Am J Clin Exp Urol 2019;7(3):170-177
www.ajceu.us /ISSN:2330-1910/AJCEU0097022

Original Article

Void sorcerer: an open source, open access framework for mouse uroflowmetry

Zunyi Wang[1], Emmanuel Contreras Guzman[4], Amit Nimunkar[4], Kimberly P Keil[2], Chad M Vezina[3], William A Ricke[5], Jill Macoska[6], Dale E Bjorling[1]

[1]Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA; [2]Department of Molecular Biosciences, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA; [3]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA; [4]Department of Biomedical Engineering, College of Engineering, University of Wisconsin-Madison, Madison, WI, USA; [5]Department of Urology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA; [6]Center for Personalized Cancer Therapy, University of Massachusetts-Boston, Boston, MA, USA

Received May 14, 2019; Accepted May 30, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Observational and experimental studies of rodent voiding behaviors have greatly contributed to our understanding of lower urinary tract function including the complex social, environmental, and internal stimuli that affect voiding in health and models of disease. Void spot assays (VSA), cystometry (awake or anesthetized), and uroflowmetry are techniques commonly used in rodent models to assess voiding. Uroflowmetry is non-invasive and can be performed multiple times in the same freely moving animals and can be used to generate synchronized video corresponding to each void to characterize micturition patterns (e.g., droplets versus solid stream). However, approaches to evaluate uroflowmetry in rodent models vary widely across laboratories. Most importantly, an open access software to run these tests is not freely available (although complete systems are commercially available), limiting use of this important assay. We developed the Void Sorcerer, an uroflowmetry system for mice for reliable determination of frequency, voided volume, voiding duration, interval times between micturitions, and flow rate. This report provides a detailed description of how to build this system and includes open access software for developing uroflowmetry capability in their laboratories and improve upon it in a cost-effective manner. Our goals are to improve access, increase reproducibility among laboratories, and facilitate standardizing testing procedures.

Keywords: Uroflowetry, micturition, mouse, Raspberry Pi 3, voiding, open access platform

Introduction

Uroflowmetry is widely used clinically in humans as a simple, inexpensive, and non-invasive method to measure voided volume and maximal (Qmax) and average (Qave) urinary flow rate throughout micturition. These parameters help to assess lower urinary tract function and can assist in diagnosis of common lower urinary tract disorders, predict response to treatment, and to determine whether more invasive tests are required [1]. A reduced flow rate is diagnostic for bladder outlet obstruction, reduced bladder contractility, or both [2]. The urinary flow rates Qmax and Qave may also decrease as patients age [3].

Mammals, particularly rodents, have proven essential for investigating cellular and molecular mechanisms underlying physiological and pathophysiological processes of lower urinary tract (LUT) function. Quantifying voiding events in awake, unrestrained animals provides information on a variety of factors and their interactions that regulate voiding in health and disease [4-6]. Rodents have anatomical and physiological similarities to humans and are cost-effective for investigating normal and abnormal LUT function [4, 5]. Rats have frequently been used in these studies, but mice are increasingly used as animal models for investigating LUT function, mainly due to availability of a variety of genetically modified mice that can provide valuable mechanistic insights in regulation of LUT function [4]. However, reliable characterization of LUT function in mice remains a technical challenge due to the small bladder size and low void volumes, as well as the somewhat tortuous course of the male mouse urethra. Void spot assays (VSA), cystometry and uroflowmetry are

techniques commonly used in rodent models to assess voiding. Catheter placement for performance of cystometry in males is by necessity invasive, and although catheters can be passed transurethrally into the bladders of females, the presence of the catheter within the urethra may create another confounding variable. VSA, while non-invasive, does not provide real-time recording of voiding events. Non-invasive experimental approaches, such as uroflowmetry [6], also referred to as metabolic cage measurement [7, 8], frequency volume measurement [9], or continuous urine weight recording [10], may evoke less physical and psychological stress than invasive testing in mice. However, approaches to evaluate uroflowmetry in rodent models vary widely across laboratories, and most importantly open access software to run these tests is not freely available. We redesigned a previously-described uroflowmetry system [6] to enhance the efficiency of data collection, increase throughput, and make this technology widely available to investigators in the field.

Our uroflowmetry system was developed as part of the Rodent Urinary Function Testing (RUFT) core of the University of Wisconsin-Madison/University of Massachusetts-Boston O'Brien Benign Urology Center. The purpose of this report (and the associated website) is to provide step-by-step fabrication instructions for a mouse uroflometry device to improve transparency in technology and enhance rigor as other laboratories engage in similar studies. We also provide access to open software to capture uroflowmetry data, and we provide validation data using a cohort of six male mice. We designed this system to be a simple and cost effective method for non-invasive evaluation of LUT function in conscious, unrestrained mice. Our system allows reliable determination of frequency, voided volume, voiding duration, interval times between micturitions, and flow rate from the uroflowmetry data. Viewing synchronized video corresponding to each void allows characterization of micturition patterns and has been previously described [6, 11]. Further, this software collects 30 seconds of video surrounding the voiding event, beginning 10 seconds prior to voiding and continuing for an additional 20 seconds. This allows discrimination between defecation and urination and also allows characterization of the voiding pattern (i.e., stream vs. droplets). Our center previously optimized VSA and provided an open source software for data analysis [12]. Here, we extend upon this work by encouraging more laboratories to perform uroflowmetry to address rigor, reproducibility, and transparency of this and other techniques used to evaluate lower urinary tract function in mice.

