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Characterization of Lower Urinary Tract Dysfunction after Thoracic Spinal Cord Injury in Yucatan Minipigs

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Abstract

There is an increasing need to develop approaches that will not only improve the clinical management of neurogenic lower urinary tract dysfunction (NLUTD) after spinal cord injury (SCI), but also advance therapeutic interventions aimed at recovering bladder function. Although pre-clinical research frequently employs rodent SCI models, large animals such as the pig may play an important translational role in facilitating the development of devices or treatments. Therefore, the objective of this study was to develop a urodynamics protocol to characterize NLUTD in a porcine model of SCI. An iterative process to develop the protocol to perform urodynamics in female Yucatan minipigs began with a group of spinally intact, anesthetized pigs. Subsequently, urodynamic studies were performed in a group of awake, lightly restrained pigs, before and after a contusion-compression SCI at the T2 or T9-T11 spinal cord level. Bladder tissue was obtained for histological analysis at the end of the study. All anesthetized pigs had bladders that were acontractile, which resulted in overflow incontinence once capacity was reached. Uninjured, conscious pigs demonstrated appropriate relaxation and contraction of the external urethral sphincter during the voiding phase. SCI pigs demonstrated neurogenic detrusor overactivity and a significantly elevated post-void residual volume. Relative to the control, SCI bladders were heavier and thicker. The developed urodynamics protocol allows for repetitive evaluation of lower urinary tract function in pigs at different time points post-SCI. This technique manifests the potential for using the pig as an intermediary, large animal model for translational studies in NLUTD.

Keywords: animal model; lower urinary tract; neurogenic bladder; spinal cord injury; urodynamic studies

Introduction

In developed countries, the prevalence of traumatic spinal cord injury (SCI) ranges from 250 to 906 per million,[1] and up to 84% of individuals will exhibit at least some degree of bladder dysfunction, which can cause severe morbidity and mortality.[2] Neurogenic lower urinary tract dysfunction (NLUTD) can lead to frequent urinary tract infections (UTI), autonomic dysreflexia, and potential upper urinary tract deterioration if the bladder is not managed properly.[3] As a result, restoration of bladder function consistently ranks as one of the most important health priorities in this population.[4,5] Given the clinical significance of NLUTD after SCI, current levels of research activity and emerging translational approaches have begun to take advantage of large animal models such as the pig, as these can be a powerful research tool to further understanding of human organ systems and clinically relevant disease states.

The current gold standard for measuring detrusor function following NLUTD after SCI is urodynamics. Urodynamic studies (UDS) are a series of procedures and tests that yield quantitative

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bladder pressure, external urethral sphincter (EUS) activity, and volume data during both the filling (storage) and voiding phases.[6,7] UDS in rodents have played a critical role in our understanding of NLUTD after SCI from the acute to the chronic stages.[8–10] However, implications for human translation are limited by multiple biological and physiological factors. For example, although the general patterns of EUS activity in rats resemble those of humans when compared with clinical outcomes that utilize similar needle electrode recording techniques,[11] EUS activity during the voiding phase in uninjured rats exhibits short bursts, which does not occur in humans,[12–14] a difference that needs to be factored into the identification and interpretation of detrusor-sphincter dyssynergia (DSD) post-SCI.[15]

The pig has been used increasingly in urological research because of the anatomical and physiological similarities between the human and pig lower urinary tract (LUT).[16–19] Previous research on the function and anatomy of the pig's LUT have suggested that it is, potentially, a representative model for human LUT diseases.[20–24] Anatomically, the presence of a slit-like urethral meatus and the transitional epithelium at the level of the bladder neck has been reported to be similar in the pig and the female human.[18] Physiologically, female pigs demonstrate a drop in intra-urethral pressure just prior to voiding, and this resembles the drop in intra-urethral pressure observed during voiding in healthy humans.[18,25] Further, the feasibility of performing UDS in minipig models using clinically applicable techniques and obtaining interpretable data has been previously demonstrated.[26,27]

In 2013, a porcine model of thoracic SCI model using Yucatan minipigs (described by Lee and coworkers[28] and more recently reviewed by Kim and coworkers[29]) was established at the University of British Columbia (UBC). The biomechanics of the injury, the hindlimb locomotor outcomes, and the histological damage to the spinal cord were initially characterized.[28] Although the model has been used for a number of different experimental questions and various aspects of spinal cord dysfunction have been described,[30–37] we have not previously examined the NLUTD that results from thoracic SCI in the pig model. Hence, the aim of this study was to develop a protocol to perform UDS in SCI minipigs, and to characterize the urodynamic outcomes and histopathological changes in the porcine bladder after SCI. As the UBC pig model was shared with SCI researchers at the University of Louisville (UofL) who were also interested in evaluating LUT function after SCI in a large animal model, this article describes our combined efforts, experiences, and findings. The results of this combined effort indicate that the pig model of SCI may be a useful intermediary model to serve as a translational tool for the evaluation of the safety and utility of novel human-sized devices or treatments that aim to improve the lives of SCI individuals with NLUTD.

Methods

The data in this study were acquired as part of a collaborative effort between UBC in Vancouver, British Columbia, Canada and UofL in Louisville, Kentucky, USA. This study involved work at both campuses using the shared UBC porcine model of SCI. All animal protocols and procedures were approved by the Animal Care Committees of UBC and UofL and were in accordance with both the Canadian Council and the United States Office of Laboratory Animal Welfare on Animal Care and Institutional Animal Care and Use Committee guidelines.

Animals and experimental design

To develop the UDS setup protocol, 40 female Yucatan minipigs were used ($n = 30$ with a thoracic SCI, and $n = 10$ uninjured pigs). The animals were obtained from either Sinclair Bio-resources, Auxvasse, Missouri, USA or S&S farms, Ramona, California, USA. To characterize NLUTD in SCI minipigs, a comprehensive study in groups of animals with different SCI severities and injury levels was performed with an emphasis on examining the changes in detrusor pressure (P_{det}) and EUS electromyography (EMG) activity. Fifteen of these animals underwent contusion/compression SCIs at either the T2 ($n = 9$, 16.73 cm drop, 50 g midline contusion) or the T10 ($n = 6$, 20 cm drop, 50 g midline contusion) spinal level. After the initial weight drop contusion injury, an additional 100 g static weight was placed on top of the 50 g impactor to impart 5 min of compression. One additional T10 SCI group was included ($n = 15$) that had a 100 g weight drop contusion from 10 cm, followed by 5 min of compression with the same weight. The UBC Impactor described in 2013[28] was used at both UBC and UofL for inducing the contusion/compression injury. After SCI, the bladders were managed with an indwelling catheter that was inserted prior to the surgery and connected to a urine collection bag for 7–10 days. Afterwards, the catheter was removed, and the bladder was allowed to drain spontaneously during the remaining study period. Further details of the injury, surgical procedure, post-operative veterinary monitoring, and care can be found in articles by Lee and coworkers[28] and Kim and coworkers.[29]

The iterative development and implementation of a standardized urodynamics protocol in the pig model of SCI occurred in three experimental phases, with several protocol modifications being made to the setup procedure and initial training between the three.

Experiment 1: UDS in anesthetized minipigs (pilot study)

Previous studies performing UDS on small animal models have been conducted successfully using anesthesia.[38–40] Several pilot experiments were initially performed to evaluate the feasibility of performing UDS on anesthetized uninjured minipigs ($n = 4$) using propofol (8–20 mg/kg/h) and fentanyl (22–45 μg/kg/h). UDS in fully anesthetized conditions were obtained with the animals suspended in the air using a hammock-style sling.

Experiment 2: UDS in awake SCI minipigs, with transient sedation during transurethral catheter placement into the bladder

Previous reports have highlighted the potential effects of anesthesia on LUT function such as inhibition of the pontine micturition center and voluntary cortical control of the bladder, as well as suppression of detrusor contractions and the micturition reflex.[41–44] Therefore, a protocol to perform UDS in awake but partially restrained SCI minipigs ($n = 15$ of which 6 received T10 SCIs and 9 received T2 SCIs; study performed at UBC) was developed as the next step. A hammock-style sling was utilized to partially restrain the animals during UDS (described in more details in the subsequent paragraphs). Sedation (dexmedetomidine intramuscular; 0.05 mg/kg) was only used during placement of the transurethral urodynamic catheter. Dexmedetomidine was reversed with atipamezole (0.2 mg/kg) given intramuscularly, and the volume was adjusted based on the alertness of the animal because the sedative wore off with time. Each pig was sedated for ≤90 min.

Experiment 3: UDS in awake SCI minipigs without procedural sedation

Lastly, a training protocol for repeated UDS pre-/post-SCI was developed to eliminate the need of sedation during placement of transurethral urodynamic catheters. Differences in LUT function between pigs that received sedation and reversal pre-urodynamics and uninjured pigs that did not receive sedation with (suspended) or without the hammock-style sling (standing) were examined. For

Table 1. Anesthesia Protocol for Experiment 1

Pig # (ID #)	*Age (days)*	*Weight (kg)*	*Anesthesia protocol*
1 (9196)	164	22	Propofol (8–20 mg/kg/h) and fentanyl (22–45 μg/kg/h)
2 (9088)	177	21	Propofol (8–20 mg/kg/h) and fentanyl (22–45 μg/kg/h)
3 (9207)	162	21	Propofol (8–20 mg/kg/h) and fentanyl (22–45 μg/kg/h)
4 (9125)	161	21	Propofol (8–20 mg/kg/h) and fentanyl (22–45 μg/kg/h)

The anesthesia protocol for all uninjured animals is shown in this table. All studies were performed at the University of Louisville with the anesthetized animals suspended in a custom hammock-style sling.

this experiment, 15 animals underwent contusion SCI (10 cm drop, 100 g midline contusion with 5 min of compression) at the T10 spinal level. This study was performed at UofL.

Summary of experiments

The anesthesia protocol for animals in Experiment 1 (anesthetized) are summarized in Table 1. Urodynamics equipment and injury parameters for animals in Experiment 2 (procedural sedation during urodynamic catheter placement) and Experiment 3 (no procedural sedation during urodynamic catheter placement) are summarized in Table 2.

Hammock-style restraining unit and urodynamics training regimen

To ensure safe placement of the transurethral urodynamic catheters during UDS (either with or without sedation), the pigs were acclimated to being suspended comfortably in a hammock-style sling with four leg openings inside a metal frame (Fig. 1A). This form of restraint required training for the animals to become accustomed to it. We developed a training regimen, which consisted of teaching the animal to learn target touching and accommodate to restraining and bladder catheterization procedures. Establishment of the training procedure for performing UDS in awake pigs occurred in three stages, which are described in the following paragraphs.

Stage 1: Targeting. Minipigs were first trained to recognize their given names and to also look up at the trainers when their names were called. Second, they were trained to approach and touch a handheld target (such as a small rubber ball fastened onto the end of a wooden dowel) using food, clicker, and verbal cues; and third to follow the target in different directions. Successful completion of the given commands resulted in the animal hearing a click and receiving a food reward with verbal praise. The behavior was considered mastered once the animals successfully responded to verbal commands including their names, ''touch/target,'' ''stay,'' and ''follow.'' Subsequently, the animals were target and clicker trained daily to freely enter and stay for 5–10 min in the metal frame with the sling on the ground. Once the animal mastered this task, Stage 2 restraint training began.

Stage 2: Restraint training. The next step in the training process was to acclimate the pigs to the sling restraint setup. The pigs were brought into the testing room and taught to walk into the sling restraint setup. Once the pig's legs were in the holes of the sling, the sling was raised to one of two heights using the winch mechanism. The first sling height supported the pig's weight and allowed the hindlimbs to stand on the surface. The second sling height completely suspended the pig's limbs in the air. While restrained, positive reinforcement (food, petting, and verbal praise) was used to calm the animal. At the end of the session, the animal was hand fed while the sling was lowered. Generally, 30 training sessions were needed until the maximum time of restraint was achieved ($\sim$10 min in duration).

Stage 3: Handling and restraint training for awake transurethral catheterization (Experiment 3 only). Potential challenges in placing urodynamic catheters in conscious, awake pigs without sedation could include discomfort, stress, and anxiety for the pigs during the procedure. To minimize stress and allow the catheterization procedure to be performed safely, training began by suspending the pigs using a hammock-style sling and by desensitizing the pigs to manual touch, particularly around the vulva area. While suspended, the pig's name was spoken and the ''stay'' command was given to the pig to focus on the trainer and remain still.

Table 2. SCI Protocol and Urodynamics Setup Differences between Experiments 2 And 3

Description	*Experiment 2: Awake urodynamics sedated catheterization*		*Experiment 3: Awake urodynamics non-sedated catheterization*
• Injury level	T10	T2	T9–T10, T10–T11
• Drop height (cm)	20	16.73	10
• Compression weight (g)	150	150	100
• Compression time (min)	5	120	5
Age at time of injury (days)	196 ± 21	165 ± 5	206 ± 8
Body weight (kg)	31 ± 4	26 ± 1	25 ± 1
Animal conciousness during catheterization	Sedated		Awake
• Size (P_{ves}/P_{abd}) (Fr)	(7/9)		(7/10)
• Pressure sensor system	Fluid-filled		Fiberoptic
Animal conciousness during urodynamics	Awake		Awake
• Pre-SCI position	Standing or suspended		Standing or suspended
• Post-SCI position	Suspended		Standing or suspended
Urodynamics equipment	Delphis, Triton (Laborie)		Lumax TS Pro (CooperSurgical)
Saline infusion	Infusion pump		Gravity
Infusion rate	1 mL/min per kg of body weight		34.5 mL/min (range = 25–44 mL/min)

This table highlights the differences in the details of the injury, setup protocol (sedation vs. no sedation), urodynamics equipment, and settings used between the two experiments.

