Journal of Visualized Experiments

Detrusor Underactivity Model in Rats by Conus Medullaris Transection

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61576R3
Full Title:	Detrusor Underactivity Model in Rats by Conus Medullaris Transection
Section/Category:	JoVE Neuroscience
Keywords:	underactive bladder; detrusor underactivity; conus medullaris; laminectomy; model; transection
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Additional Information:	
Question	**Response**
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Dear editor/reviewers,

We submit our manuscript (research article) entitled "A Rat Model Of Conus Medullaris Transection For Studying Lower Urinary Tract Function Of Detrusor Underactivity" to JoVE. The manuscript focuses on establishing a detrusor underactivity (DU) model of rat through laminectomy and to understand the pathophysiology, and treatment. In this study, we explore a new way for establishing DU model, since the models build before can't simulate the happening and processing of DU successfully. But in our study, we found an easy, cheap, and repeatable to establish animal model of DU.

Few articles about animal models of DU were published in recent years, so even the ICI-RS meeting in Bristol in 2010 called for more attention of researchers to be paid on DU.

Since then, only a few UAB models have been reported, for example, (1) spinal cord transection or contusion injury, (2) supraspinal injury (decerebration, local lesions, and middle cerebral artery occlusion), and (3) systemic (e.g., cyclophosphamide) or intravesical administration of irritant or inflammatory agents (e.g., acrolein, acid and lipopolysaccharide).

Of all the methods stated above, only the spinal cord transection or contusion injury method can be used in establishing animal model of DU.

But this method was not applied to establish DU animal model, because the damaged spinal cord inducing OAB or DU is different and researchers are still confused about of pathogenesis of DU.
So in this study, we opted for laminectomy to establish rat model successfully. It was performed at the L4-L5 level to divide the spinal cord totally. We also investigated the maximum cystometric capacity (MCC), detrusor opening pressure (DOP), and compliance of bladder.

We submit our manuscript as a Research Article.

And neither the entire paper nor any part of its content has been published or has been accepted elsewhere. It is not being submitted to any other journal. We hope the manuscript could be considered for publication in JoVE.

Thank you very much for your reading. We are looking forward to hearing from you.

Best regards,
Sincerely.
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TITLE:

Detrusor Underactivity Model in Rats by Conus Medullaris Transection

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KEYWORDS:

underactive bladder, detrusor underactivity, conus medullaris, laminectomy, model, transection

SUMMARY:

We present a method for establishing a detrusor underactivity model by conus medullaris transection in rats. Detrusor underactivity was successfully stimulated in these animals. The model can be used for studying urinary tract function.

ABSTRACT:

The goal of the presented protocol was to establish a detrusor underactivity (DU) model in the rat through conus medullaris transection. Laminectomy was performed in a total of 40 female Wistar rats (control group: 10 rats; test group: 30 rats) weighing 200–220 g, and the conus medullaris was transected at the L4–L5 level in the test group. All the rats were housed and fed under the same environmental conditions for six weeks. In the test group, urine voiding was performed twice daily for six weeks, and mean residual urine volume was recorded. A cystometrogram was performed in both groups. Maximum cystometric capacity (MCC), detrusor opening pressure (DOP), and compliance of the bladder were recorded and calculated. The test group showed significant urinary retention after the surgery, both during and after the spinal shock. However, no abnormality was observed in the control group. When compared to the control group, the MCC and compliance of bladder in the test group was significantly higher than that of the test group (3.24 ± 2.261 mL versus 1.04 ± 0.571 mL; 0.43 ± 0.578 mL/cmH_2O versus 0.032 ± 0.016 mL/cmH_2O), whereas DOP in the test group was lower than control (20.28 ± 14.022

cmH_2O versus 35 ± 13.258 cmH_2O). This method of establishing an animal model of DU by the conus medullaris transection offers an excellent opportunity to understand DU's pathophysiology in a better manner.

INTRODUCTION:

Detrusor underactivity (DU) is a typical lower urinary tract dysfunction that has remained under studied. Even though DU has been defined by the International Continence Society (ICS)[1], numerous different terminologies are used to refer to this disease, e.g., "detrusor failure," "acontractile bladder," "detrusor areflexia"[2]. DU, as defined by the International Continence Society (ICS) in 2002, is a contraction of reduced strength and duration, which results in prolonged increase in time for bladder emptying, thereby resulting in failure to achieve complete bladder emptying within a normal period.

DU may affect 48% of men and 12% of women (aged >70 years)[3] with lower urinary tract symptoms. It seems to be multifactorial, and no effective treatment exists. It is reported that DU is ubiquitous in patients with neurogenic bladder dysfunction, such as multiple sclerosis[4], diabetes mellitus[5], Parkinson's disease[6], or cerebral stroke[7]. DU can also be caused by iatrogenic nerve damage, such as laparoscopic hysterectomy, prostatectomy, or other surgical interventions in the small pelvis[8]. The pathophysiology changes and available treatments of DU are still confusing because of the lack of an appropriate animal model for study.

