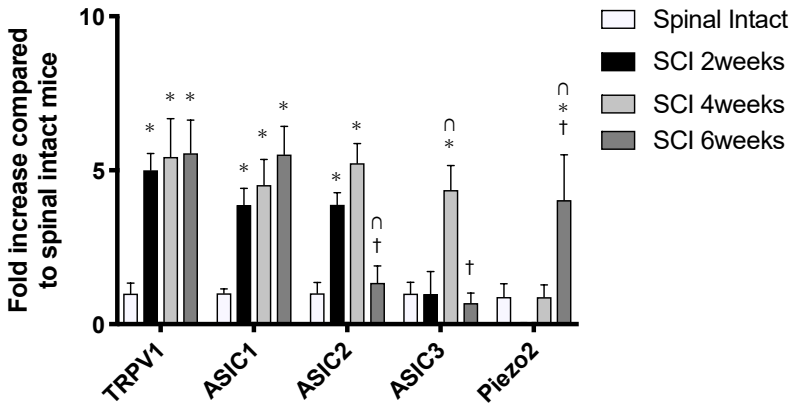

Fig. 2. Results of RT-PCR of L6-S1 DRG. TRPV1 and ASIC1 showed an increase of mRNA levels in 2-, 4-, 6 weeks post spinal cord injury (SCI) compared to spinal intact (SI). ASIC2 showed an increase at 2- and 4-weeks post SCI, and a decrease at 6weeks. ASIC3 showed an increase at 4-weeks post SCI and a decrease at 6weeks. Piezo2 showed an increase only at 6weeks post SCI. ∗: P<0.05 vs SI. ∩: P<0.05 vs 2week. †: P<0.05 vs 4week. N=7-8 mice at each group

[Results] RT-PCR of L6-S1 DRG showed increased mRNA levels of TRPV1 and ASIC1-3 in SCI mice compared to spinal intact mice, with a later decrease of ASIC2 and 3 at 6 weeks compared to 4-weeks SCI mice. However, Piezo2 showed a slow increase at 6 weeks of SCI compared to 4-weeks SCI mice (Fig. 2). A C-fiber marker, TRPV1, was continuously increased at 2-6 weeks post SCI.

[Conclusions] These results indicate that that ASIC and Piezo2 mechanosensitive channels may be involved in the establishment of DSD in early (2-4 weeks) and late phases (4-6 weeks) of SCI, respectively. These data with functional results above

would help us to understand the progression mechanisms of SCI-induced LUTD. We presented these functional and molecular results (Figs. 1 & 2) at the 2020 AUA Annual meeting (see Appendix), and also published the data up to 4 weeks after SCI, including bladder BDNF expression, in American J Physiology (Wada et al. 2019) and Neurourology & Urodynamics (Wada et al., 2020).

3. Role of BDNF overexpression in lower urinary tract dysfunction after SCI

Because we have shown BDNF upregulation in the bladder from an early phase of SCI in previous years, we examined the effects of early-started, short- or long-term anti-BDNF treatments on lower urinary tract dysfunction after SCI.

[Methods] Using the same mouse model of SCI, anti-BDNF antibody (Ab) (10 μg/kg/h) were subcutaneously administered using osmotic pumps from day 3 after SCI, and single-filling cystometry was performed at 10 and 30 days (7 and 27 days of treatment, respectively) after SCI.