SCI, spinal cord injury; P_{ves}/P_{abd}, intravesical pressure/abdominal pressure.

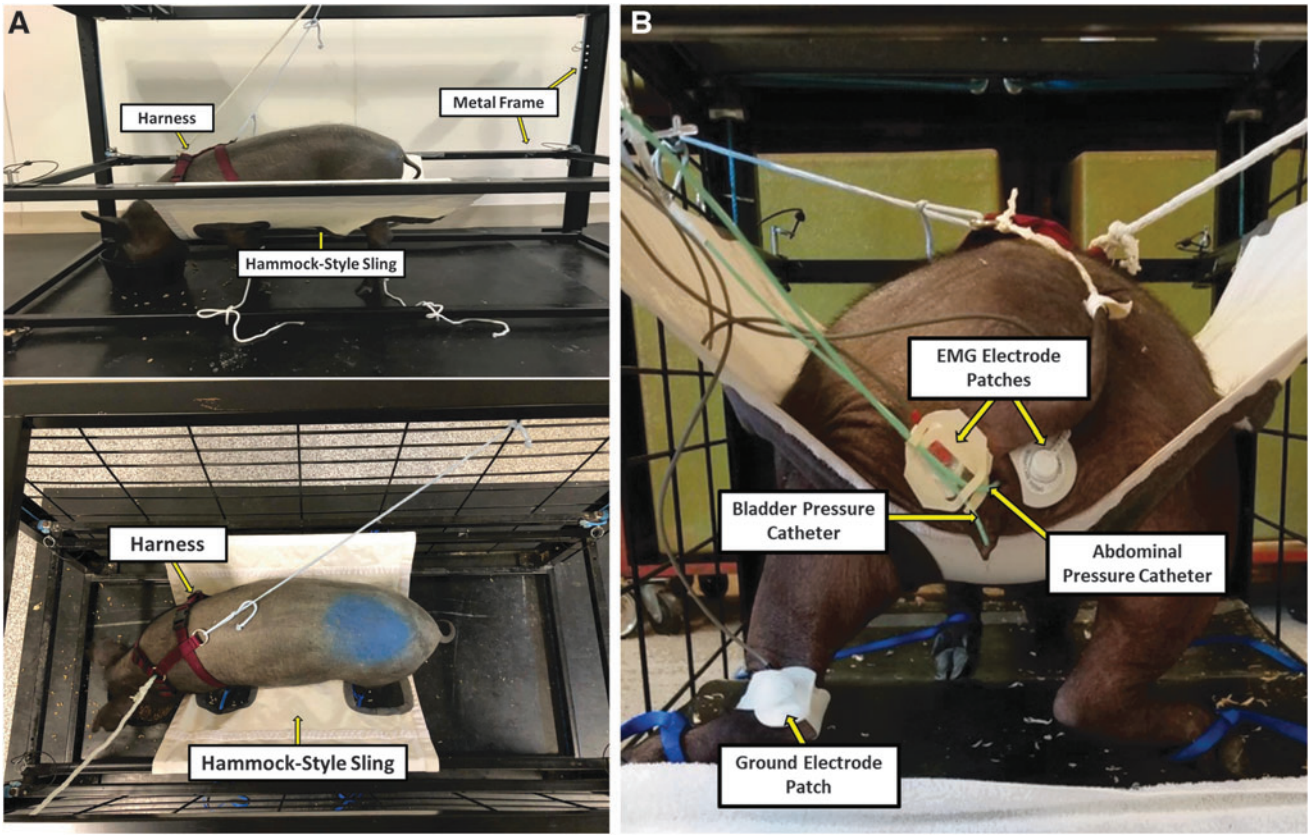

FIG. 1. **(A)** Urodynamics setup design. A hammock-style sling was placed into the middle of a metal frame (length: 152 cm, width: 68 cm, height: 88 cm). There were four small leg holes in the hammock-style sling for the animal. Once the animal was fitted into the sling, it was slowly elevated using a winch mechanism just before the sling touched the abdomen. This was done to give space for the animal to squat. The legs were loosely tied to the frame to prevent the animals from lifting their legs out of the sling. A harness worn by the pig was also tied to the frame to prevent the uninjured animals from jumping out of the sling. Both uninjured and spinal cord injured (SCI) animals stood on all four limbs; a cohort was fully suspended in the air. SCI animals that had paralyzed hindlimbs were supported in an upright posture to promote positioning of partially extended hindlimbs beneath the body. **(B)** Placement of urodynamics equipment. The intravesical pressure catheter was inserted transurethrally into the bladder. The abdominal pressure catheter was placed in the rectum. The catheters were subsequently taped to the side of the left buttock. Electromyography (EMG) patches were placed in the perianal region at the 9 and 3 o'clock positions, with a ground EMG placed on the bony protrusion of the left ankle or onto the superior edge of the iliac crest.

When the animal became restless and agitated, a second trained animal attendant applied gentle but firm pressure onto the hips and shoulders until the animal stopped moving. As soon as the animal became still, the pressure was immediately released, and a clicker sound was made followed by a food reward. On average, nine training sessions were required to train the animals to acclimate to the handling procedures associated with catheterization without exhibiting behaviors indicative of fear or stress.

Urodynamics equipment and set-up

Urodynamic catheters were placed in either awake or sedated pigs suspended with the hammock-style sling. With the animal suspended in an upright position, feces were removed using digital stimulation to prevent defecation during testing. Using aseptic technique, a 7-Fr dual lumen catheter was passed transurethrally into the bladder for filling and to measure intravesical pressure (P_{ves}). Another dual lumen catheter (9-Fr or 10-Fr) was passed into the rectum to measure abdominal pressure (P_{abd}). In situations in which transurethral catheter insertion was difficult, an extra small KleenSpec LED speculum (Welch Allyn, New York, USA) was utilized to visualize the urethral opening (only in sedated pigs). Entry into the bladder was confirmed by the presence of urine flow. After successful placement of the transurethral catheter, the bladder was emptied, and a urine sample was collected for urinalysis for uninjured and SCI pigs. The external pressure transducers were zeroed at atmospheric pressure using the level of the symphysis pubis as the reference height (Experiment 2 only). Two EMG electrode patches (Conmed, New York, USA) were subsequently placed on the skin around the external anal sphincter at the 9 and 3 o'clock positions, which is thought to be a surrogate of EUS activity.[45] The ground EMG patch was placed and taped onto either the bony protrusion of the ankle of the left hindlimb or onto the superior edge of the iliac crest (Fig. 1B). The EMG data collected in this study was not filtered for analysis or presentation.

For animals that were sedated, atipamezole (0.1–0.2 mg/kg) was intramuscularly administered to reverse the sedation at a volume dependent on the alertness of the animal (typically a quarter or half dosage). Filling of the bladder commenced once the animal recovered from sedation (eye blinking, eating from food bowl, making noises, increased heart rate), which generally occurred within 8–13 min after the injection.

UDS protocol

All awake UDS evaluations (Experiment 2 post-sedation, and Experiment 3 without any sedation) were performed with the animal in an upright posture with or without sling support (with the latter

allowing for a more natural squatting position during voiding). With the bladder drained and the pressure transducers zeroed, sterile saline was continuously infused into the bladder. It is important to note that infusion rates were equipment dependent and, therefore, differed between the two institutions. At UBC, body temperature saline solution (0.9%) was infused into the bladder via an infusion pump at a rate equivalent to the weight of the animal (1.0 mL/min per kg of body weight). At UofL, the bladder was filled with body temperature saline solution by gravity, with an average filling rate of 34.5 mL/min (range: 25–44 mL/min]. Saline infusion was stopped when a void or leakage of urine was visibly seen from the vulva of the pig. Urine was collected in a beaker placed on top of a uroflow meter placed underneath the pig to measure the flow rate of urine as well as volume of urine voided. At UofL, the flow rate of urine was not measured.

UDS lasted for a period of 30–60 min from the start of the filling phase to the end of the voiding phase. While awake, animals were hand fed using food as positive reinforcement. All procedures were performed between 9 a.m. and 2 p. m. to minimize the potential effects of circadian changes in voiding function.[46,47] Following data collection, the urodynamic catheters and surface patch EMGs were removed. To prevent overdistension of the bladder, any residual volume was removed via the Pves catheter or manual expression of the bladder was performed with the Crede's maneuver to ensure that the bladder was at least partially emptied prior to transporting the animal back to the pen.

Definitions of UDS outcome measures

The UDS parameters studied during the filling phase were: P_{ves}, P_{abd}, detrusor pressure ($P_{det} = P_{ves} - P_{abd}$), cystometric capacity (maximum infusion volume reached at the end of filling) and EUS EMG activity. During the voiding phase, the maximum urine flow rate (Q_{max}), the detrusor pressure at maximum flow ($P_{det\text{-}}Q_{max}$), the detrusor pressure recorded immediately before the isovolumetric contraction ($P_{detopen}$), voided volume (VV), post-void residual (PVR) volume, voided percentage [VV/(PVR+VV) × 100%, voided%], and bladder compliance (change in bladder volume/change in P_{det} during that change in bladder volume; expressed as mL/cm H_2O). The P_{det} at which involuntary expulsion of water/urine occurred was considered the detrusor leak point pressure (DLPP). Neurogenic acontractile detrusor was defined by the absence of a detrusor contraction during voiding. Neurogenic detrusor overactivity (NDO) was characterized by involuntary detrusor contractions during the filling phase. We refrained from diagnosing DSD, because EUS activity was recorded with EMG patch electrodes without concurrent voiding cystourethrography.

All parameters defined are in consonance with the metric units and definitions established by the International Continence Society (ICS).[48–51] For example, bladder compliance values <20 mL/cm H_2O were determined to be indicative of low bladder compliance.

Bladder histology

At the end of the study, after being sedated deeply with Telazol (4–6 mg/kg, intramuscular), animals were euthanized with an intravenous overdose of sodium pentobarbital (120 mg/kg). The bladder was then removed and fixed with 10% formalin in 0.1 M phosphate buffer with a volume to tissue ratio of 20:1 for a minimum of 72 h at 4°C within 30 min of harvesting. Subsequently, specimens were processed, and paraffin-embedded by Wax-It Histology (Vancouver, BC, Canada). Tissues embedded in paraffin blocks were then sectioned (5 μm) and slides were stained with hematoxylin and eosin (H&E)[52] and Masson's trichrome stain (Abcam, Toronto, Ontario, Canada).[53] The slides were imaged at 5 × using a conventional light microscope (ZEISS Axio Imager M2, ZEISS, Germany) and then examined for general morphological and histopathological changes.[53] Muscle-to-collagen ratio was quantified on the Masson's trichrome stained cross-sections using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) and averaged from two cross sections taken from the dome, body, and neck regions of the bladder as well as from the urethra. With the color threshold plugin, a threshold was set to only showing areas occupied by collagen (blue) or muscle (red). The relative area (%) occupied by collagen and muscle was then calculated. Tissue thickness measurements were taken by drawing 10 lines across the tissue while ensuring that the lines spanned across the bladder wall (using ZEN software; ZEISS, Germany). The length of the perpendicular lines was averaged and taken as the thickness (in millimeters, mm). This was then compared with control specimens taken from the bladder wall of Yucatan female pigs with no SCI or with acute SCI (< 12 h). Age at euthanasia, body weight, and bladder weight of these pigs are provided in Table 3. Not every bladder from a control or SCI animal was collected for histological processing, but all bladder weights were recorded.

Statistical analysis

All statistics were calculated using GraphPad (GraphPad Software, Inc., California, USA). The values are represented in mean ± standard error of the mean (SEM). *T* tests were performed to compare UDS parameters and injury parameters between groups as well as for the animal age, body weight, bladder weight, wall thickness, percent of muscle content, percent of collagen content, and muscle-to-collagen ratio of the bladder wall. A one-way analysis of variance (ANOVA) was performed to compare UDS parameters for Experiment 2 animals across time (4, 8, and 10–17 weeks post-injury). A Kruskal–Wallis test with Dunn's multiple comparison test was performed to compare the UDS parameters between anesthetized (Experiment 1), pre-UDS sedated (Experiment 2), and non-sedated (Experiment 3) uninjured animals.

Table 3. Age, Body Weight, and Bladder Weight of Pigs Whose Bladders Were Harvested for Histological Analysis

Pig # (ID#)	*Injury level*	*Age*	*Weight*	*Bladder weight*
		Days	*kg*	*g*
1 (6134)	T2	234	36	50
2 (6135)	T2	237	32	42
3 (6792)	T2	250	33	22
4 (6785)	T2	267	33	18
5 (7924)	T2	234	37	50
6 (7914)	T2	237	31	N.D.
7 (7928)	T2	267	30	32
8 (7932)	T2	272	35	40
9 (6789)	T10	284	48	38
10 (6794)	T10	292	38	38
Mean ± SEM		**257 ± 7**	**35 ± 2**	**37 ± 4**
Control 13 (7915)	T10 (< 12 h)	157	27	24
Control 14 (8563)	T10 (< 12 h)	149	24	12
Control 15 (8539)	T10 (< 12 h)	152	22	12
Control 16 (1868)	No SCI	126	25	7
Mean ± SEM		**146 ± 7*** **	**24 ± 1****	**14 ± 4****

This table contains the injury level, age at euthanasia, body weight, and bladder weight of pigs whose bladders were processed for histological examination.

Significant difference between control and SCI age, body, and bladder weight, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

N.D., not determined; SEM, standard error of the mean; SCI, spinal cord injury.