The micturition reflex is controlled by spino-bulbospinal pathways that combines the pontine micturition center, sacral parasympathetic nucleus, and more senior cortex centers[9]. Activation and maintenance of the micturition reflex mainly depend on the regular transport of sensory signals from the bladder to more senior cortex centers. It may be postulated that sensory dysfunction contributes to DU.

Most experimental animal studies related to lower urinary tract dysfunctions have focused on overactive bladder (OAB) models[10]. These models provide a reasonable understanding of OAB pathophysiology and prognosis. However, only a few DU models have been reported, e.g., supraspinal injury (local lesions, decerebration, and middle cerebral artery occlusion), spinal cord transection or contusion injury, systemic (e.g., cyclophosphamide) or intravesical administration of irritant or inflammatory agents (e.g., acid, acrolein, and lipopolysaccharide)[11-14]. Among these methods, only the spinal cord transection or contusion injury method can be used in establishing an animal model of DU[13]. Attempts involving the injury of the pontine micturition center and higher cortex centers were abandoned because of the severe trauma. So, increased attention is being paid to find an accurate location in the micturition reflex center to induce the DU with minimum side effects.

As mentioned previously, one of the mechanisms of inducing DU is to injure the spinal cord to damage the signaling pathway of the micturition reflex. Allen's weight-drop method was

developed to establish laboratory animals with injured spinal cords[15]. However, there are no further experimental data available on this method. Moreover, since parts of the animals recovered spinal function after stroke without DU, it cannot be considered as a perfect method for generating a DU animal model[16].

In 1987, Bregman excogitated a process of transecting the spinal cord for generating the DU animal model and acquired experimental data[17]. Nevertheless, this method was not applied to establish the DU animal model. At that time, researchers were still confused about the pathogenesis of DU. As locations in the spinal cord associated with the induction of OAB or DU are adjacent to each other, they were unable to find the accurate site of damage to the spinal cord to induce DU[17]. OAB and DU were introduced either together or separately by this method. So, although this method introduced DU, it was imprecise and could not be used for the understanding of DU's occurrence and processing.

As stated above, the lack of a suitable animal model of DU is one of the main obstacles for the study of DU. Researchers are continuously looking for an accurate and manageable model that can simulate the pathology of DU. Even the treatment options for DU have not significantly improved during the last 20 years. Collectively, there is a great need to describe a standard protocol for establishing an animal model of DU.

So, in this paper, we describe a method to successfully establish a rat model of DU by conus medullaris transection. Transection was performed at the L4–L5 level to separate the conus medullaris. The maximum cystometric capacity (MCC), detrusor opening pressure (DOP), and compliance of the bladder were recorded and analyzed to validate the protocol. The protocol stated below combines both feasibility and reliability in a standardized manner to establish the DU animal model, simulating the occurrence and processing of DU. The protocol can be used as a technique for further study of DU.

PROTOCOL:

All rats were used according to protocols approved by the Animal Experimental Committee of Beijing Friendship Hospital, Capital Medical University.

1. Surgical preparation, anesthetization, and surgical techniques

NOTE: A total of 40 female Wistar rats, weighing 200–220 g, were commercially obtained for the present study. Of the 40 rats, 10 were randomly selected as the control group, and the rest were treated as the test group. All animals were housed in a sterile environment in the animal facilities of Beijing Friendship Hospital, Capital Medical University.

1.1. Perform general anesthesia by administering sodium pentobarbital intraperitoneally (40 mg/kg). Then, place the rat on the surgical platform.

1.2. Check for the depth of anesthesia by the lack of response to the toe pinch. Shave the fur from the whole back area with a razor.

1.3. Sterilize the back with an alcohol prep pad twice. Secure the limbs with surgical tape and make a median incision of about 3 cm on the back with surgical scissors.

1.4. Deepen the incision through the subcutaneous tissues using the #15 surgical scalpel blade and cut off the muscles attached to the spine.

1.5. Visually identify and expose the 13th rib (the intervertebral space connected to that rib is interval T13–L1). Mark the 13th rib using a suture.

1.6. After identification, carefully resect the muscles attached to the spine and expose the vertebral column. Resect the supraspinous ligament and interspinous ligament for an accurate identification of the vertebral column. Expose the level of L4–L5 with a #15 surgical scalpel blade and mini-blades.

NOTE: The supraspinous ligament can be identified easily because of the presence of thin subcutaneous tissue. After the resection of supraspinous ligament, the ligament between spinous process is interspinous ligament.

1.7. Carefully dissect away the L4–L5 vertebral spinous process and parts of the transverse process by rongeur to expose the spinal cord (**Figure 1**).

1.8. Completely expose the conus medullaris at the L4–L5 level and transect the conus medullaris totally with iridectomy scissors. Insert some tissue packing to block the recovery of the spinal cord.

1.9. Close the overlying muscle and skin on the outer skin layer using 4-0 non-absorbable suture.

1.10. For the control group, perform steps 1.1–1.7, and leave the conus medullaris intact. Close the incision according to step 1.9.