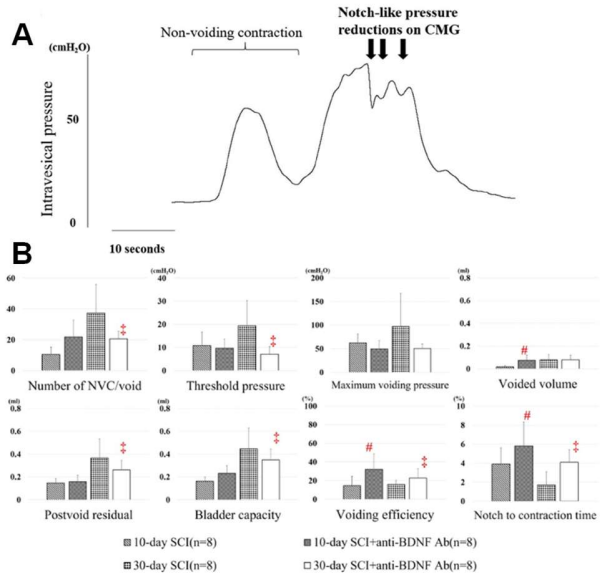

Fig. 3. Effects of anti-BDNF treatment on SCI induced voiding dysfunction
A: a representative trace of cystometry showing the voiding reflex. B: Effects of early-started, 10-days or 30days anti-BDNF antibody treatment. Note that voiding efficiency and notch-like reduction duration during voiding bladder contractions were improved after short-and long-term anti-BDNF treatments. #: P<0.05 versus 10-day SCI mice, ‡: P<0.05 versus 30-day SCI mice

[Results] Voiding efficiency was lower at each time point after SCI than that of spinal intact mice. In both 10- and 30-days SCI groups treated with anti-BDNF Ab, voiding efficiency was improved, and the duration of notch-like intravesical pressure reductions during voiding bladder contractions was prolonged (Fig. 3). The number of NVC was significantly decreased only in 30-days SCI mice with 27-days anti-BDNF treatment (Fig. 3).

[Conclusions] These results indicate that the early-started, short- and long-term inhibition of BDNF both improved voiding dysfunction associated with DSD; however,

the long-term BDNF inhibition was required to reduce the later-phase development of DO after SCI. Thus, it is likely that, in SCI mice, overexpressed BDNF in the bladder is involved in inefficient voiding and also in the emergence of NVC during the storage phase. These results were presented in a peer-reviewed article published in Neurourology & Urodynamics, 2020 (Wada et al., 2020).

4. Role of Aδ-bladder afferent pathways in lower urinary tract dysfunction after SCI

Because our data obtained during the third year of this funded project suggest that BDNF-dependent Aδ-bladder afferent pathways, which showed the upregulation of mechanosensitive ASCI (1-3) and Piezo2 after SCI, may be involved in SCI-induced lower urinary tract dysfunction, especially in DSD/inefficient voiding, we further evaluated if inhibition of Aδ-fiber afferent pathways through double mutant glycine (G2M) receptors delivered by replication-defective herpes simplex virus (HSV) vectors driven by an A-fiber-targeting neurofilament 200 promotor (NF200p) can improve DSD and inefficient voiding in 4-weeks SCI mice. Newly transduced G2M receptors were chemogenetically activated by exogenous application of ivermectin, an FDA-approved anti-parasitic drug.

[Methods] Female C57BL/6N mice were used, and the Th8/9 spinal cord was transected. Two weeks after SCI, NF200p-driven HSV vectors expressing G2M or wild-type glycine receptors (WT-GlyR) were inoculated into the bladder wall. One week after vector inoculation, ivermectin (IVM; 50μl of 1μM solution) was intra-peritoneally injected daily for 7 days. Thereafter, SCI mice underwent single-filling cystometry (CMG) and external urethral sphincter (EUS) electromyogram (EMG) under an awake condition.

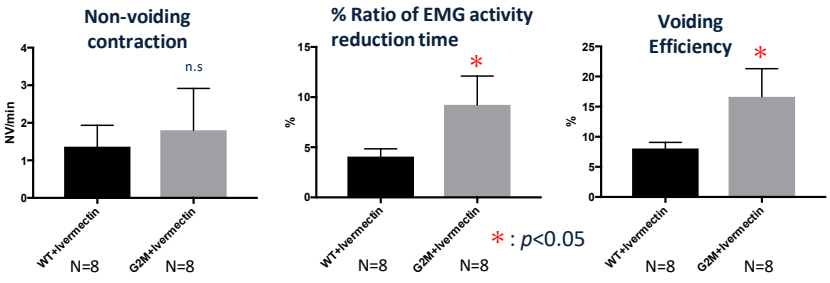

Fig. 4. Effects of HSV vector-mediated delivery of G2M receptors to Aδ-fiber bladder afferents with chemogenetic activation by IVM on SCI-induced lower urinary tract dysfunction

IVM-induced activation of G2M receptors significantly increased EMG activity reduction time during voiding bladder contractions in association with improvement of voiding efficiency. However, DO evident as non-voiding contractions during the storage phase

was not affected by IVM-mediated G2M activation in Aδ-fiber bladder afferent pathways.