Results

Experiment 1: UDS in anesthetized uninjured pigs (UofL)

All anesthetized animals ($n=4$) demonstrated inhibition of the voiding reflex characterized on the UDS tracing by the absence of a detrusor contraction and overflow incontinence. Furthermore, these animals had elevated PVR volumes (711 ± 356 mL) with small voided volumes (15 ± 7 mL), which suggested that the anesthetics were impacting bladder function. When comparing the UDS parameters between anesthetized animals and either uninjured pigs that received pre-UDS sedation (Experiment 2) or uninjured pigs that received no sedation (Experiment 3), there were significant differences in the DLPP ($p=0.01$), volume voided ($p=0.002$), PVR volume ($p=0.0001$), and voided percentage ($p=0.0001$). There were no significant differences in the cystometric capacity ($p=0.09$) and bladder compliance ($p=0.07$) (Fig. 2).

A UDS tracing from an uninjured, propofol- and fentanyl-anesthetized animal is depicted in Figure 3. At an infused volume of 281 mL, the voiding reflex was suppressed, and a void (1 mL) occured at a DLPP of 13 cm H_2O with no apparent detrusor contraction. During the filling phase, the EMG activity appeared to be largely silent and there was no apparent guarding reflex observed in response to increased bladder volume.

Following this, we proceeded with the development of a protocol in awake pigs, either initially sedated to facilitate placement of the urodynamic catheters (Experiment 2), or without the use of sedation (Experiment 3), which is also more analogous to how UDS are performed in humans.

Experiment 2: Awake UDS with sedation during catheterization (UBC)

Procedural Observations. Most animals successfully learned targeting behavior and tolerated the awake UDS procedure very well. The pigs acclimated to the restraint procedure in ~30 sessions. Overall, 84 UDS were attempted (38 pre-SCI and 46 post-SCI), with some animals having repeat UDS pre- and post-SCI. In total, 64 UDS (29 pre-SCI and 35 post-SCI) were analyzed, with 20 UDS being excluded for a variety of reasons. Many of these issues occurred at the beginning stages as the UDS protocol was first being developed, and were eventually resolved with further refinement of the protocol. In eight cases, we were unable to catheterize the bladder (this was later solved with the use of the LED speculum), and in another eight cases, the transurethral

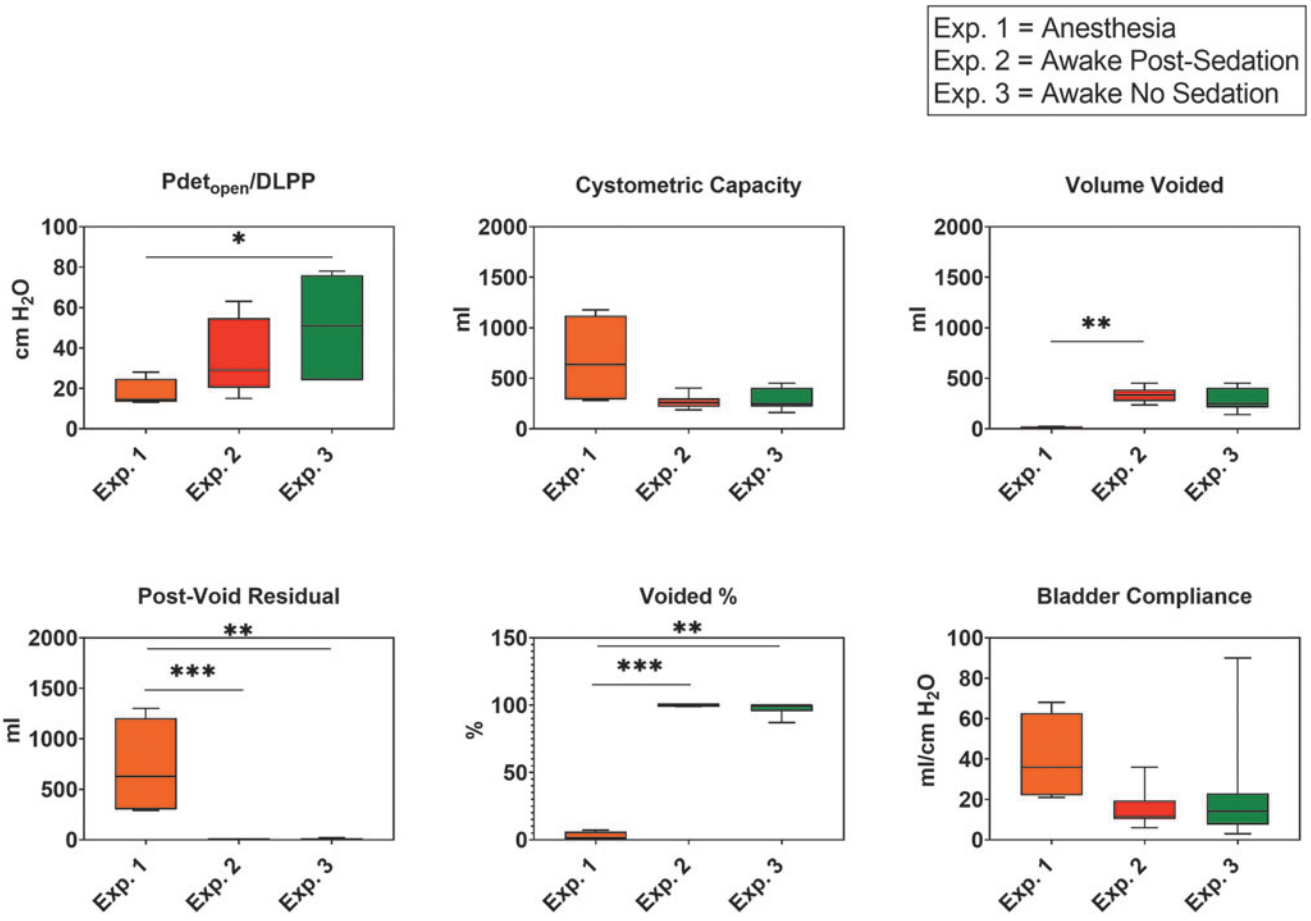

FIG. 2. Effect of anesthesia on urodynamic parameters. Uninjured pigs underwent urodynamics either under anesthesia (Experiment [Exp.] 1, $n=4$), with pre-urodynamics sedation (Exp. 2, $n=5$), or fully awake with no sedation (Exp. 3, $n=8$). All animals were suspended in the sling shown in Figure 1. Animals under anesthesia demonstrated inhibition of the voiding reflex with large cystometric capacities and high post-void residual volumes. A Kruskal–Wallis with Dunn's multiple comparison test was performed to compare the differences between animals under anesthesia (Exp. 1) and those that received pre-urodynamics sedation (Exp. 2) as well as those that were fully awake with no sedation (Exp. 3). *$p<0.05$, **$p<0.01$, ***$p<0.001$.

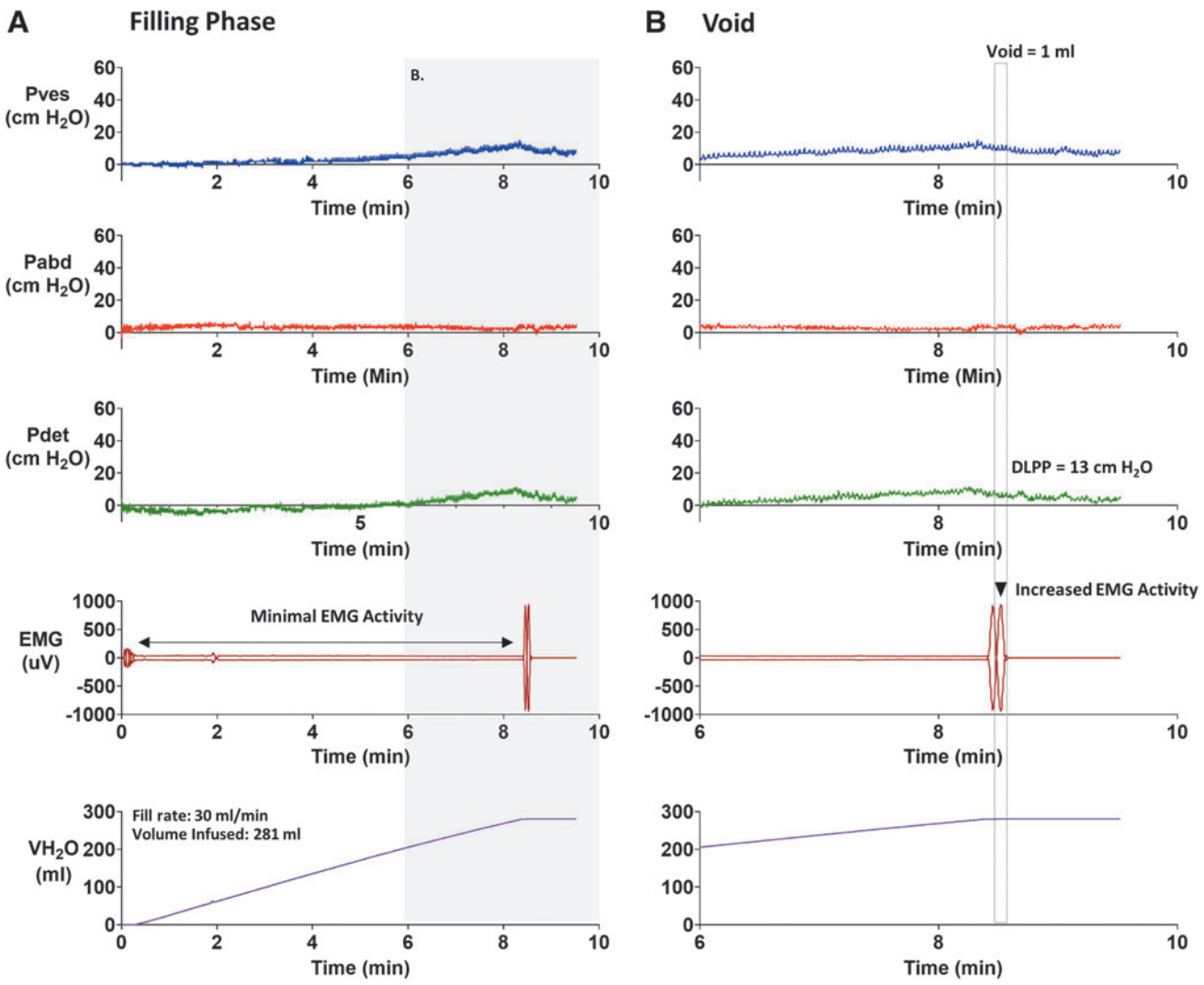

FIG. 3. Urodynamics tracing from an anesthetized, uninjured pig. **(A)** Filling phase. With propofol (8–20 mg/kg/h) and fentanyl (22–45 μg/kg/h) anesthesia, the bladder was filled at 30 mL/min. During the filling phase, electromyography (EMG) activity was largely silent and no apparent guarding reflex could be seen in response to increased bladder volume. A zoom in on the voiding phase is shown in **(B). (B)** Void. Expansion of the voiding phase through minutes 6–10. At 281 mL, a void occurred at a detrusor leak point pressure (DLPP) of 13 cm H_2O, and 1 mL was voided. An increase in EMG activity during the void was observed (arrowhead). From top to bottom: intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), detrusor pressure (P_{det}), EMG of the external anal sphincter, and volume infused (VH_2O).

catheter fell out during the filling period due to movement or the animal voided out the catheter. In four cases, the animals were deemed to have a concomitant UTI, as determined by a positive urinalysis test post-UDS with significant bacteriuria and pyuria along with visual observations of behaviors signifying pain, such as loss of appetite and quiet behavior, as well as signs of fever, foul-smelling urine, cloudy urine, and hematuria.[54] Supplementary Figure S1 shows a urodynamics tracing from a SCI animal that had a concomitant UTI during UDS.

Awake UDS in uninjured pigs. After the reversal of the sedation used to place the urodynamic catheters, the awake uninjured animals ($n=17$) had UDS performed in either a suspended position using the hammock-style sling or in a standing/squatting position. Using this approach, two distinct voiding patterns were observed. The first pattern ($n=7$; 9/29 UDS, 31%) was characterized by a marked detrusor contraction during the voiding phase (Fig. 4A and B). The contraction of the detrusor continued and the EUS remained relaxed until voiding was complete. The second voiding pattern, ($n=12$; 20/29 UDS, 69%), was characterized by a rapid increase in P_{det} during the filling phase (Fig. 5A) without a detrusor contraction during urine expulsion (Fig. 5B). These animals typically demonstrated lower bladder compliance.

When comparing the UDS data between animals displaying voiding pattern 1 and those displaying voiding pattern 2 (irrespective of voiding position), the cystometric capacity was significantly greater in animals displaying voiding pattern 1 than in those displaying voiding pattern 2 (632 ± 92 mL vs. 429 ± 48 mL, respectively, $p=0.04$) (Fig. 6). Bladder compliance was highly variable (ranging from 6 to 442 mL/cm H_2O), but there was a significant difference in compliance between animals displaying pattern 1 and animals displaying pattern 2 (115 ± 47 mL/cm H_2O vs. 41 ± 10 mL/cm H_2O, respectively, $p=0.04$). There were no significant differences in the voided percentage, $P_{detopen}$, P_{det}-Q_{max}, Q_{max}, and PVR.