2. Animal recovery

2.1. Keep the rats in a temperature-controlled incubator (30 °C) during the first hour post-operation and monitor it until it has regained consciousness.

NOTE: It takes about half an hour for total recovery.

2.2. Transfer the animal to a clean cage with sufficient food and water. Keep the rats in separate cages.

NOTE: The transection's success is indicated when the rats in the test group move only with the help of forelegs, whereas the rats in the control group could walk normally.

3. Post-operation management

3.1. Inject Penicillin G, an antibiotic (50,000 U/mL per animal) intraperitoneal and inject buprenorphine (0.05 mg/kg) subcutaneously at 24 h and 48 h time points post-operation.

3.2. Compress the urinary bladder at the hypogastrium to help with the voiding. Perform this twice daily at the same time (8 am and 8 pm) for six weeks.

NOTE: The loss of normal constriction of detrusor is the symbol of DU.

3.3. House all rats in metabolic cages, each containing a urine collection funnel placed over a previously weighed absorbent paper to monitor the micturition and incontinence.

3.4. Collect and note the weight change of absorbent paper, which indicates the voided volume (VV), and the residual urine volume separately.

4. Urodynamic testing

4.1. At six-weeks post-operation, perform a cystometrogram, using urodynamic measurement equipment as follows.

4.1.1. Anesthetize rats by injecting 10% chloral hydrate into the peritoneal cavity (3 mL/kg).

4.1.2. Compress the bladder for voiding, then fix the rat to the surgical platform using a tape.

4.1.3. Insert the epidural catheter (3F) into the bladder and connect the urodynamic measurement equipment, epidural catheter, and infusion pump by the three-limb tube.

4.1.4. Pump physiological saline at a speed of 0.2 mL/min for urodynamic measurement (see **Table of Materials**). Record the MCC and DOP, and compliance of the bladder (calculated by dividing δ bladder volume with δ pressure of the detrusor).

5. Statistical analysis

5.1. Perform statistical analysis using commercially available software.

5.2. Use Kolmogorov-Smirnov test to test the normality of data.

5.3. Express the normally distributed variables as mean values with standard deviations. Use the two-tailed paired Student's *t*-tests to compare the parameters of cystometrogram in both groups.

NOTE: $p < 0.05$ indicates that the difference had statistical significance.

REPRESENTATIVE RESULTS:

The entire procedure of the conus medullaris transection can be completed within 45 min by experienced surgeons. Our laboratory has performed over 100 cases of conus medullaris transection surgeries. The success rate is over 95%, as defined by the rats' survival and successful induction of DU. The urodynamic test confirmed the induction of DU.

Based on our experience, the induction of DU can be preliminarily evaluated by the residual urine volume. The retention of urine was observed immediately after the surgery. In the test group, the peak point of volume appeared on the second-day post-operation, and the decrease in the volume gradually sustained for about ten days. Ten days after surgery, the volume reached a steady level (**Figure 2**). It was observed that during the first ten days after surgery, the mean residual urine volume was 2.09 ± 1.05 mL, which was reduced to 0.67 ± 0.21 mL on the 10^{th} day after surgery. However, no abnormality was observed in the control group.

To confirm the induction of DU, the urodynamic test needs to be performed. The representative pressure-volume profile of the test group and the control group are shown in **Figure 3** and **Figure 4**. When compared with the control group, the MCC and compliance of bladder in the test group significantly higher in the test group (1.04 ± 0.571 mL vs. 3.24 ± 2.261 mL, $p < 0.001$ and 0.032 ± 0.016 mL/cmH_2O vs. 0.43 ± 0.578 mL/cmH_2O, $p < 0.05$, respectively) whereas DOP in test group decreased significantly (35 ± 13.258 cmH_2O vs 20.28 ± 14.022 cmH_2O; $p < 0.01$). See **Table 1**.

FIGURE AND TABLE LEGENDS:

Figure 1: Method for conus medullaris transection. (**a**) Exposing the 13th rib (black arrow). (**b**) Exposing L4 and L5 vertebral arches. The vertebral plate was destroyed by rongeur to unmask the spinal cord (black arrow).

Figure 2: Time course of the changes in voiding behavior parameters in the test group. Values are represented as mean ± SD.

Figure 3: Representative cystometric traces in the test group. (**a**) Representative tracings from a rat exhibiting significantly elevated bladder volume and low detrusor pressure. (**b**) Representative tracing from a second rat exhibiting elevated bladder volume and slightly lower detrusor pressure

than usual. With the fixed infusion speed, the infusion time in the test group is quite different. However, the infusion time of all the rats in the test group increased significantly, which means an enlarged bladder.

Figure 4: Representative cystometric traces in the control group. (**a**) A rat with normal bladder volume and gradually elevating bladder pressure with the infusion. (**b**) A rat with normal bladder volume and gradually elevating bladder pressure with the infusion. With a fixed infusion speed, the infusion time in the control group for nearly 6 min means indicates the same bladder volume across the control group.