[Results] Compared to the WT-GlyR and IVM group, treatment with G2M and IVM in SCI mice significantly increased the EMG activity reduction time, leading to improvements of voiding efficiency and residual volume without affecting non-voiding contractions during bladder filling (Fig. 4). RT-PCR analyses showed reductions of ASIC 1-3 & Piezo2, but not TRPV1, in L6-S1 DRG (data not shown).

[Conclusions] These results indicate that IVM-mediated activation of G2M receptors in Aδ-fiber bladder afferent pathways after NF200p-driven HSV vector inoculation improves DSD and inefficient voiding in SCI. An abstract describing these results has been accepted for podium presentation at 2020 International Continence Society (ICS) meeting held in Nov, 2020.

- In the third year of the project, we successfully completed the Major Tasks 5-6 of the SOW (characterization of lower urinary tract dysfunction in 4-weeks SCI mice) and the major portion of the Major Tasks 7-8 of the SOW (functional and molecular analyses of 6-weeks SCI mice). However, we could not completely finish the Major Tasks 7-8 because of the COVID-19 pandemic that slowed down our research activity in the last 5 months since March 2020.
- We also performed the experiments using early-started anti-BDNF antibody treatment to test if BDNF upregulation in the bladder directly contributes to lower urinary tract dysfunction after SCI
- We further investigated directly the role of BDNF-dependent Aδ-finer bladder afferent pathways in voiding dysfunction and DSD using chemogenetic gene transfer approaches. The results of these experiments will be used for preliminary data of the SCIRP Expansion Award application (W81XWH-20-SCIRP-EA) of this project.
- The findings in third year study of this project were/will be presented at the 2020 AUA and ICS meetings. Also, we published the time-course and anti-BDNF treatment results in American Journal of Physiology Renal Physiology and Neurourology & Urodynamics this year.

- **What opportunities for training and professional development has the project provided?**
  - Nothing to Report.

- **How were the results disseminated to communities of interest?**
  - Nothing to Report.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - During the 6-months NCE period, we will continue to perform more experiments with an increased number of animals using the functional and molecular techniques to identify the underlying mechanisms of bladder and urethral sphincter dysfunctions after SCI, especially focusing on a (6 weeks) phase of SCI, as outlined in the SOW Major Tasks #7-8. Also, we plan to prepare a couple of more publications to finalize the project (SOW Major Task #9).

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Nothing to Report.
- **What was the impact on other disciplines?**
  - Nothing to Report.
- **What was the impact on technology transfer?**
  - Nothing to Report.
- **What was the impact on society beyond science and technology?**
  - Nothing to Report.

5. **CHANGES/PROBLEMS:**

- Because the COVID-19 pandemic, our research activity was considerably slowed down in the last 5 months since March 2020, we could not complete the last portion of the Major Tasks 7-8. Thus, we requested the 6-months No-Cost extension till Jan, 2021, which has been approved in July, 2020.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  - **Publications**

1. Wada, N., Shimizu, T., Shimizu, N., Kurobe, M., de Groat, W.C., Tyagi, P., Kakizaki, H., Yoshimura, N.: Therapeutic effects of inhibition of brain-derived neurotrophic factor (BDNF) on voiding dysfunction in mice with spinal cord injury. American Journal of Physiology Renal Physiology, 317: F1305-F1310, 2019. acknowledgement

of federal support (yes) https://journals.physiology.org/doi/abs/10.1152/ajprenal.00239.2019

2. Shimizu, N., Hashimoto, M., Suzuki, T., Takaoka, E., Kwon, J., Shimizu, T., Hirayama, A., Uemura, H., Kanai, A.J., de Groat, W.C., Yoshimura, N.: Role of p38 MAP kinase signaling pathways in storage and voiding dysfunction in mice with spinal cord injury. Neurourology and Urodynamics, 39: 108–115, 2020. acknowledgement of federal support (yes) https://onlinelibrary.wiley.com/doi/full/10.1002/nau.24170
3. Wada, N., Yoshimura, N., Kurobe, M., Saito, T., Tyagi, P., Kakizaki, H.: The early, long-term inhibition of brain-derived neurotrophic factor (BDNF) improves voiding and storage dysfunctions in mice with spinal cord injury. Neurourology and Urodynamics. 39: 1345-1354, 2020. acknowledgement of federal support (yes) https://onlinelibrary.wiley.com/doi/abs/10.1002/nau.24385