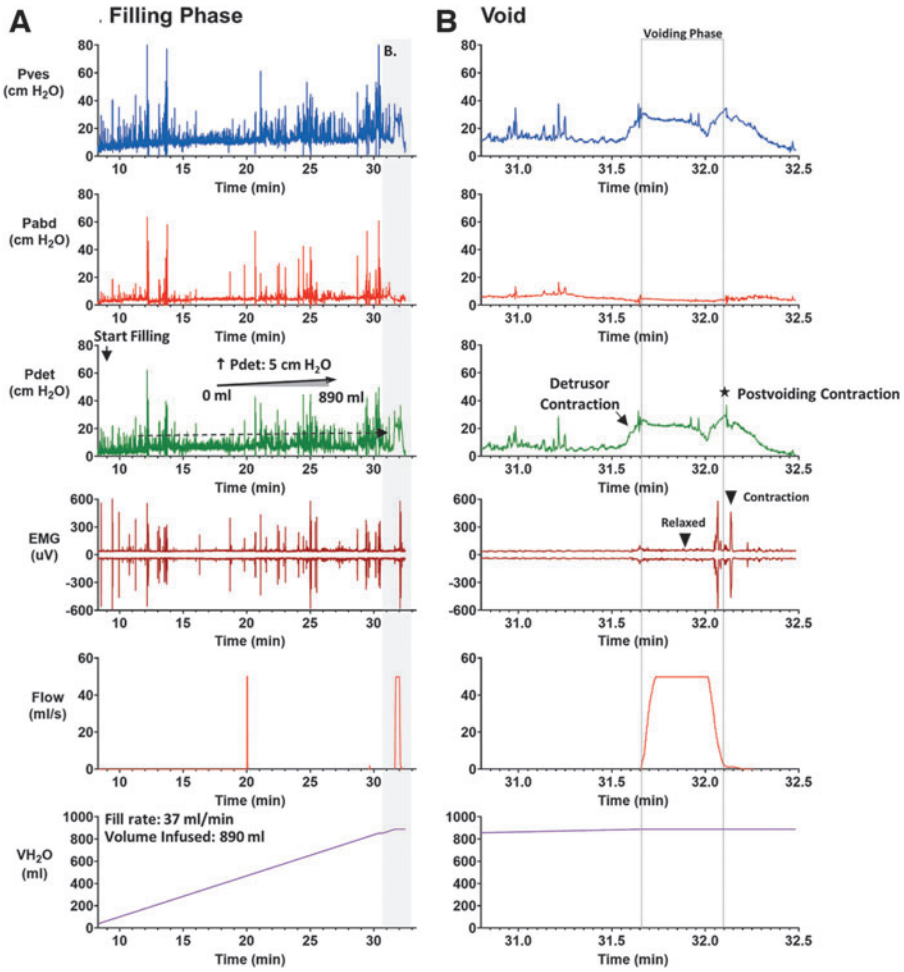

FIG. 4. Urodynamics tracing from an uninjured pig (Experiment 2) demonstrating voiding pattern 1. **(A)** Filling phase. The fill rate was 37 mL/min and the total volume infused until the void was 890 mL. The change in detrusor pressure (P_{det}) was 5 cm H_2O over the entire filling cycle, indicating good bladder compliance (dashed arrow). The shaded region highlights the zoomed-in view of the voiding phase shown in **(B)**. **(B)** Void. At the start of the void, a detrusor contraction (arrow) is present. During the void, the detrusor continues to contract while the external urethral sphincter (EUS) remains relaxed (arrowhead) until the void is complete. Near the end of the void, a post-voiding contraction (star) occurs. At the end of the void, an increase in electromyography (EMG) activity is seen signifying contraction of the EUS (arrowhead). It is also important to note that the flow curve plateaus because the flow meter does not read measurements beyond 50 mL/sec. From top to bottom: intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), detrusor pressure (P_{det}), EMG of the external anal sphincter, flow rate, and volume infused (VH_2O).

When comparing the UDS parameters between those animals in a standing/squatting position ($n=12$; 19/29 UDS [66%]; $n=7$ displayed voiding pattern 1, n = 7 displayed voiding pattern 2, and some animals demonstrated both voiding patterns 1 and 2) and those were that were fully suspended ($n=5$; 10/29 UDS [34%]; $n=5$ displayed voiding pattern 2), $P_{detopen}$ was significantly lower in the standing animals than in the suspended animals (23 ± 1 cm H_2O vs. 35 ± 6 cm H_2O, respectively, $p=0.02$). This may reflect the pressure being locally applied to the bladder by the sling itself during suspension. Further, the cystometric capacity was significantly smaller in the fully suspended animals (609 ± 53 mL vs. 270 ± 21 mL, $p=0.0001$).

Awake UDS in SCI Pigs ("16.73/20-cm drop"). A total of 15 animals received a thoracic SCI, of which 6 (40%) had a T10 injury and 9 (60%) had a T2 injury. Details on the injury and the biomechanical parameters related to the injury are provided in Table 4. With this injury severity, none of the animals were able to support their body weight during UDS, and therefore the sling technique for supporting the hindlimbs and lower trunk was used.

NDO was observed in all T10 and T2 SCI animals, characterized by involuntary detrusor contractions during bladder filling (Fig. 7A and B). The onset of NDO was observed as early as 3 weeks after SCI (earliest time point UDS was performed), and was still observed at 17 weeks after SCI (latest time point UDS was performed). The amplitude and frequency of the contractions varied across SCI animals. NDO events started at $\sim 53 \pm 6\%$ of the cystometric capacity ($\sim$ 295 mL on average).

There were insufficient T10 and T2 animals at weeks 4 and 8 to perform a valid statistical comparison of the UDS parameters against the uninjured animals. Therefore, we combined these animals into a single thoracic group for comparison against the pre-injury ($n=17$, from Experiment 2) and the SCI pigs that did not receive procedural sedation ($n=12$, from Experiment 3).

Analysis of the UDS parameters of T10 (10–17 weeks post-SCI) and T2 (12 weeks post-SCI) animals combined showed no

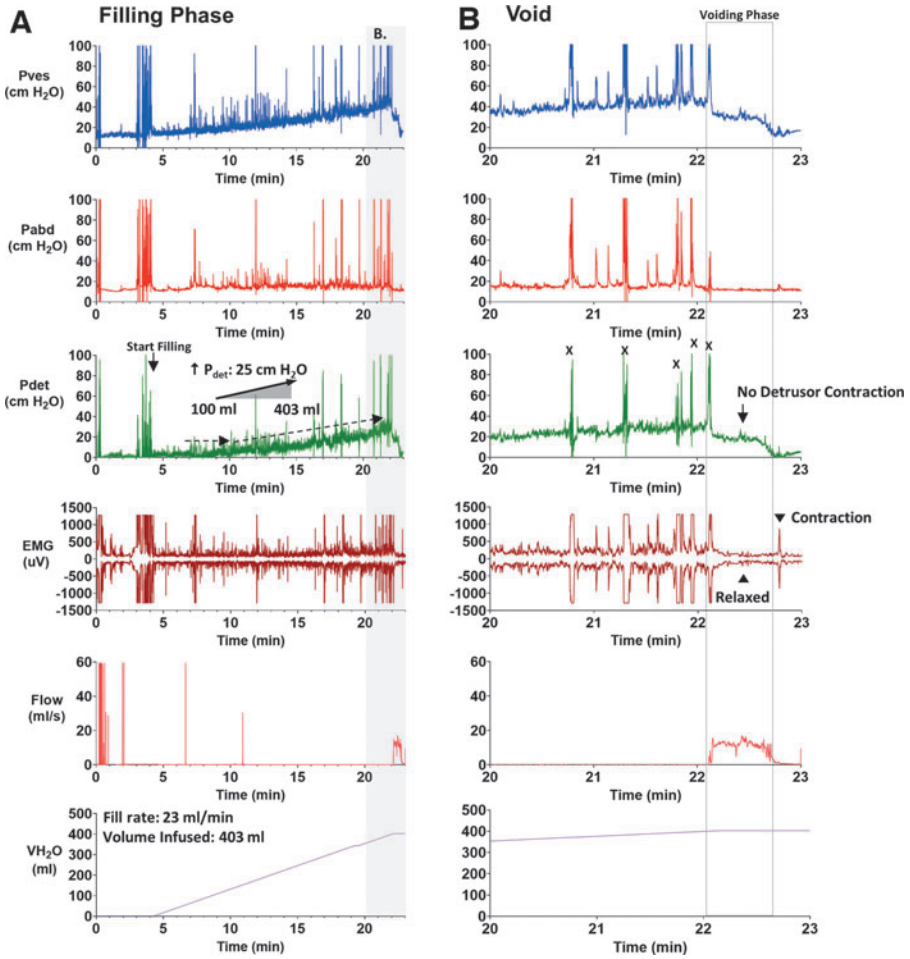

FIG. 5. Urodynamics tracing from an uninjured pig demonstrating voiding pattern 2. **(A)** Filling phase. The fill rate was 23 mL/min, and the total volume infused until the void was 403 mL. From ~100 to 403 mL, poor bladder compliance (dashed arrows) was observed characterized by a rapid rise in detrusor pressure (P_{det}) (↑ 25 cm H_2O). The shaded region highlights the zoomed-in view of the voiding phase shown in **(B)**. **(B)** Void. Moments prior to the void, sudden movements from the pig caused spikes in the P_{det} and electromyography (EMG) channels (X's – movement artifact and not a true detrusor contraction). During the void, a detrusor contraction was not observed (arrow) but the external urethral sphincter (EUS) remained relaxed (arrowhead) throughout the duration of the void, resulting in complete emptying of the bladder. At the end of the void, an increase in EMG activity (arrowhead) is observed indicating contraction of the EUS. From top to bottom: intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), detrusor pressure (P_{det}), EMG of the external anal sphincter, flow rate, and volume infused (VH_2O).

significant differences in cystometric capacity post-SCI (Fig. 8) compared with the uninjured group irrespective of voiding position or pattern (646 ± 72 mL vs. 492 ± 46 ml, $p = 0.07$). Voiding in SCI animals occurred at a significantly lower Q_{max} (8 ± 3 mL/sec vs. 27 ± 2 mL/sec, $p < 0.0001$) compared with the uninjured group. In addition, the voided percentage was significantly lower in SCI animals (11 ± 5% vs. 97 ± 2%, $p < 0.0001$). As a result, SCI animals demonstrated a significantly greater PVR volume compared to the uninjured group (593 ± 80 mL vs. 24 ± 10 mL, $p < 0.0001$). Bladder compliance was not significantly different compared to the uninjured group (50 ± 9 mL/cm H_2O vs. 64 ± 17 mL/cm H_2O, $p = 0.59$), nor was $P_{detopen}$/DLPP significantly different (24 ± 3 cm H_2O vs. 27 ± 2 cm H_2O, $p = 0.40$). UDS performed at earlier time points (3–4 and 7–8 weeks), also revealed significantly lower voided volume (3–4 weeks: $p < 0.0001$, 7–8 weeks: $p < 0.0001$) and significantly greater post-void residual volume (3–4 weeks: $p < 0.0001$, 7–8 weeks: $p < 0.0001$) in the T2/T10 SCI animals relative to uninjured controls. Across time, there was no biological significance in the UDS parameters in the Experiment 2 SCI group.

Experiment 3: Awake UDS without sedation during catheterization (UofL)

Procedural observations. Using our training protocol, the pigs acclimated to the restraint procedure in approximately nine sessions. All animals were successfully catheterized while awake and suspended in the sling without the use of sedation. Notably, all animals were tolerant of and were also very cooperative during the awake catheterization procedure. A total of 32 UDS (17 pre-SCI, 15 post-SCI) were conducted with an overall bladder catheterization success rate of 100%. One UDS recording session (3%) was not included in the analysis because of confirmation of a clinical UTI post-UDS. One (3%) other session's data were not included because of high artifact interference in the urodynamic profile and in addition, the urodynamic catheter fell out, both of which was caused by animal movement. Overall, 30 UDS were analyzed (17 pre-SCI, 13 post-SCI).

Awake UDS in uninjured pigs. All animals were able to void with a marked detrusor contraction (i.e., pattern 1) during all UDS

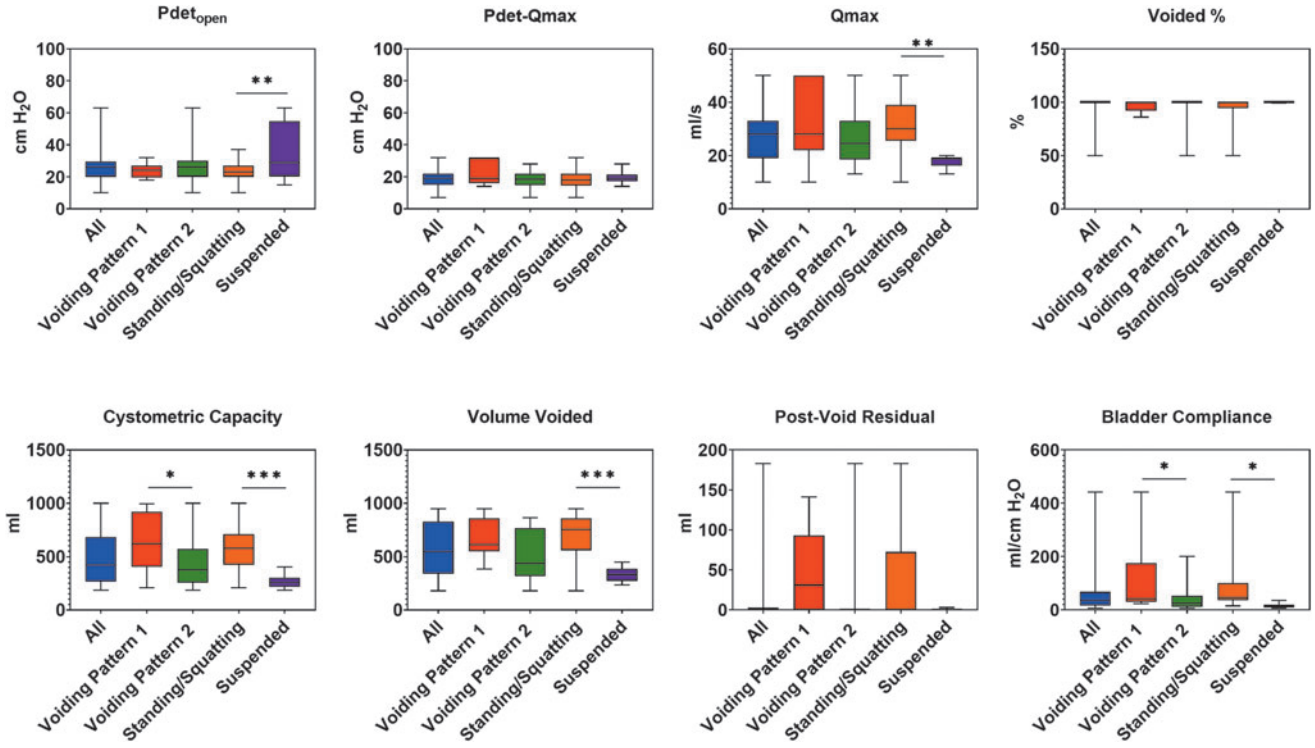

FIG. 6. Experiment 2 (awake urodynamics with sedation during catheterization): urodynamic parameters of uninjured, awake animals. Two distinct voiding patterns were observed: animals that had a clear detrusor contraction (pattern 1) and animals that had no clear contraction (pattern 2). These two distinct voiding patterns were seemingly dependent on the posture of the animal during urodynamics, either standing/squatting or suspended in the sling. A *t* test was performed to compare the differences between animals that demonstrated voiding pattern 1 and those that demonstrated voiding pattern 2, as well as between suspended and standing/squatting animals. Significant difference between animals displaying voiding pattern 1 and those displaying voiding pattern 2 or between animals that performed cystometry in a standing/squatting versus a suspended position, $*p<0.05$, $**p<0.01$, $***p<0.001$.