Table 1: The representative pressure-volume profiles of two groups.

DISCUSSION

DU is a common cause of lower urinary tract symptoms in both men and women. It is a complex constellation of symptoms with few treatment options that can significantly diminish the quality-of-life (Qol) of those affected[18]. Although it is believed that DU is multifactorial, the understanding of its pathogenesis remains rudimentary. Studies have shown that the pathogenesis of DU might be related to myogenic and neurogenic factors.

In the myogenic hypotheses, it was observed that individuals with DU might experience a more significant decline in detrusor contractility than those with healthy aging. It was found that detrusor contractility diminishes with age and is probably affected by other factors like metabolic or neurogenic diseases. Data from urodynamic assessment showed that DU and post-void residuals were associated with aging[19]. A study showed that 22.1% of men and 10.8% of women (all aged > 60 years) reported difficulties with bladder emptying[3]. Furthermore, the leading cause behind this was decreased detrusor contractility. Studies in diabetic bladders have displayed similar changes like those found in DU[20]. The decrease of the muscle to collagen ratio leading to widened spaces between muscle cells may cause the diminishing detrusor contractility. Age-related increase in circulating norepinephrine has also been found in most neurogenic bladders[21,22]. Therefore, there have been attempts to induce DU by establishing diabetes mellitus in the animal model. But these failed because of the lack of accurate control of the blood sugar levels and other complications of diabetes mellitus. However, in the neurogenic hypotheses, DU was classified into three groups: obstacle in the efferent signals of the micturition reflex, obstacle of afferent signals initiating the reflex, and defective integrative control[23]. So, many researchers paid attention to establishing the animal model by an accurate injury of the neurogenic system components. Because of the neurogenic system's complicated function, it is difficult to pinpoint the position inducing DU. Unfortunately, numerous attempts to use neurogenic system injury to induce DU have failed.

Our protocol is the first report of establishing the DU animal model by transection of the conus medullaris. In the present study, the spinal cord was transected at the level of L4–L5 to induce damage of the lower sacral nerves.

The most critical step of the surgery is identifying the spinal cord at the level of L4–L5 because the conus medullaris of the rat is long and thin, and ranges from the upper side of L1 to the lower side of the L4. If the spinal cord is transected above the L4, it is possible to induce damage to higher sacral nerves. On the contrary, if transection occurs below L5, it may not eradicate the micturition center. So, performing transection surgery at the level of L4–L5 can make sure that both the afferent and efferent pathways of the micturition center are destroyed, which makes this method unique.

In the test group, urine retention emerged immediately after the surgery, and the variation profile of the residual urine volume corresponded to the change in micturition function during or after the shock stage of spinal cord injury. Simultaneously, the classic reflex incontinence aftershock stage was not observed, which indicated that the efferent nerve to bladder had been damaged.

We also found an increase in residual urine in the first week after surgery and a significant decrease after the first week. The change of residual urine is likely caused by the impaired coordination of outlet/sphincter/pelvic floor function. So, in the first week after surgery, the sudden disruption leads to an increase in residual urine, and when the compromise of outlet/sphincter/pelvic floor function is rebuilt to some extent, the residual urine decreased to a stable level.

As per the meaning of DU conceived by ICS: (1) too feeble detrusor contraction power and (2) too short detrusor contraction span, it is connected to deficient bladder emptying (diminished voiding effectiveness), diminished sensation, and lower urinary tract symptoms. Upon comparing the urodynamic data of the two groups, we found that the maximum cystometric capacity and the compliance of the test group’s bladder increased dramatically by six weeks post-operation, while the detrusor opening pressure decreased. With the help of these data, it is clear that the contractility of detrusor decreased after six weeks, causing the bladder’s inability to contract to induce micturition.

As shown in the bladder pressure-volume profile, with the increased maximum cystometric capacity, the micturition did not emerge, although the detrusor's pressure was also exaggerated. The absence of micturition indicated that the surgery blocked afferent signals, which induce micturition, by causing bladder afferent nerve dysuria. Furthermore, these profiles correspond with the pathophysiological change of DU.

There are also limitations to this research. For example, intensive care should be taken to prevent infection after the surgery. From our experience, the conus medullaris transection might lead to

impaired motivation of the lower hindlimbs. Moreover, the leakage of retained urine (due to incontinence) may be challenging to be found quickly resulting the constant contact between a moist cage bed wetted by urine and animal lower body. This can lead to severe cutaneous or urinary tract infection, which might be fatal. This protocol demand that surgeons with limited microsurgical experience undergo extensive surgical training to master the technique, especially the accurate identification of conus medullaris.