Our intention is that this will facilitate standardization of procedures to allow greater fidelity in comparison of data generated among laboratories. The software developed for this purpose and the detailed instructions for assembling the system with photographs are available at https://obrien.urology.wisc.edu/core-resources/uroflowmetry/. This report describes this system and its use to evaluate LUT function in male Senescence Accelerated Mouse Prone 6 (SAMP6) mice [13], demonstrating the capacity of this system to characterize micturition patterns and determine urodynamic parameters in an efficient and reliable manner.

Materials and methods

Animals

All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. Three month old male SAMP6 mice generated as part of a previous study [13] were used. A breeding colony was established using SAMP6 mice obtained from Harlan Laboratories, Indianapolis, IN (reference or link to Harlan Labs).

Uroflowmetry

A detailed description of parts, software, and assembly of the system can be found at https://obrien.urology.wisc.edu/core-resources/uroflowmetry/. The metabolic cages and balances were placed inside plexiglass enclosures to prevent fluctuations in recording caused by airflow in the room. Metabolic cages (Model 650-0322, Nalgene, Rochester, New York) with rat floor grids (10 × 33 mm openings) to minimize urine retention were used. A balance stage was placed under the metabolic cage to minimize the distance between the floor grid and balance while also preventing contact of the mouse tail with the waste collecting plate. A

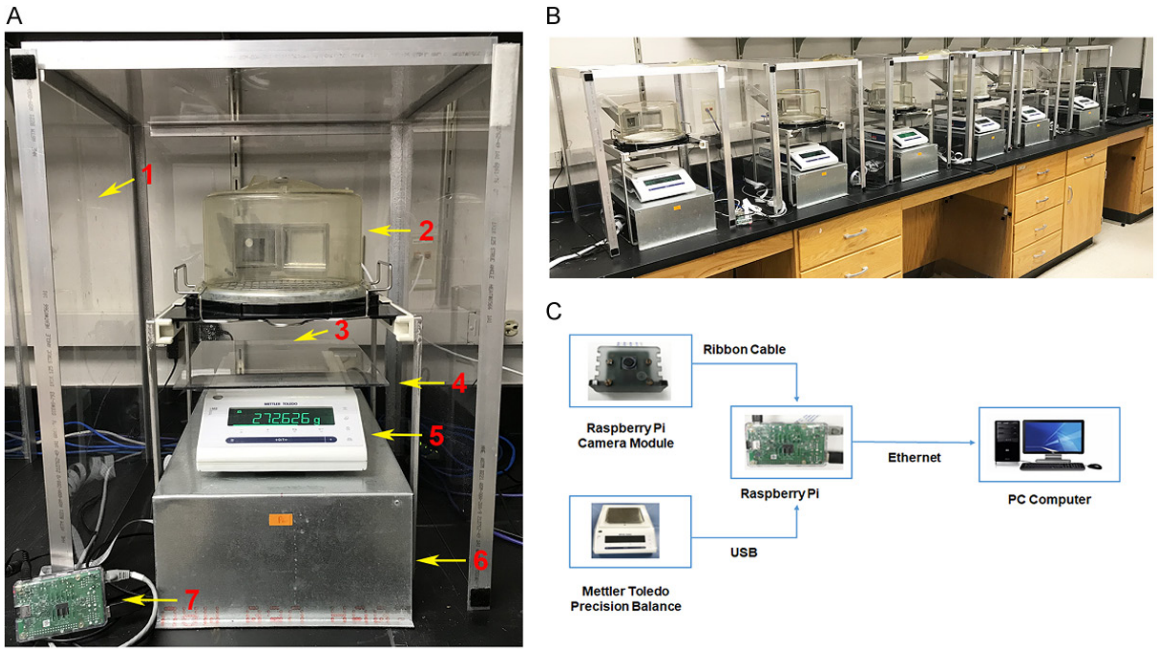

Figure 1. A: Illustration of an uroflowmetry setup. 1. Enclosure; 2. Metabolic cage; 3. Location of Raspberry Pi camera module (hidden by waste collection plate); 4. Waste collection plate; 5. Mettler Toledo precision balance; 6. Balance stage; 7. Raspberry Pi 3. B: Assembly of six uroflowmetry setups. C: Diagram of uroflowmetry components. A Raspberry Pi camera module and a Mettler Toledo precision balance were connected to Raspberry Pi processor using customized software. Data collected by Raspberry Pi processors are transmitted simultaneously to a desktop computer for downloading and subsequent analysis.