- **Presentations**

1. Saito, T., Kurobe, M., Gotoh, D., Igarashi, T., Ishizuka, O., Yoshimura, N.: Post-injury time course of lower urinary tract dysfunction after spinal cord injury (SCI) in the mouse model (Abstract #PD24-19). Online poster presentation at 2020 American Urological Association (AUA) Annual Meeting, May 16, 2020. Status of publication (published in Journal of Urology, 203: 4S Supplement, e535-536); acknowledgement of federal support (yes).
2. Saito, T., Goins, W., Kurobe, M., Gotoh, D., Igarashi, T., Glorioso, J., Ishizuka, O., Yoshimura, N.: Ivermectin-induced improvement of voiding dysfunction and molecular and electrical properties of bladder afferent neurons in spinal cord injured mice with gene delivery of mutant glycine receptors using herpes simplex virus vectors driven by the subpopulation specific neurofilament 200 promoter (Abstract #454). Accepted for podium presentation at 2020 International Continence Society (ICS) annual meeting, November 18-21, 2020. Status of publication (will be published in Neurourology & Urodynamics); acknowledgement of federal support (yes).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Naoki Yoshimura
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8070-1664
Nearest person month worked:	2
Contribution to Project:	Dr. Yoshimura has performed work in the areas of overall study design, study quality control, data analysis and preparation of papers/abstracts.
Funding Support:	Supported by this award

Name:	Pradeep Tyagi
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-6586-4545
Nearest person month worked:	1
Contribution to Project:	Dr. Tyagi has performed work in the areas of study quality control, molecular data analysis and editing of papers/abstracts.
Funding Support:	Supported by this award

Name:	Masahiro Kurobe
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	Not available
Nearest person month worked:	4

Contribution to Project:	Dr. Kurobe has performed work in the areas of data generation in functional (cystometry, EUS-EMG) & molecular (ELISA, RT-PCR) experiments, data analysis and editing of abstracts and publications.
Funding Support:	Supported by this award

Name:	Janet Okonski
Project Role:	Research Specialist
Researcher Identifier (e.g. ORCID ID):	Not available
Nearest person month worked:	4
Contribution to Project:	Ms. Okonski has assisted Research Associate/Scholars to perform work for data generation in functional & molecular experiments and data analysis.
Funding Support:	Supported by this award

Name:	Naoki Wada
Project Role:	Visiting Research Scholar
Researcher Identifier (e.g. ORCID ID):	Not available
Nearest person month worked:	2
Contribution to Project:	Dr. Wada has performed work in the areas of data generation in functional & molecular experiments, data analysis and drafting/editing of abstracts and manuscripts.
Funding Support:	No salary paid as he was a self-supported visiting scientist.

Name:	Tetsuich Saito

Project Role:	Visiting Research Scholar
Researcher Identifier (e.g. ORCID ID):	Not available
Nearest person month worked:	2
Contribution to Project:	Dr. Saito has performed work in the areas of data generation in functional & molecular experiments, data analysis and drafting/editing of abstracts.
Funding Support:	No salary paid as he was a self-supported visiting scientist.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - Nothing to Report.
- **What other organizations were involved as partners?**
  - Nothing to Report.