TABLE 4. EXPERIMENT 2 (AWAKE URODYNAMICS WITH SEDATION DURING CATHETERIZATION) INJURY PARAMETERS

Pig # *(ID#)*	*Injury level*	*SCI*	*Age* *days*	*Weight* *kg*	*Force* *Kdynes*	*Displacement* *mm*	*Impulse* *Kdynes × sec*	*Velocity* *mm/sec*
1 (6502)	T10	Drop height: 20 cm	263	44	2558	3.5	9.9	1803
2 (6504)	T10	Impact weight: 50 g	258	43	3171	3.6	10.3	1807
3 (9524)	T10	Compression: 5 min	165	25	3066	4.3	11.6	1643
4 (9514)	T10	with 100 g	169	25	3338	4.4	11.4	1668
5 (4344)	T10		168	26	3201	3.5	11.5	1802
6 (5042)	T10		153	27	3355	3.5	11.7	1800
Mean ± SEM			**196 ± 21**	**32 ± 4**	**3115 ± 120**	**3.8 ± 0.2**	**11.1 ± 0.3**	**1754 ± 31**
7 (6134)	T2	Drop height: 16.73 cm	149	25	3612	3.9	12.1	1915
8 (6135)	T2	Impact weight: 50 g	153	26	2377	3.6	11.0	1772
9 (6792)	T2	Compression: 120 min	159	25	3178	3.9	12.0	1953
10 (6785)	T2	with 100 g	175	31	2914	4.2	12.6	1927
11 (6790)	T2		172	30	N.D.	N.D.	N.D.	N.D.
12 (7924)	T2		150	25	1859	3.3	9.5	1707
13 (7914)	T2		153	23	2314	3.7	10.7	1869
14 (7928)	T2		183	23	2920	1.9	19.6	1923
15 (7932)	T2		188	28	4279	3.6	17.3	1833
Mean ± SEM			**165 ± 5**	**26 ± 1**	**2932 ± 273**	**3.5 ± 0.2**	**13.1 ± 1.2**	**1862 ± 30***

Measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. After SCI, biomechanical data acquired for each impact were collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact.

Significant difference between SCI animals with injury at the T10 level and those with injury at the T2 level, $*P<0.05$.

N.D., not determined; SEM, standard error of the mean; SCI, spinal cord injury.

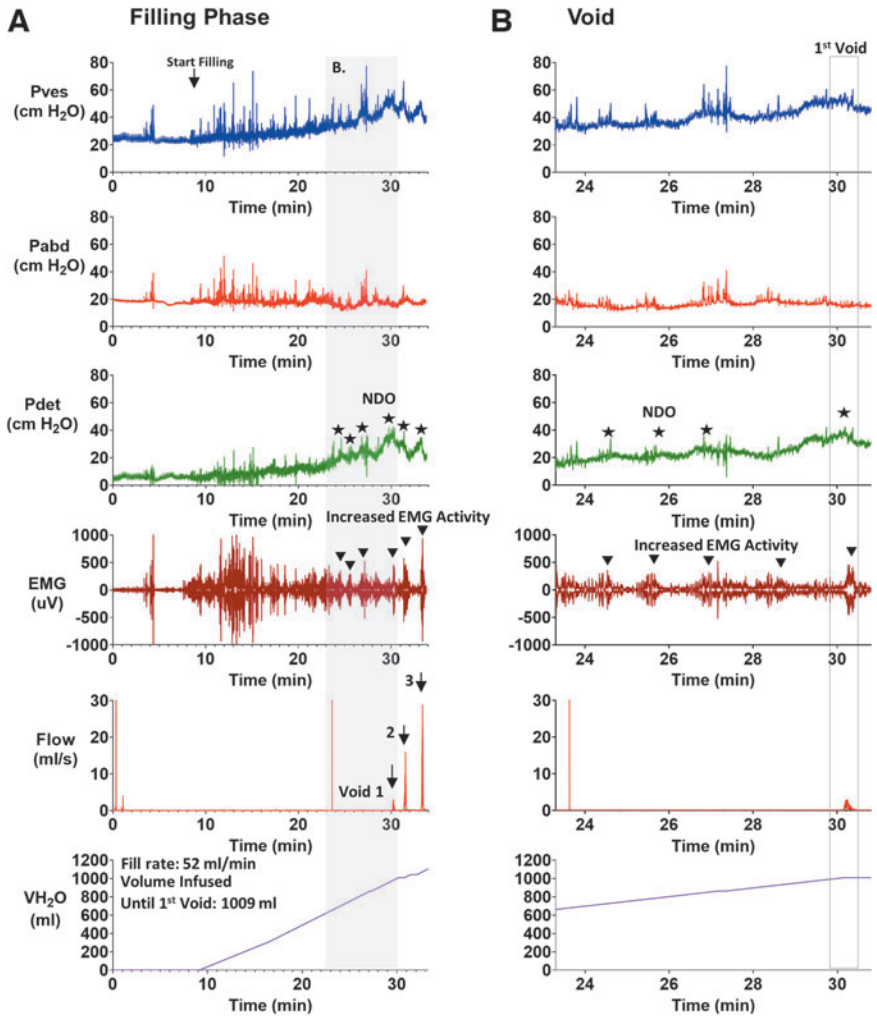

FIG. 7. Urodynamics tracing (awake urodynamics with sedation during catheterization) from a T10 SCI pig at 17 weeks post-injury demonstrating neurogenic detrusor overactivity (NDO) with possible detrusor-sphincter dyssynergia (DSD). **(A)** Filling phase. The fill rate was 52 mL/min and the infused volume until the first void was 1009 mL. At ~700 mL, NDO events (stars), which are characterized by a waveform appearance in the detrusor pressure (P_{det}) channel, can be seen leading up to the first void. During these NDO events, there are bursts of electromyography (EMG) activity (arrowheads), which could possibly signify DSD. The shaded region highlights the zoomed-in view shown in **(B)**. **(B)** Void. Possible DSD events prior to the first void can be seen. At an infused volume of 1009 mL, the first void of the study occurs during a possible DSD event resulting in incomplete emptying of the bladder. Two more subsequent voids were captured in this study, both of which appear to occur during a possible DSD event. From top to bottom: intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), detrusor pressure (P_{det}), EMG of the external anal sphincter, flow rate, and volume infused (VH_2O).

sessions ($n=15$; 17/17 UDS sessions, 100%) irrespective of voiding position (standing or squatting) (Fig. 9). Conversely, only 7/17 (41%) of the uninjured animals from Experiment 2 demonstrated this pattern. When comparing the UDS parameters between uninjured pigs from Experiments 2 and 3 displaying pattern 1, regardless of voiding position, the cystometric capacity of uninjured pigs from Experiment 2 was significantly greater than from those in Experiment 3 (632 ± 92 mL vs. 301 ± 42 mL, $p=0.001$) (Fig. 10). Further, the $P_{detopen}$ was significantly lower by ~22 cm H_2O (24 ± 2 cm H_2O vs. 46 ± 5 cm H_2O, respectively, $p=0.003$). Voided percentage was not significantly different, with both experiments exhibiting voiding efficiencies of ~96% ($p=0.75$). Bladder compliance was significantly higher in Experiment 2 animals than in Experiment 3 animals (115 ± 47 mL/cm H_2O vs. 23 ± 5 mL/cm H_2O, respectively, $p=0.01$). These differences were also observed in Experiment 2 animals that demonstrated voiding pattern 2.

No significant differences in the UDS parameters were observed between animals that voided in a standing/squatting position ($n=8$) and those were that were fully suspended ($n=8$). One animal performed UDS in both a standing/squatting and a fully suspended position.

Awake UDS in SCI pigs (10-cm drop). For the subsequent part of the experiment, we utilized the 100 g weight with a drop height of 10-cm followed by 5 min of compression with the same weight. Details on the injury and the biomechanical parameters related to the injury are provided in Table 5. Two animals that had pre-injury UDS performed had complications after SCI surgery and were euthanized.

Overall, 5/12 (42%) of the post-SCI animals demonstrated NDO at 11–13 weeks post-injury with NDO occurring at ~90 ± 4% of the cystometric capacity (~ 336 mL on average). A urodynamics

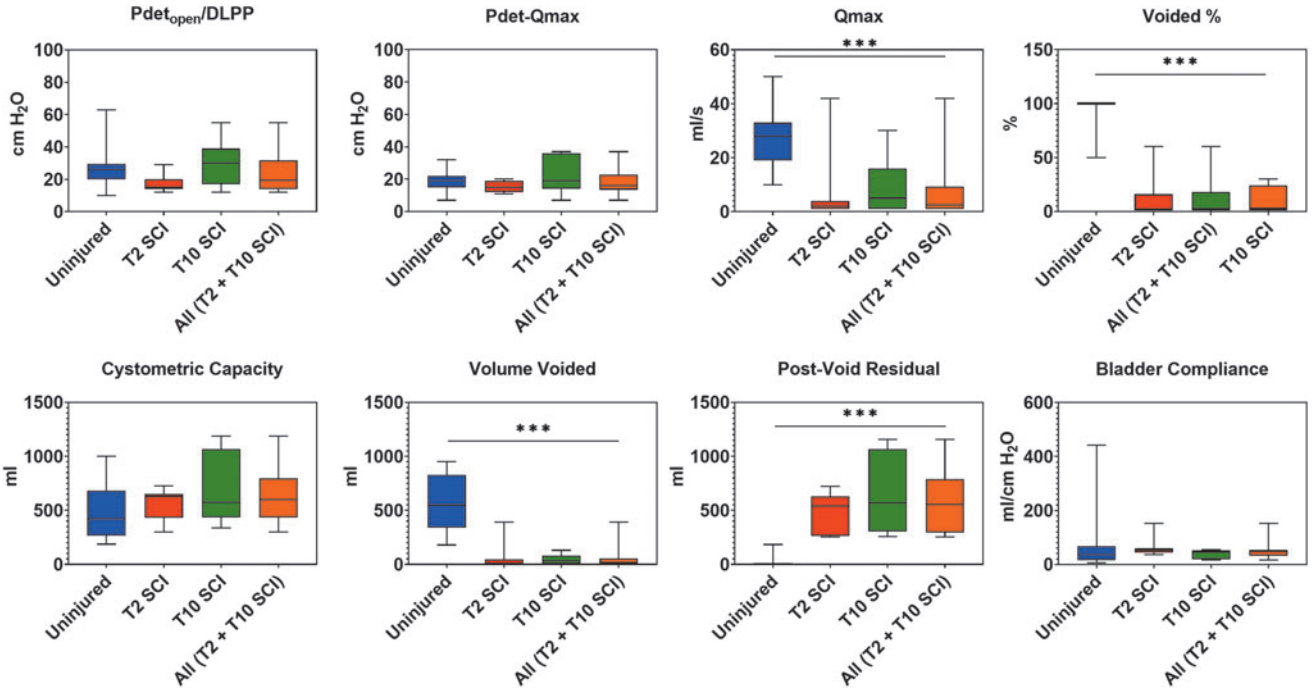

FIG. 8. Experiment 2 (awake urodynamics with sedation during catheterization): urodynamic parameters of spinal cord injured (SCI) pigs 10–17 weeks post-injury. All animals received a 20/16.73-cm drop contusion injury with 5 or 120 min of 150 g of compression at either the T10 ($n=3$) or T2 level ($n=7$). As none of the animals were able to support their body weight as a result of the injury severity, their hind legs were supported (toes were in contact with the ground) or fully suspended using a hammock-style sling. There were no significant differences between SCI animals with an injury at the T10 level and those with an injury at the T2 level. Similar findings were found at the 3–4- and the 7–8-week post-injury time points. There was no biological significance in the urodynamic parameters across time. A *t* test was performed to compare the differences in the urodynamic parameters between the uninjured and SCI animals. There were no significant differences between T2 and T10 SCI animals. Significant differences between all uninjured animals from Experiment 2 ($n=17$), ***$p<0.001$.

tracing from a SCI animal that was awake during catheterization at 11 weeks post-injury is shown in Figure 11.