As the clinical highlights of impeded bladder emptying (e.g., decreased urinary flow rate, elevated postvoid residual [PVR]) may emerge because of DU yet may likewise happen because of bladder outflow obstruction (BOO) (e.g., benign prostatic hyperplasia, urethral stricture). As such, regularly testing to recognize DU and BOO without invasive pressure-flow studies[24] is required. However, in our model, no micturition is observed in the urodynamic test caused by the impaired detrusor constriction ability. It is challenging to analyze the BOO factor simultaneously, which is also a limitation of the model.

In conclusion, setting up the animal model of DU by transecting the conus medullaris provides a desirable animal model for further understanding of DU. With proper training and practice, this surgery can be performed with a success rate greater than 95%.

ACKNOWLEDGMENTS:
None.

DISCLOSURES
The authors have nothing to disclose.

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Figure1

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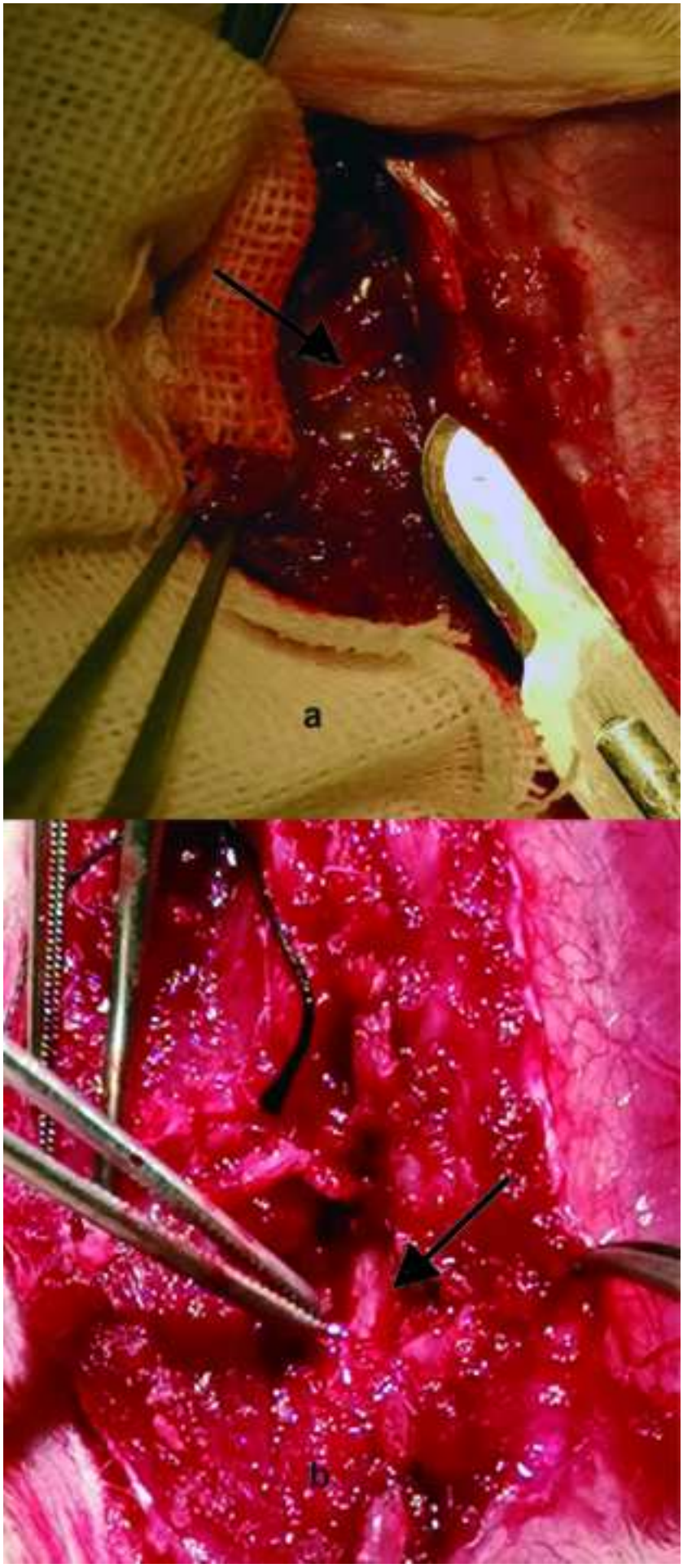