8. **APPENDICES:**

Published Abstract:

1. Saito, T., Kurobe, M., Gotoh, D., Igarashi, T., Ishizuka, O., Yoshimura, N.: Post-injury time course of lower urinary tract dysfunction after spinal cord injury (SCI) in the mouse model (Abstract #PD24-19). Online poster presentation at 2020 American Urological Association (AUA) Annual Meeting, May 16, 2020. Status of publication (published in Journal of Urology, 203: 4S Supplement, e535-536)

Table 2. Treatment outcome based on different cystoscopic phenotype

Phenotype	GRA ≤ 1	GRA ≥ 2	Total
Glomerulation= 0,1, MBC ≥ 760 mL	19 (41.3%)	27 (58.7%)	46 (19.3%)
Glomerulation= 0,1, MBC < 760 mL	28 (60.9%)	18 (39.1%)	46 (19.3%)
Glomerulation= 2,3, MBC ≥ 760 mL	5 (25.0%)	15 (75.0%)	20 (8.4%)
Glomerulation= 2,3, MBC < 760 mL	66 (58.4%)	47 (41.6%)	113 (47.5%)
With Hunner's lesion	7 (53.8%)	6 (46.2%)	13 (5.5%)
Total	125 (52.5%)	113 (47.5%)	238 (100%)

P= 0.024

Table 3. Changes of symptom scores and urodynamic parameters after intravesical Botox injection in IC/BPS patients with successful treatment outcome (GRA ≥ 2) and failed treatment outcome (GRA ≤ 1)

Urodynamic parameters	Time point	GRA ≤ 1 (n= 125)	GRA ≥ 2 (n= 113)	Total (n=238)
ICSI	BL	12.2±3.70	12.6±3.77	
	FU	9.41±4.71*	4.97±3.88*#	
ICPI	BL	11.5±3.07	12.0±3.39	
	FU	9.45±4.41*	4.59±4.23*#	
OSS	BL	23.7±6.39	24.6±6.64	
	FU	18.9±8.56*	9.56±7.77*#	
VAS	BL	4.48±32.42	5.19±2.77	
	FU	3.87±3.38	2.15±2.74*#	
First sensation (mL)	BL	112±50.5	117±51.9	115±51.2
	FU	126±67.9*	130±58.2*	128±63.2*
Full sensation (mL)	BL	177±74.2	184±73.3	180±7.7
	FU	190±91.1	205±90.0	197±90.6*
Urge sensation (mL)	BL	216±86.6	229±90.1	222±88.4
	FU	222±110	238±110	230±110
Detrusor pressure (cmH_2O)	BL	20.4±12.7	21.9±14.9	21.1±13.8
	FU	21.4±25.7	18.2±14.4*	19.9±20.9
Maximum flow rate (mL/s)	BL	12.0±6.51	12.5±4.94	12.3±5.79
	FU	10.5±5.97	12.4±6.30	11.4±6.19
Voided volume (mL)	BL	232±113	268±130	249±123
	FU	227±139	253±131	240±135
Post-void residual volume (mL)	BL	39.4±71.3	26.9±53.4	33.2±63.3
	FU	70.4±106*	48.0±82.1*	59.3±95.2*
Cystometric bladder capacity (mL)	BL	273±109	297±126	285±118
	FU	290±147	304±126	297±137
Bladder compliance (mL/cmH_2O)	BL	63.4±67.0	60.0±61.8	61.7±64.4
	FU	62.7±60.0	79.7±88.5	71.1±75.7

Source of Funding: None

PD24-08

A RANDOMIZED CONTROLLED TRIAL OF "BAG-SQUEEZE" TO MINIMIZE DISCOMFORT IN MALE OUTPATIENT FLEXIBLE CYSTOSCOPY

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INTRODUCTION AND OBJECTIVE: Among the most common symptoms of patients undergoing flexible cystoscopy are pain and discomfort. Hydrodistension of prostate is a commonly utilized, yet unproven technique used by physicians to minimize outpatient flexible cystoscopy induced discomfort. This maneuver involves the health professional performing a "bag squeeze" of the irrigation fluid as one traverses the distal urinary sphincter until passing through the bladder neck. We herein report results from a single center, single-blinded randomized trial to determine whether utilization of the "bag squeeze" technique during flexible cystoscopy significantly alters pain scores.

METHODS: Consenting participants were all male, outpatients, ambulatory and not expected to have any further secondary procedures (e.g. stent, tumor fulgarization etc.) during the cystoscopy. Furthermore, this should not be the first scope and any patient who previously had a stricture or bladder neck contracture was excluded. They were randomized to Group A - Bag Squeeze or Group B - no Bag Squeeze. Following the procedure, all men responded to a Likert-scale pain grid. Data were compiled as means with two sided probability value of 0.05 using a Students T-test.