There were no significant differences observed in cystometric capacity at 11–13 weeks after SCI compared with pre-injury values (503 ± 102 mL vs. 301 ± 42 mL, $p=0.06$). Similar to Experiment 2, SCI resulted in significantly elevated PVR volumes (353 ± 71 mL vs. 6 ± 4 mL, $p<0.0001$), and reduced voided percentage (28 ± 8% vs. 95 ± 3%, $p<0.0001$). Bladder compliance was not significantly different versus pre-SCI (25 ± 6 mL/cm H_2O vs. 23 ± 5 mL/cm H_2O, $p=0.89$), nor was $P_{detopen}$/DLPP significantly different (59 ± 12 cm H_2O vs. 46 ± 5 cm H_2O, $p=0.26$).

Although this SCI severity resulted in obvious hindlimb impairments, 6/12 (50%) animals were able to have UDS performed in a stand/squat position with minimal support (with an animal attendant physically holding the hindlimbs). When comparing UDS parameters between those SCI pigs in a stand/squat position and those that were fully suspended, the voided percentage was significantly higher in standing SCI pigs than in SCI suspended animals (43 ± 12% vs. 11 ± 5%, respectively, $p=0.002$) (Fig. 12). $P_{detopen}$ was also significantly higher (78 ± 18 cm H_2O vs. 34 ± 6 cm H_2O, respectively, $p=0.03$).

There were no significant differences in the UDS parameters between Experiment 2 ($n=10$) and 3 ($n=6$) suspended SCI animals at the 10–17-week time points. This could suggest that the use of procedural sedatives or injury severity may not have had a notable influence on overall UDS outcomes in chronic SCI animals.

SCI severity on LUT dysfunction

Experiment 3 SCI animals (10-cm injury severity, 100 g weight compression) had a higher voided percentage (11–13 weeks post-injury [WPI]) compared to Experiment 2 SCI animals (16.73/20-cm injury severity, 150 g weight compression) at the 10–17 WPI time point, but this difference was not significant (28 ± 8% vs. 11 ± 5%, respectively, $p=0.07$). When examining the volume-related urodynamic parameters, there was no significant difference in the cystometric capacity between the 16.73/20-cm and the 10-cm injury severity animals (646 ± 72 mL vs. 503 ± 102 mL, respectively, ($p=0.26$). However, the PVR volume was significantly elevated in the 16.73/20-cm injury severity animals versus the 10-cm injury severity animals (592 ± 80 mL vs. 353 ± 71 mL, respectively, $p=0.04$). This suggested that the bladders of the 10-cm injury severity animals were slightly better at emptying than their 16.73/20-cm severity counterparts.

Gross and histological features of the bladder after SCI

The wet weight of Experiment 2 SCI bladders ($n=24$) was significantly increased compared with those of control animals ($n=22$) (euthanized <12 h post-SCI, no SCI) (36 ± 2 g vs. 18 ± 1 g, $p<0.0001$). Further, in those animals that underwent histological analysis, the body of the bladder in SCI animals ($n=10$) tended to be thicker than those of the controls ($n=4$) (5.47 ± 0.34 mm vs. 4.41 ± 0.28 mm, $p=0.09$). Although the dome and urethra of the

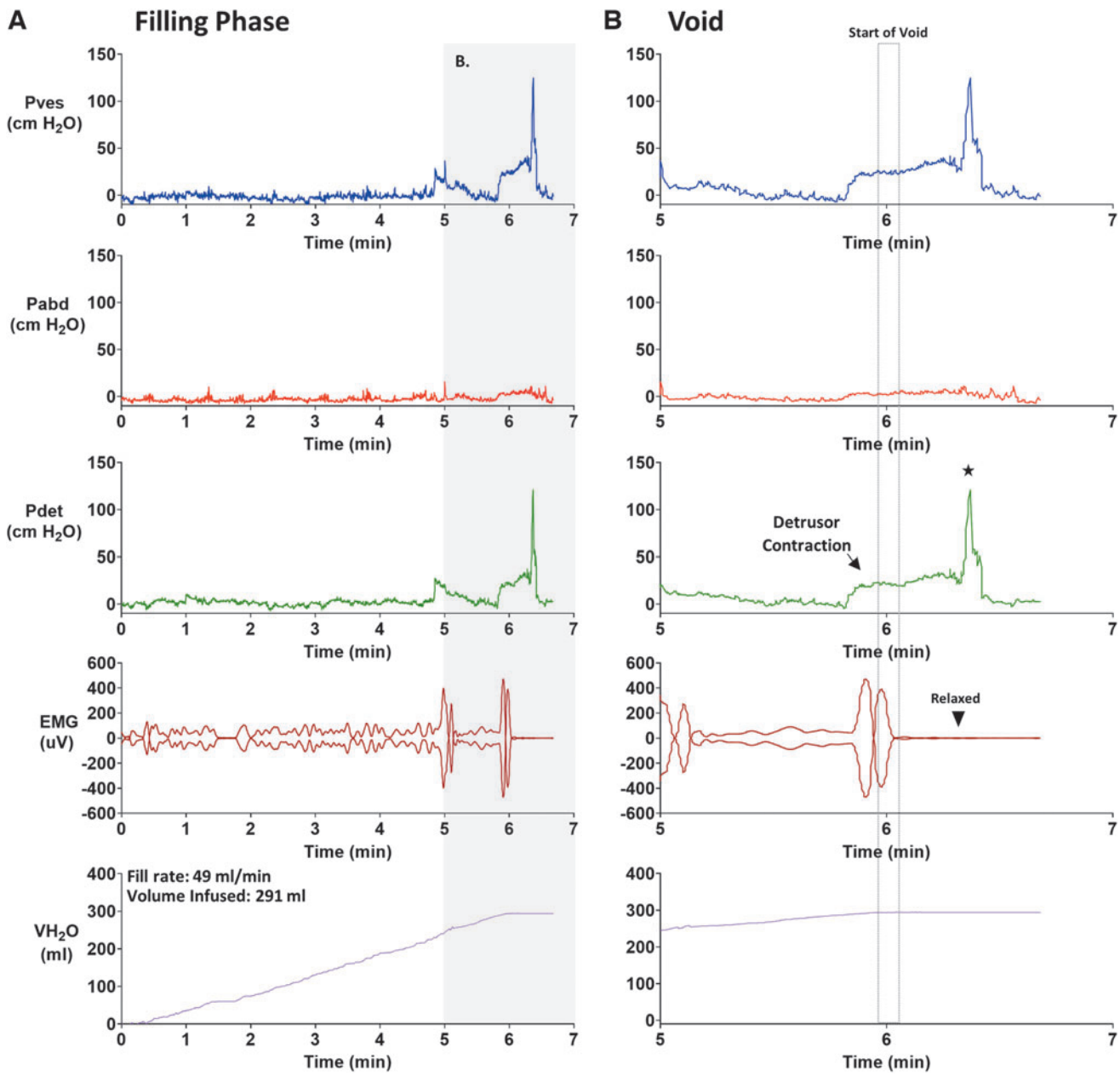

FIG. 9. Urodynamics tracing from an uninjured pig (Experiment 3) demonstrating voiding pattern 1. **(A)** Filling phase. The fill rate was 49 mL/min and the total volume infused until the void was 291 mL. The shaded region highlights the zoomed-in view of the voiding phase shown in **(B)**. **(B)** Void. At the start of the void, a detrusor contraction (arrow) is present. During the void, the detrusor continues to contract while the external urethral sphincter (EUS) remains relaxed (arrowhead) until the void is complete. A larger second detrusor contraction occurs after the initial contraction (star). From top to bottom: intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), detrusor pressure (P_{det}), electromyography (EMG) of the external anal sphincter, and volume infused (VH_2O).

SCI animals were thicker than those of the controls, this difference was not significant (dome: 5.28 ± 0.31 mm vs. 4.80 ± 0.30 mm, $p = 0.38$; urethra: 3.31 ± 0.21 mm vs. 2.83 ± 0.17 mm, $p = 0.17$). Interestingly, the neck of the bladder of the SCI animals was thinner than that of the controls (3.58 ± 0.23 mm vs. 4.04 ± 0.15 mm, $p = 0.26$), but this difference was also not significant.

All SCI animals, regardless if they had a T2 or T10 SCI, demonstrated noticeable detrusor hypertrophy in the dome and body regions of the bladder (Fig. 13). Dense patches of collagen (Masson's Trichrome) within the detrusor layer were observed in a few cases, but significant fibrosis was not observed.

The percent of muscle was significantly increased in the dome and body regions of the bladder of SCI animals compared with the control (dome: $50.0 \pm 0.8\%$ vs. $40.3 \pm 3.0\%$, $p = 0.0010$; body: $54.4 \pm 1.4\%$ vs. $44.8 \pm 1.0\%$, $p = 0.0017$). The percent collagen in the dome region was similar between the SCI animals and the controls ($39.9 \pm 1.9\%$ vs. $40.6 \pm 1.6\%$, $p = 0.85$). However, the percent of the collagen tended to be lower in the body region of the bladder of SCI animals than in the controls ($41.2 \pm 1.7\%$ vs. $43.5 \pm 1.3\%$, $p = 0.42$) but this difference was not significant. The muscle-to-collagen ratio in the body region was significantly different between the SCI animals and the controls (1.34 ± 0.07 vs.

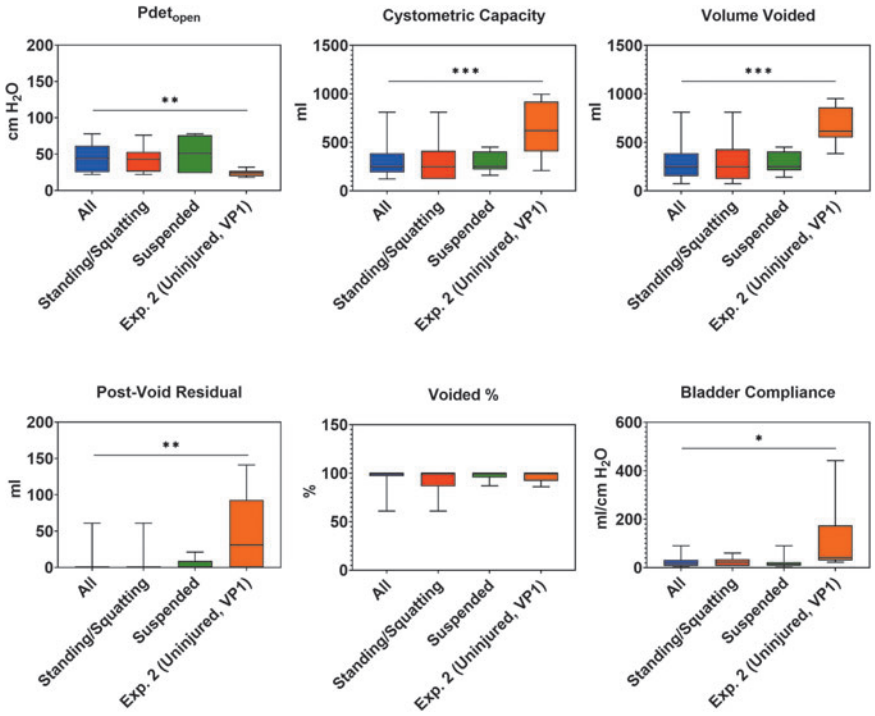

FIG. 10. Experiment 3 (awake urodynamics with no sedation during catheterization): urodynamic parameters of uninjured, awake animals. Animals predominantly displayed a clear detrusor contraction during voiding (pattern 1). A *t* test was performed to compare the differences in the urodynamic parameters between all uninjured Experiment (Exp.) 3 animals and uninjured Exp. 2 animals that demonstrated voiding pattern 1 (VP1) as well as between suspended and standing/squatting uninjured Exp. 3 animals. There were no significant differences between animals in a standing/squatting and those in a suspended position. Significant differences between all Exp. 3 and all Exp. 2 animals that demonstrated VP1, $*p<0.05$, $**p<0.01$, $***p<0.001$.

TABLE 5. EXPERIMENT 3 (AWAKE URODYNAMICS WITH NO SEDATION DURING CATHETERIZATION) INJURY PARAMETERS

#	*Injury level*	*SCI*	*Age* *Days*	*Weight* *kg*	*Force* *Kdynes*	*Displacement* *mm*	*Impulse* *Kdynes × sec*	*Velocity* *mm/sec*
1 (9364)	T9–T10	Drop height: 10 cm	242	29	1983	1.4	7.7	876
2 (9480)	T9–T10	Impact weight: 100 g	267	35	2175	1.9	8.2	1095
3 (59076)	T9–T10	Compression: 5 min	269	30	1823	2.1	8.5	1064
4 (58)	T10–T11		197	24	2122	2.1	8.5	1140
5 (75)	T10–T11		191	25	2130	1.8	9.1	1086
6 (194)	T10–T11		204	26	1939	1.8	7.8	996
7 (182)	T10–T11		208	24	1699	1.8	7.3	979
8 (566)	T10–T11		178	19	1957	2.3	8.0	1115
9 (981)	T11		188	23	N.D.	N.D.	N.D.	N.D.
10 (1011)	T11		185	23	1780	2.0	7.9	1067
11 (1003)	T10–T11		178	24	765	0.4	3.9	353
12 (1101)	T10–T11		197	26	2287	2.3	8.6	1262
13 (1121)	T10–T11		198	25	1863	2.3	7.9	1090
14! (1121)	T10–T11		198	25	1863	2.3	7.9	1090
15! (575)	T10–T11		N.D.	N.D.	4656	N.D.	N.D.	N.D.
Mean ± SEM			**207 ± 8*** **	**26 ± 1***	**2074 ± 221****	**1.9 ± 0.1*** **	**7.8 ± 0.3*** **	**1016 ± 61*** **

Measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. After SCI, biomechanical data acquired for each impact were collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact.