Figure2

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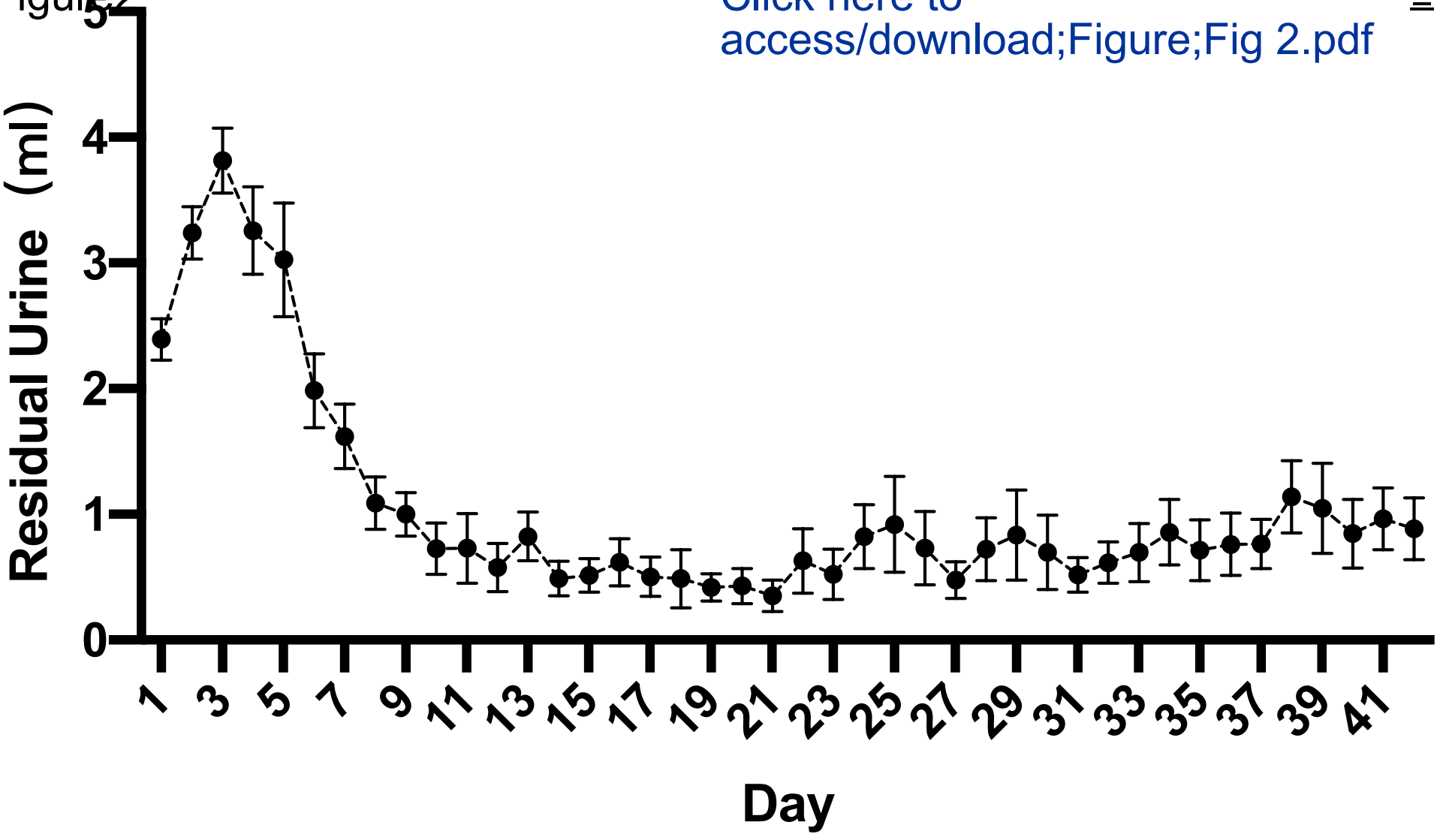