RESULTS: 152 patients were recruited and underwent a flexible cystoscopy. Four were deemed ineligible as they required secondary procedures. Among the 148 eligible patients, mean pain scores was 4.47 in the no Bag Squeeze arm and 3.01 in the Bag Squeeze arm ($p<0.005$).

CONCLUSIONS: This study showed a statistically and clinically significant decrease in pain score when the bag is squeezed during a flexible cystoscopy and accordingly should be considered standard of care.

Source of Funding: No funding was required for this study.

PD24-09

POST-INJURY TIME COURSE OF LOWER URINARY TRACT DYSFUNCTION AFTER SPINAL CORD INJURY (SCI) IN THE MOUSE MODEL

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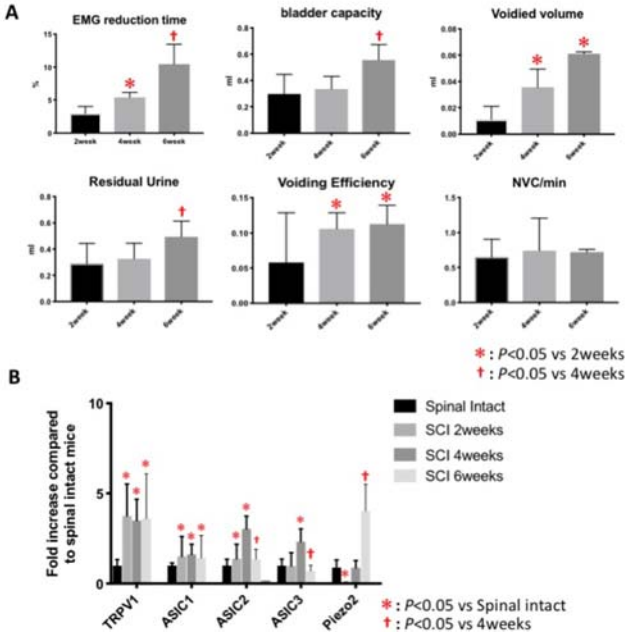
INTRODUCTION AND OBJECTIVE: Lower urinary tract dysfunction (LUTD) due to SCI causes detrusor overactivity (DO) and detrusor sphincter dyssynergia (DSD) leading to inefficient voiding with increased residual urine. Animal models of SCI have been used to study these conditions; however, the adequate timing of evaluation after SCI in mouse models has not been explored. Thus, this study evaluated the time-course changes of bladder and external urinary sphincter (EUS) activity as well as mechanosensitive channels in L6-S1 dorsal root ganglia (DRG).

METHODS: Female C57BL/6N mice were used and SCI was induced by Th8/9 spinal cord transection. The mice were divided into 3 groups; A: 2 weeks post SCI, B: 4weeks post SCI and C: 6 weeks post SCI, and evaluated by single-filling cystometery and EUS-electromyography (EMG) under an awake condition. After evaluation, L6-S1 DRG were harvested to measure mRNA expression of TRP channels and mechanosensitive ion channels such as ASICs and Piezo.

RESULTS: In SCI mice, non-voiding contractions (NVC) during bladder filling were confirmed at 2 weeks post-SCI and did not increase over time to 6 weeks (Fig. 1A). In EUS-EMG measurements, DSD was observed at 2 weeks, but periodic EMG reductions during bladder contraction, resulting in urination, were not observed in most 2-weeks SCI mice, thereby leading to urinary retention (Fig. 1A). At 4 weeks, SCI mice showed increases of EMG activity reduction time with increased voiding efficiency (VE). At 6 weeks, SCI mice exhibited further increases in bladder capacity, residual volume and EMG reduction time compared to groups A and B without significant changes in VE compared to 4 weeks (Fig. 1A). RT-PCR of L6-S1 DRG showed increased mRNA levels of TRPV1 and ASIC1-3 in SCI mice with a decrease of ASIC2-3 at 6 weeks compared to 4 weeks while Piezo2 showed a slow increase at 6 weeks (Fig. 1B).