Significant difference between SCI animals with a 10 cm drop and 100 g of compression and those with a 20 cm drop (T10 level only) and 150 g compression (Experiment 2, Table 4), $*p<0.05$, $**p<0.01$, $***p<0.001$. ! = animals that had complications after SCI surgery and had to be euthanized.

N.D., not determined; SEM, standard error of the mean; SCI, spinal cord injury.

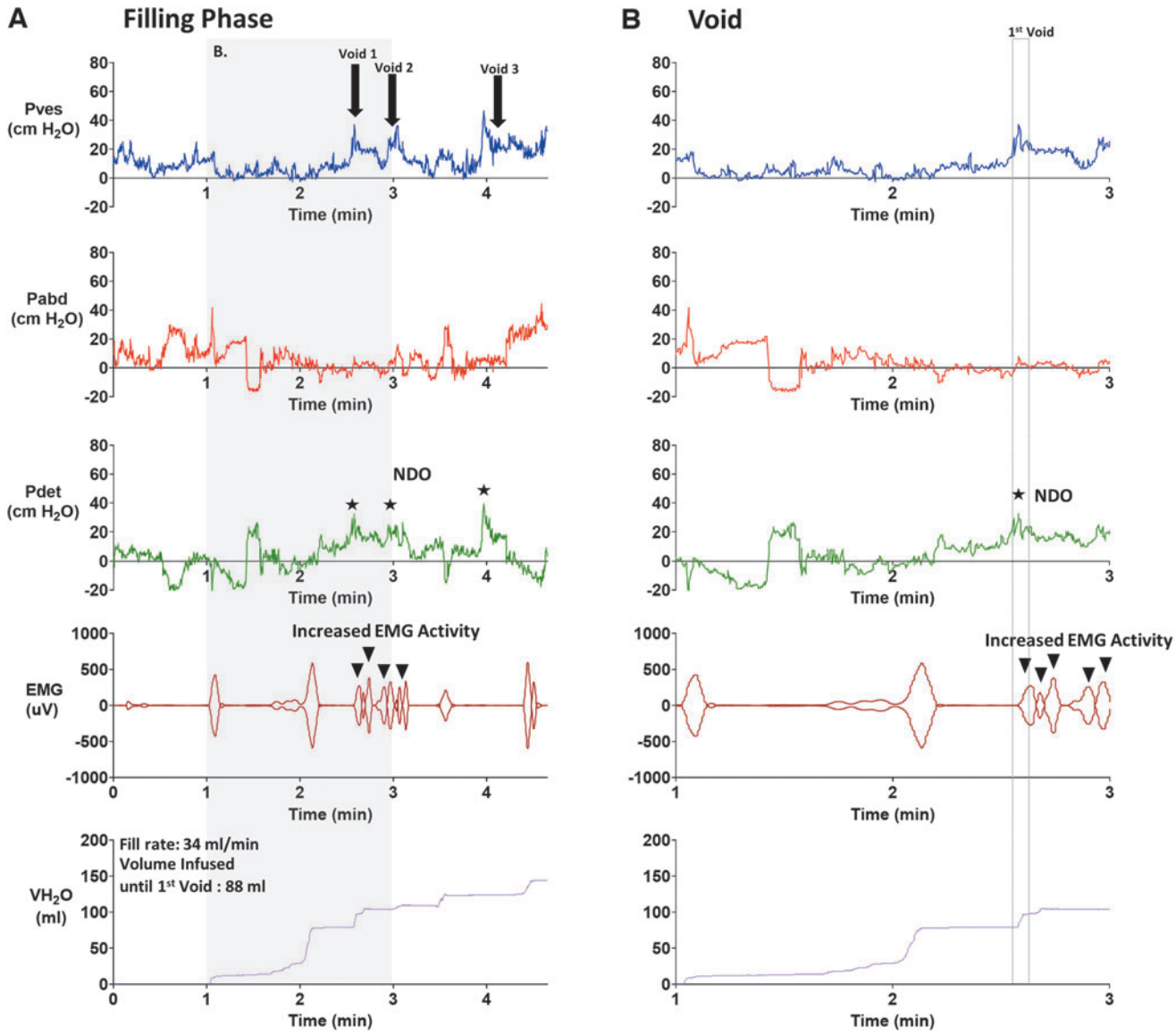

FIG. 11. Urodynamics tracing (awake urodynamics without sedation during catheterization) from a T10 spinal cord injured (SCI) pig at 11 weeks post-injury demonstrating neurogenic detrusor overactivity (NDO) with possible detrusor-sphincter dyssynergia (DSD). **(A)** Filling phase. The fill rate was 34 mL/min, and the infused volume until the first void was 88 mL. A terminal NDO event occurs and during the first two voids, there are bursts of electromyography (EMG) activity (arrowheads), which could possibly signify DSD. The shaded region highlights the zoomed-in view shown in **(B)**. **(B)** Void. At an infused volume of 88 mL, the first void of the study occurs during a possible DSD event (increase in EMG activity [arrowheads]) resulting in incomplete emptying of the bladder during the void. Two more subsequent voids were captured in this study and occurred during terminal NDO (star). From top to bottom: intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), detrusor pressure (P_{det}), EMG of the external anal sphincter, and volume infused (VH_2O).

1.03 ± 0.03, $p = 0.019$). In the dome region, the muscle-to-collagen ratio of SCI bladders was almost significantly larger than in the controls (1.28 ± 0.07 vs. 1.00 ± 0.11, $p = 0.0529$).

At the neck and urethra, there were no significant differences in the percent of muscle between SCI animals and the controls (neck: 38.2–1.4% vs. 35.2–3.1%, $p = 0.33$; urethra: 30.5–1.3% vs. 26.3–2.3%, $p = 0.12$) or percent of collagen (neck: $42.9 \pm 1.5\%$ vs. $39.3 \pm 2.4\%$, $p = 0.23$; urethra: $50.6 \pm 1.6\%$ vs. $53.5 \pm 2.6\%$, $p = 0.36$). There was a significant difference in the muscle-to-collagen ratio in the SCI urethras compared with the controls (0.60 ± 0.02 vs. 0.49 ± 0.04, $p = 0.020$) but not in the neck region (0.90 ± 0.05 vs. 0.91 ± 0.12, $p = 0.92$).

Hallmark features of SCI bladders included thinning of the lamina propria and mild to moderate chronic inflammation, identified by the presence of lymphoplasmacytic infiltration of the lamina propria in H&E stained sections. Mild edema was observed in some cases. The presence of focal acute inflammation was also identified by the presence of neutrophils and eosinophils within the urothelium. Additionally, the urothelium of the SCI bladders showed more prominent folding than the controls. In the SCI urethras (T2 and T10), there was also mild to moderate chronic inflammation (focal neutrophils in the urothelium), but no other distinct pathological differences were observed between SCI and control urethras.

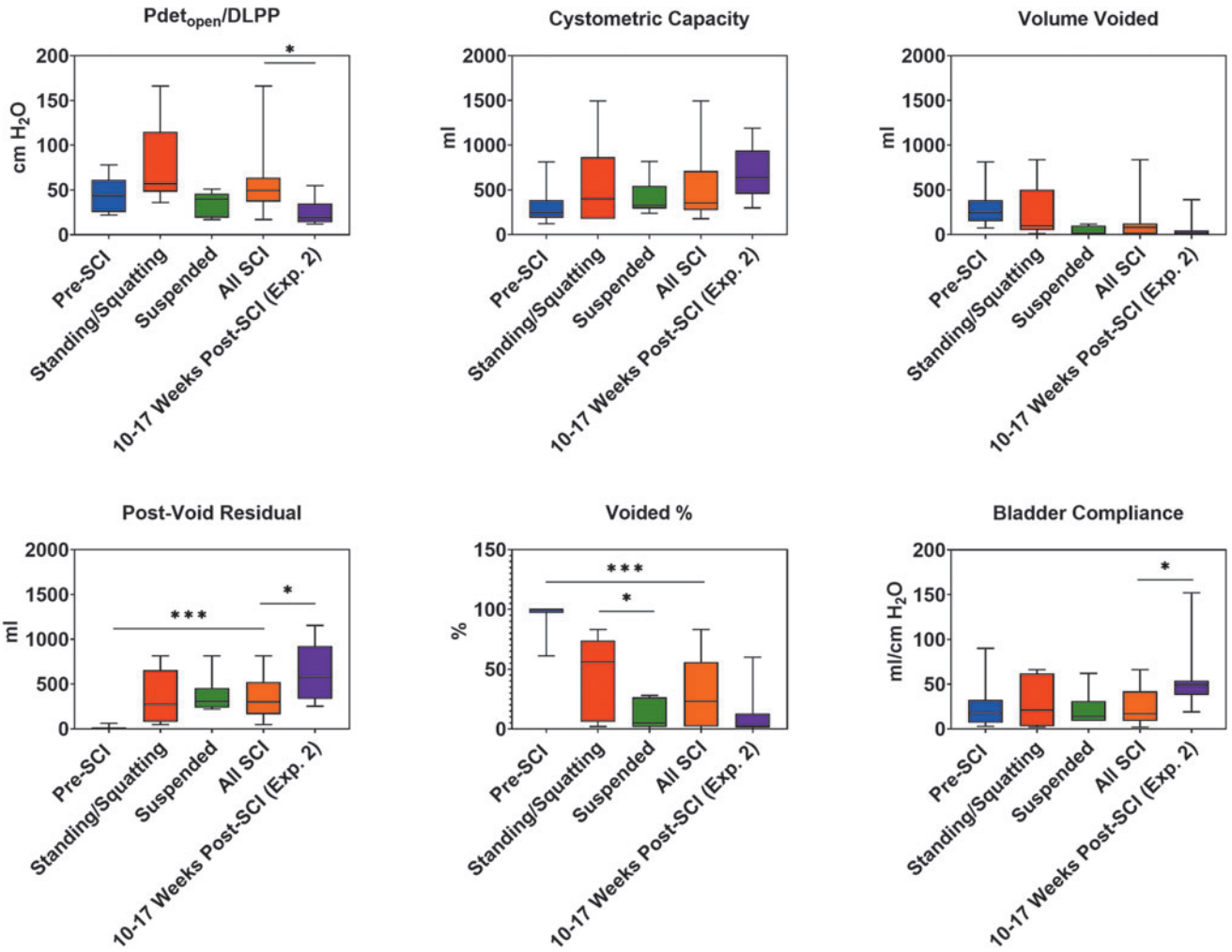

FIG. 12. Experiment 3 (awake urodynamics with no sedation during catheterization): urodynamic parameters of spinal cord injured (SCI) pigs 11–13 weeks post-injury. All animals received a 10-cm drop contusion injury with 5 min of 100 g compression between the T9 and T10 level ($n = 12$). Although this injury severity resulted in substantial hindlimb impairments, 6 out of 12 animals were able to perform urodynamics in a standing/squatting position with minimal support (with someone physically holding the hindlimbs). Urodynamics in the remaining animals was performed in a suspended position using a custom-built sling. A *t* test was performed to compare the differences in the urodynamic parameters between SCI pigs in a standing/squatting and those in a suspended position as well as between all Experiment 3 and 2 SCI animals at similar time points. Significant difference between SCI animals and pre-SCI animals from Experiment 3 or between SCI animals in a standing/squatting and those in a suspended position, $*p < 0.05$, $***p < 0.001$.

Discussion

The minipig model has shown great potential for urologic research.[20–24] The anatomical and physiological similarities of the LUT of humans and pigs have been well described in several studies.[16–19] Most recently, the minipig model was used for a NLUTD study by Keller and coworkers, in which they performed UDS on post-SCI pigs to assess if sacral neuromodulation would ameliorate bladder dysfunction. In their study, they noted that catheterizing awake healthy minipigs was difficult. To circumvent this issue, the authors tried using sedatives (propofol or xylazine) to place the urodynamic catheters and then perform UDS. However, they stated that this procedure gave ''poor information'' during UDS analysis, and attributed this to the influence of the sedative agents.[27]

In this collaborative study between UBC and UofL using the shared Yucatan minipig SCI model and the same impactor, we went through an iterative process to develop and adjust procedures to enable conscious, awake UDS without the need for sedation during catheterization. This achievement is accredited to the training protocol developed by our animal trainers. Our training and UDS setup protocol allows for reliable and reproducible assessments of LUT function with animals in an awake state with minimal restraints. Moreover, it also allows for us to longitudinally follow and characterize the changes in the pig's LUT function before and after SCI.

Major findings in our study include demonstrating that during voids, the EUS of uninjured pigs is relaxed, and that this is similar to how the human EUS behaves during voiding.[11,55] Following SCI, 15/22 (68%) pigs demonstrated NDO at the latest UDS time point (10–17 weeks post-injury) which parallels findings in humans with chronic traumatic SCI.[56,57] The potential to perform longitudinal studies repeatedly in the same animal opens promising avenues to investigate NLUTD over time and in response to intervention.