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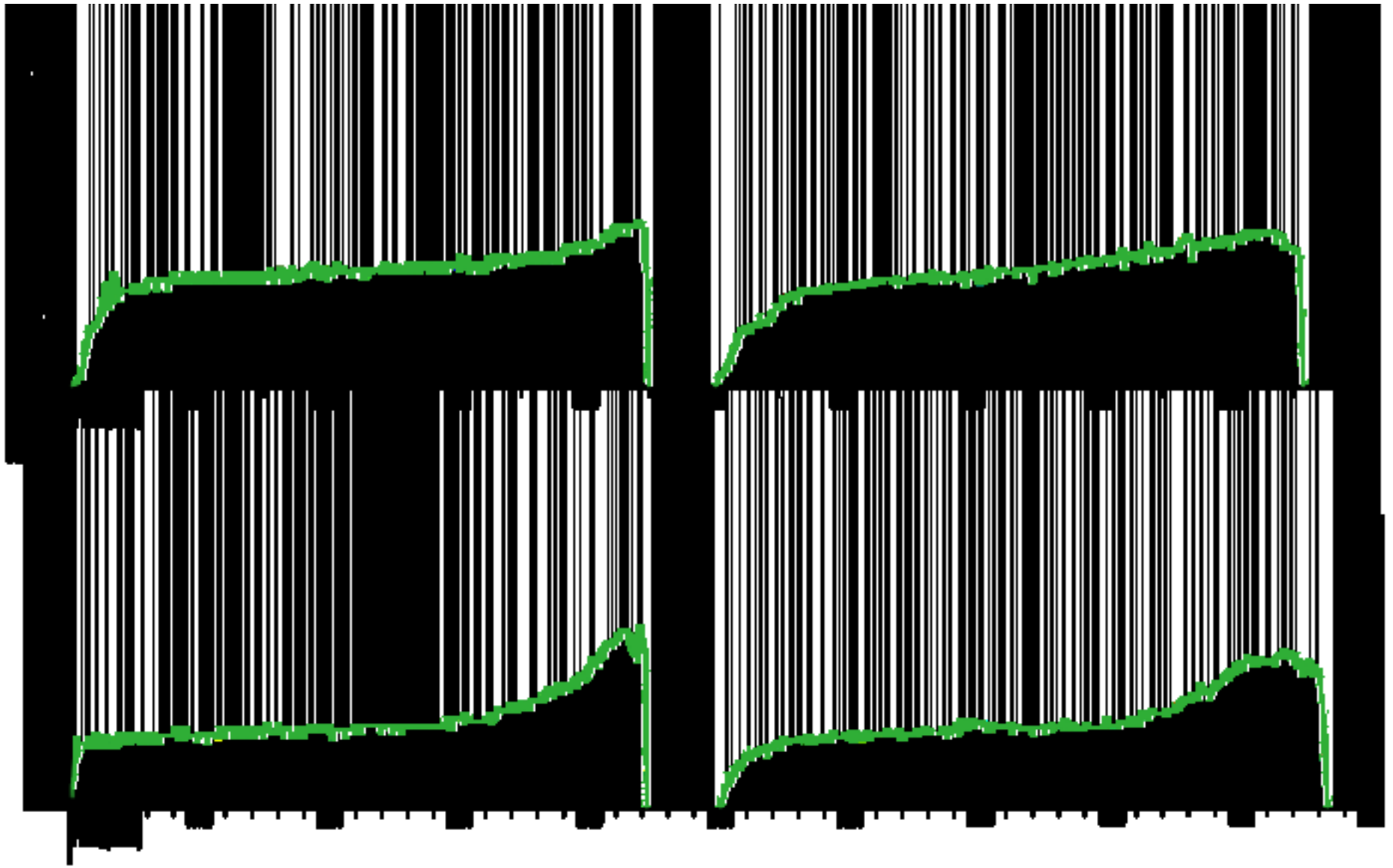

Figure4

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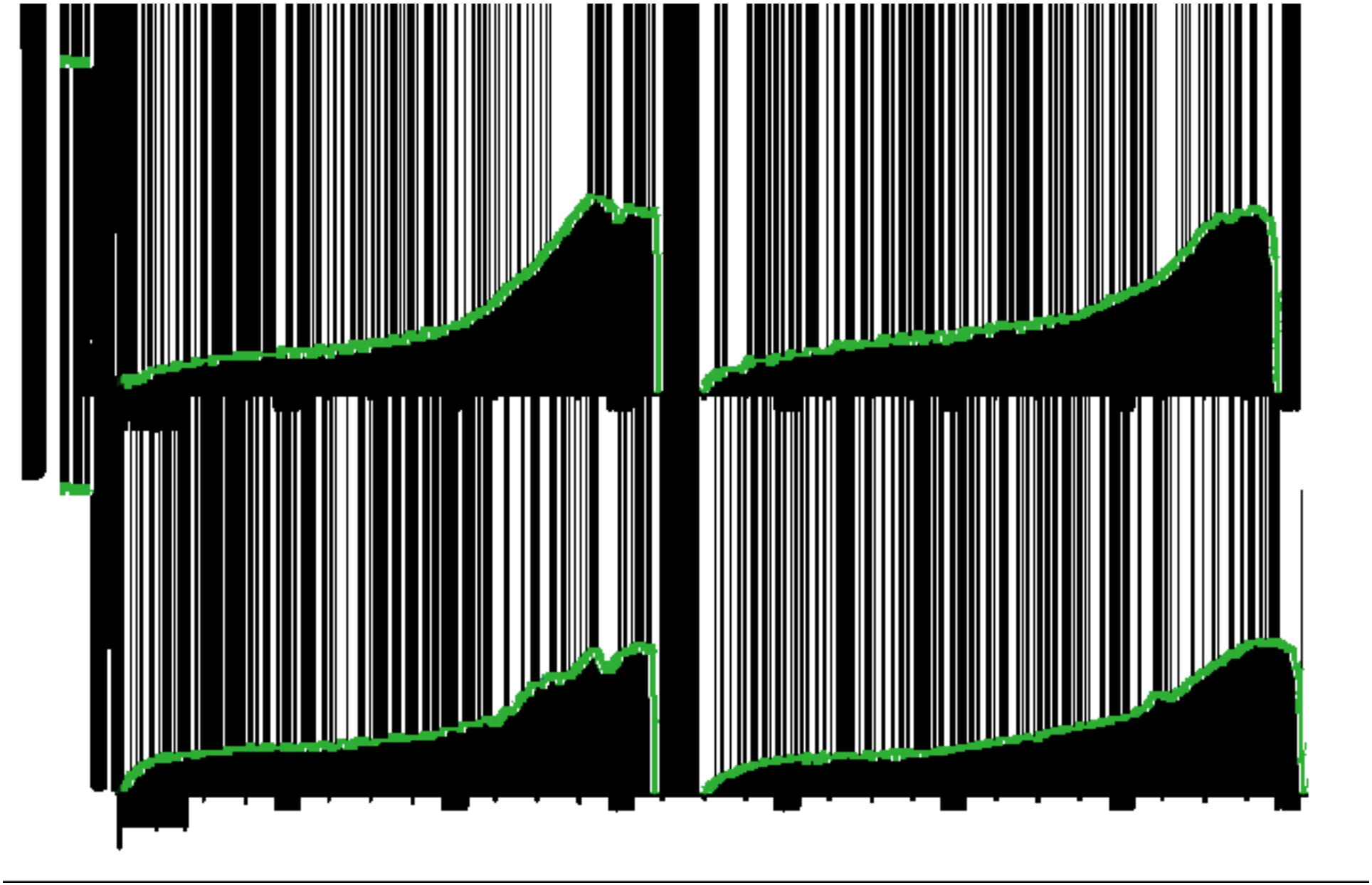