CONCLUSIONS: These results indicate that DO evident as NVC is established in the early phase (2weeks) whereas DSD is completed later at 4weeks with a slight improvement evident as increased EMG reduction time at 6 weeks post SCI, and that ASIC and Piezo2 mechanosensitive channels may be involved in the establishment of DSD in early (2-4 weeks) and late phases (4-6 weeks) of SCI, respectively. These data would help us to understand the progression mechanisms of SCI-induced LUTD.

Fig. 1



Source of Funding: DOD W81XWH-17-1-0403

PD24-10

NICOTINIC RECEPTORS ON NERVE TERMINALS INDUCE ACETYLCHOLINE RELEASE IN CANINE BLADDER

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INTRODUCTION AND OBJECTIVE: 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 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% O2/5% CO2 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.

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. The $\alpha7$ nAChR-selective agonist AR-R17779 had no effect and the $\alpha7$ selective antagonist MLA had no effect at $\alpha7$ selective concentrations on epidbatidine induced contractions. The $\alpha4\beta2$ selective agonist TC2559 also had no effect whereas the $\alpha3\beta4$ selective agonist NS3861 and the non-selective ganglionic receptor agonist DMPP induced small contractions. Also, the $\alpha3\beta4$ selective antagonist SR-16584 (1-3 uM) blocked eipibatidine induced contractions by 50% . At relatively high concentrations, the skeletal muscle neuromuscular junction nicotinic receptor antagonists atracurium besylate (5 uM) and tubocurarine (0.1 uM) blocked epibatidine induced contractions. At concentrations reported to be selective for ganglionic nicotinic receptors, the neuronal nicotinic receptor antagonists hexamethonium (100 uM) and mecamylamine (10uM) blocked epibatidine induced contractions.

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. Based on the pharmacological data, the $\alpha3\beta4$ receptor subtype is likely involved in contraction of the canine bladder.

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PD24-11

CHRONIC MEALTIME SHIFT DISTURBS METABOLIC AND URINARY FUNCTIONS IN MICE

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INTRODUCTION AND OBJECTIVE: The circadian clock is an endogenous oscillator that harmonizes various physiological processes, including urinary function. We showed that each of the three bladder tissues and lumbar spine had a circadian clock. In addition, mice with 24-hour cycle genes show changes in water intake and excretion rhythms, suggesting a functional clock-dependent nature of micturition function. However, the effect of chronic circadian disturbance on urinary function and the exact mechanism is not yet clear. Previously, we reported that early mealtime shift in mice led to severe metabolic disturbance. In the present study, we investigated the effect of chronic meal time shifts on the daily cycle patterns of food intake, water consumption, and urine excretion in young adult male mice.

METHODS: We found that shifts in meal time significantly altered the circadian pattern of water intake and urine excretion. Next, we identified the activity of the *Per2* promotor in the *ex vivo* state of young adult bladder of *Per2* :: Luc knock-in mice that were chronically shifted in time. As a result, we observed that changes in meal time increased the amplitude of *Per2* oscillations. In addition, meal time shifts clearly delayed the acrophase by delaying several hours. In order to confirm whether this phenomenon is related to clock gene, it was confirmed that the meal time has a greater effect on the clock of the bladder than the clock gene. Subsequently we confirmed that meal time shift induced an imbalance between antioxidant capacity and reactive oxygen species (ROS) levels in the body, resulting in increased oxidative damage during resting periods in mice.

RESULTS: We found that daily supplementation of antioxidants such as melatonin or C3G in ZT23 can block insulin resistance due to chronic meal shifts. Nevertheless, the same supplementation of antioxidants had no significant effect on the circadian pattern of water intake and urine excretion due to meal time shifts from 4 to 6 weeks, or the pattern of *Per2* oscillation in the *ex vivo* cultured bladder. However, the longer the shift of meal time, the more the change in urine volume and the rhythm that occurs every day was found to be weakened.

CONCLUSIONS: Our findings suggest that chronic mealtime shifts cause metabolic disorders and urinary changes through separable mechanisms.

Source of Funding: None

PD24-12

BLADDER PDGFRα^+ CELLS AND EARLY DIABETIC BLADDER DYSFUNCTION

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INTRODUCTION AND OBJECTIVE: Early diabetic bladder dysfunction is associated with an increase in non-voiding contractions (NVCs) and a decrease in compliance. We have reported that