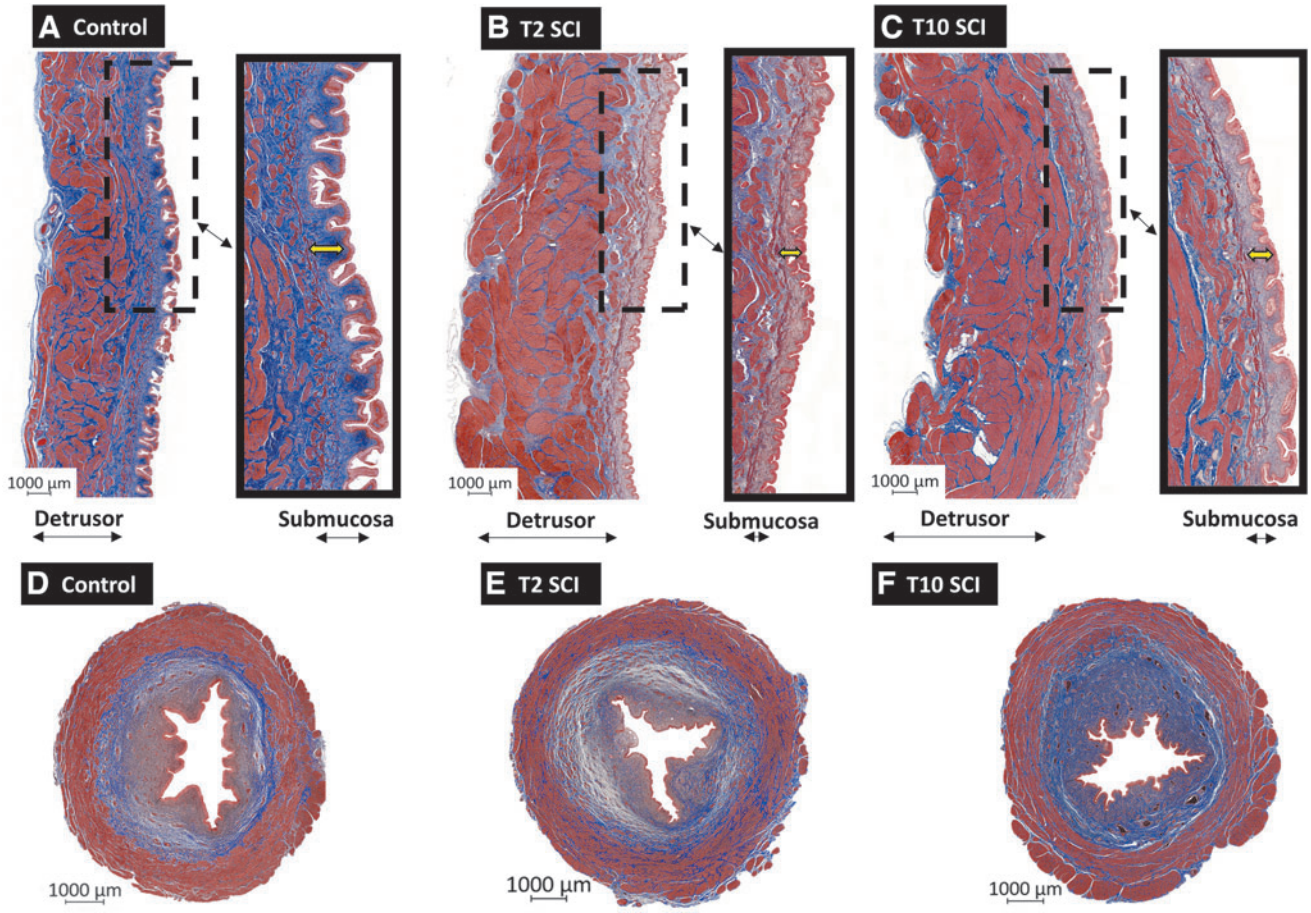

FIG. 13. Detrusor hypertrophy in spinal cord injured (SCI) bladders. Masson's trichrome staining of the body of the bladder from **(A)** a control pig, **(B)** an Experiment 2 T2 SCI pig 12 weeks post-injury (WPI), **(C)** an Experiment 2 T10 SCI pig 12 WPI, and the urethra from the corresponding animals **(D–F)**. The smooth muscle tissue is stained in red and collagen is stained in blue. There is marked detrusor muscle hypertrophy in the body of the bladder after SCI (both after T2 and T10 SCI) compared with the control. Moreover, there is a reduction of the submucosa (yellow arrows show the distance between the urothelium and the first suburothelial smooth muscle layer) in both the T2 and T10 SCI bladders compared with the control. There were no other apparent morphological differences observed between pigs with a T2 or a T10 SCI. The SCI animal's urethras were slightly thicker than those of the control, and the muscle-to-collagen ratio was significantly increased.

Influence of anesthetics on LUT function

Previous studies have shown that anesthesia can result in an inhibition of micturition and direct loss of coordination between the detrusor muscle and EUS as well as an imbalance of sympathetic and parasympathetic control of micturition.[41–44] However, the influence of anesthetics and sedatives on UDS outcomes seems to be dependent on the class of the anesthetic agents as well as the depth of anesthesia.[58–60] In Experiment 1, we observed that propofol and fentanyl inhibited the voiding reflex, which resulted in overflow incontinence in uninjured pigs. However, we could not form a definitive interpretation on how the anesthetics were influencing EUS activity. Further work is required to investigate how propofol and fentanyl influence EUS activity.

Influence of dexmedetomidine and atipamezole on LUT function

UDS results from pigs in Experiment 2 may have been influenced by the sedative and reversal (dexmedetomidine and atipamezole). There was a higher likelihood of observing a greater cystometric capacity than the previously reported female Yucatan minipig bladder capacity ($\sim$180 ml)[23] in uninjured pigs that received sedation and reversal (10/17, 59%) compared with uninjured pigs that did not receive sedation (3/15, 20%). Moreover, bladder emptying (pattern 2) that occurred without contraction of the detrusor was only prominent (12/17 pigs; 71%) in pigs that were sedated and reversed, and did not occur in pigs that did not receive sedation (0/15 pigs; 0%). However, we acknowledge that our interpretation is complicated by the methodological differences between Experiments 2 and 3, and the use of different urodynamics equipment.

The effect of dexmedetomidine and atipamezole on LUT function have been previously investigated in rodent models. Ishizuka and coworkers reported that intrathecal and intra-arterial (close to the bladder) administration of a single dose of dexmedetomidine in conscious rats reduced micturition pressure, bladder capacity, and micturition volume; whereas atipamezole reversed the effects of dexmedetomidine.[62] The same study also found a very similar result with systemic administration.[62]

In another study, Harada and Constantinou found that dexmedetomidine increased voiding frequency and overflow incontinence leading to bladder leakage, but the interpretation of this

finding was complicated by the diuretic effect of dexmedetomidine.[63] Diuresis by dexmedetomidine has also been reported in humans.[64,65] Pigs in Experiment 2 typically voided ~12% more than what was infused during the UDS, whereas pigs in Experiment 3 did not void more than what was infused, suggesting that there was diuresis in pigs that were sedated with dexmedetomidine. Other reported effects of dexmedetomidine on the LUT include reduced bladder sensation[66] in humans and inhibition of bladder contractility in animals.[67]

Harada and Constantinou also reported that the rodent's bladder capacity was decreased with dexmedetomidine, whereas atipamezole increased capacity.[63] Therefore, suggesting that greater capacities in uninjured pigs may have been caused by the effects of atipamezole. Overall, the combination of the dexmedetomidine and atipamezole may have resulted in diuresis, altered voiding patterns, and greater cystometric capacities in a proportion of Experiment 2 uninjured animals.

Although we acknowledge the paucity of data describing UDS in animal models performed with sedation, the aforementioned animal studies, studies in urethane-anesthetized rats,[63,68] and our data, highlight the potential influence of dexmedetomidine and atipamezole on the LUT function of neurologically intact pigs. The variability in the results between previous studies and ours could be associated with differences in the experimental conditions such as the species used, drug dosage, and the time between the exposure to the drug and when the animal voided or leaked. Taking all these potential issues into consideration, we plan in future studies to avoid using sedation such as dexmedetomidine for bladder catheterization, unless absolutely necessary.

Influence of the actual UDS testing procedure on LUT functional measures

Although the current gold standard for characterizing NLUTD is UDS, it has been questioned whether such procedures can be used to characterize ''normal'' bladder function in the non-SCI condition. Although in experimental studies it would only be natural to perform UDS pre-SCI to establish the ''normal baseline'' state, it should be acknowledged that the act of performing UDS with transurethral urodynamic catheters and retrograde filling at rapid rates may not replicate the ''normal'' function of the uninjured bladder. In our study, we observed large inter-animal variability in the UDS outcomes of uninjured pigs. These findings highlight the variability of urodynamics, as also observed in healthy individuals. In 1999, Wyndaele reported wide variation in all UDS parameters from a total of 28 men and 10 women with no history, symptoms, or signs of urological disease. In the study, healthy volunteers demonstrated pathological signs such as altered flow patterns, low Q_{max}, large cystometric capacities, bladder overactivity, and elevated PVR.[69] Likewise, Leitner and coworkers found that 71% (30/42) of healthy participants without LUT symptoms demonstrated some sort of pathological finding, with DSD being the most common finding during UDS.[70] Further, the healthy participants also demonstrated larger bladder capacities during UDS compared with the capacities recorded in their bladder diaries.

These previous findings allude to the fact that UDS may not necessarily be a valid tool to define ''normal'' LUT function, and that variability may result because of the non-physiological nature of the test. For example, we observed cystometric capacities in non-sedated uninjured pigs ranging from 122 to 811 mL. Note that we measured that the Yucatan minipigs voided no more than 350 mL in their own pens (our own preliminary data, not shown). With everything considered, this may suggest that the comfort level of the pigs to void during UDS could influence the final UDS outcomes.

Influence of injury severity on LUT function

The relationship between SCI lesion severity and the degree of NLUTD remains a complicated and unresolved issue. Despite inducing injuries in a controlled setting, heterogeneity in the lesions can still arise. Take, for example, the 10-cm injury severity (100 g weight compression) group where a cohort (6/12, 50%) were able to stand on their own and squat to void. Although we did not investigate the histopathological changes of the spinal cords for the animals in this study, our previous work has shown that a 10-cm injury severity will result in more white matter sparing than the 20-cm injury severity (150 g weight compression). From this work, we have also shown that white matter sparing is positively correlated to hindlimb function.[28] The retention of hindlimb function after SCI in a small cohort of 10-cm injury severity animals suggests that there may also be some retention of bladder function. This may explain why the 10-cm injury severity group had slightly better bladder outcomes than the 20-cm injury severity group. However, further work is required to investigate the unresolved relationship between the lesion severity and degree of NLUTD in this model.

Bladder wall thickening and trabeculation after SCI

Chronic SCI has been reported in other porcine[71] and rodent models[72,73] to result in increased bladder weight and wall thickness, highlighting the downstream consequences of SCI on the morphology of the bladder. Histological evaluation of SCI bladder sections revealed increased markers of chronic inflammation in the submucosa. Specifically, plasmo-lymphocytic infiltration of the lamina propria was seen as early as 3 weeks post-injury. This feature has also been found in bladder biopsies of SCI patients.[74,75] The retention of urine or the presence of foreign bacteria may have acted as a noxious stimuli and triggered chronic inflammation.[76]

Thickening of the detrusor was also another common morphological observation in the SCI bladders. This morphological alteration is the most relevant in patients with bladder outlet obstruction. In a similar fashion, DSD is a form of bladder outlet obstruction and may have resulted in detrusor hypertrophy via a combination of factors such as cyclic stretching, elevated intravesical pressure, and hypoxia.[77]

Limitations of the study

This porcine model of NLUTD was established by a collaboration between two independent institutions working on the same SCI model. Although there was a unique strength in the collaboration and some observations were compatible between the two sites, there were also differences in the methodology and approaches.

First, different urodynamics systems were used for Experiment 2 (fluid-filled pressure sensor catheters) and Experiment 3 (fiberoptic pressure sensor catheters). This complicates the comparison of the findings between the experiments, especially considering that these systems operate on different principles in terms of calibration. In fluid-filled systems, the pressure transducers are aligned with the ICS reference level (superior edge of the pubic symphysis) and

zeroed to the atmospheric pressure. In contrast, fiberoptic systems are not calibrated to the ICS reference level, and this has been suggested to result in variability in the initial detrusor pressure measurements.[78]

Second, the bladder filling rate was not controlled in a uniform and consistent fashion, ideally to the recommended rates by the ICS. The maximum physiological filling rate is estimated to be the body weight in kg divided by 4,[79] which in this case would have been ~5–10 mL/min for the pigs. Yet, there is a fine balance in choosing a rate that resembles the physiological filling rate as well as completing the study in an acceptable time for the participant. Rapid filling of the bladder has shown to increase variability in parameters such as bladder compliance,[80] detrusor pressure and contractility,[19,81,82] volume threshold to void,[83] and presence of NDO.[84] However, the exact influence of rapid and variable bladder filling on bladder function appears to be inconclusive in the literature; therefore, further investigation is required as future work.

Conclusion

Overall, with the establishment of these techniques for UDS, we contend that the Yucatan minipig model of SCI can serve as a valuable large animal model of NLUTD. The size of this animal is adequate for the development and testing of novel human-sized devices that aim to improve LUT function. A setup protocol for performing urodynamics in awake, slightly restrained Yucatan minipigs that allows for repetitive and longitudinal evaluation of LUT function before and after SCI has been described. We do not recommend performing urodynamics in anesthetized pigs, as we have demonstrated how these drugs can inhibit the voiding reflex. Instead, we recommend performing urodynamics in the pigs (either uninjured or post-SCI) without the use of sedation and reversal during catheter placement, unless absolutely necessary. This characterization of LUT function will allow for future studies to investigate different bladder management strategies post-SCI and the effect of SCI therapies, including neuromodulation on bladder function. Given the morbidity associated with LUT dysfunction after SCI, we hope that such a large animal model will facilitate advances in urological care that will ultimately improve the quality of life for persons living with SCI.

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Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1

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