Table 1. The representative pressure–volume profile of two groups.

Group	Case	Maximum Cystometric capacity (ml)	Detrusor opening pressure (cmH_2O)
Test Group	26	3.24±2.261	20.28±14.022
Control Group	10	1.04±0.571	35±13.258
T value		4.517 (p =0.000)	-2.847 (p =0.008)

Statistical analysis was employed using the t test. Data presented as mean ± SD.
A $p<0.05$ was considered statistically significant.

Compliance of bladder (ml/H_2O)
0.43±0.578
0.032±0.016
3.435 (p =0.002)

Name of Material/Equipment	Company	Catalog Number
0.9% saline	Wuhan Prosai Company	EY-C1178
10% chloral hydrate	Shandong Yulong Co., Ltd	H37022673
Buprenorphine Hydrochloride Injection	Tianjin Pharmaceutical Research Institute Pharmaceutical Co. LTD	H12020275
Epidural Catheter	Shandong Xinghua Co, Ltd	VABR3L
Penicillin G	Alta Technology Co., Ltd	1ST5637
pentobarbital	Beijing solabo Technology Co., Ltd	NK-WF0001
Suture line(4-0)	ETHICON	VCP422H
Three-limb tube	Shandong Xinghua Co, Ltd	VAB3T
Trace infusion pump	Zhejiang Smith Medical Instrument Co., Ltd	20162540335
Urodynamic measurement equipment	Medical Measurement SystemsB.V.	08-0467
Wistar Rats	HFK Biotechnology Co.Ltd,Beijing ,China	SCXK2012-0023

Comments/Description

pump for urodynamic measurement
3mL/kg, administered intraperitoneally

0.05mg/kg subcutaneously 24h and 48h postoperation
for urodynamic measurement
50,000 unit/ml per animal

40 mg/kg, administered intraperitoneally
suture the injury
for urodynamic measurement

Pump the saline at a speed of 0.2ml/min for urodynamic measurement

'suria, but also provide objective materials for treatment and therapeutic effect. It is the most commonly used examination method ir

200-220g

n clinical diagnosis and treatment of lower urinary tract functional diseases

Dear Editors,

Thank you for giving us advices about our submission. We will resubmit a revised manuscript.

As stated in the article, we performed Kolmogorov-Smirnov test to test the normality of data and the results is attached below. After the confirmation, we performed student t test.

One-Sample Kolmogorov-Smirnov Test

[illegible]			Maximum Cystometric capacity	Detrusor opening pressure	Compliance of bladder	Maximum Cystometric capacity (second circle)	Detrusor opening pressure (second circle)
test	N		26	26	26	25	25
	Normal Parameters(a,b)	Mean	3.24338	20.27692	0.43231	3.404	20.04
		Std. Deviation	2.261	14.021875	0.577977	2.663406	10.537552
	Most Extreme Differences	Absolute	0.201	0.154	0.292	0.215	0.126
		Positive	0.201	0.154	0.292	0.215	0.088
		Negative	-0.121	-0.144	-0.247	-0.146	-0.126
	Kolmogorov-Smirnov Z		1.027	0.787	1.487	1.075	0.632
	Asymp. Sig. (2-tailed)		0.243	0.566	0.124	0.198	0.819
normal	N		10	10	10	10	10
		Mean	1.04	35	0.032	1.06	31

	Normal Parameters(a,b)	Std. Deviation	0.571936	13.258121	0.016344	0.48808	12.578642
	Most Extreme Differences	Absolute	0.197	0.21	0.197	0.228	0.194
		Positive	0.197	0.153	0.197	0.228	0.194
		Negative	-0.136	-0.21	-0.123	-0.116	-0.145
	Kolmogorov-Smirnov Z		0.622	0.666	0.622	0.722	0.614
	Asymp. Sig. (2-tailed)		0.834	0.767	0.834	0.674	0.845
a Test distribution is Normal.							
b Calculated from data.							

Best regards,
Sincerely.
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