waste collecting plate was placed on an analytical balance (Mettler Toledo New Classic MF, model MS 303S) directly under the floor grid. Each monitoring and weight recording unit consisted of a Raspberry Pi 3 processor (RS Components Limited, Corby, UK), connected to a Raspberry Pi camera module V2 and a Mettler Toledo New Classic MF model MS 303S balance using customized software (**Figure 1C**). Each Raspberry Pi 3 processor was assigned a static IP address and connected to a desktop computer. Individual Raspberry Pi cameras were focused on the grid bottoms of metabolic cages to continuously monitor mouse activity by viewing the image while adjusting the camera mount. Communication between the desktop computer and Raspberry Pi 3 processors utilized PuTTY, a free network file transfer software. The video and data files were downloaded from the Raspberry Pi 3 processor to the desktop computer using FileZilla®, a free cross-platform File Transfer Protocol application. All video and weight records were time stamped. Weight on the collecting plate sensed by the balance was recorded continuously, sampled 10 times/second, and recorded data were stored as CSV file format (Excel®, Microsoft Corporation, USA). Since the specific gravity of urine is close to 1.0 g/ml, each g of weight increase was assumed to reflect 1.0 ml of voided urine. A synchronized 30 second video movie corresponding to each event detected by the balance was recorded including the 10 seconds prior to, and the 20 seconds after, each event. This minimized the time required for analysis of video images, allowed discrimination between defecation and micturition, captured a video of behavior during micturition, permitted characterization of voiding as a stream or expression of droplets, and identified those micturition events in which urine struck the bars of the cage bottom.

SAMP6 mice were tested twice, once after receiving regular water and again after receiving sweetened water for 48 hours prior to testing. The mice were offered sweetened drinking water prior to the second test to increase water intake as previously described [14]. Mice were acclimation to the testing room for 2 days, and then placed individually in metabolic cages and tested for 2 hours in the presence of regular

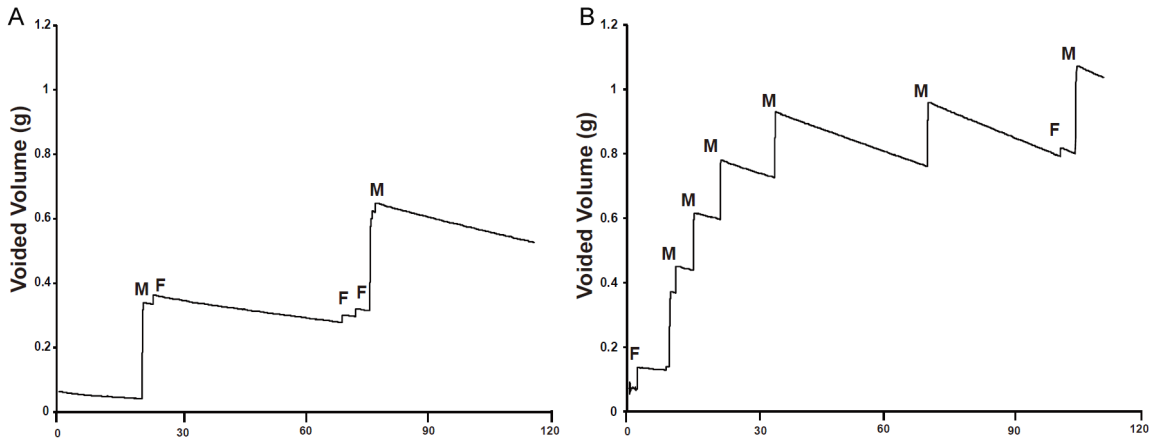

Figure 2. A graph of 2 hour recordings of weight change detected by the balance in a mouse receiving regular water (A) or sweetened water (B). Micturition frequency was significantly increased in the same mouse after receiving sweetened water (B). The time stamp is shown at the bottom of each graph and is exported from Xcel® file in which data are stored. M indicates micturition, and F denotes weight increase due to fecal pellet striking the waste collection plate.

drinking water but no food to prevent pellets or crumbs of food from striking the waste collecting plate. Two days after testing, the same mice were provided a sweetened water solution containing 3% glucose and 0.125% saccharin for 48 hours before repeating uroflowmetry testing as previously described, with the exception that sweetened water was provided during testing [11, 14]. Video recordings corresponding to each event were reviewed to verify whether the weight increase was caused by dropping of feces or urine onto the balance. The micturition pattern, defined as droplets or sustained voiding, was characterized as previously describe [15, 16]. Droplet voids are typically less than 3 seconds in duration and less than 0.100 g in detected weight. Sustained urination causes a rapid increase in detected weight that is typically no less than 100 mg. Voids passing cleanly between bars of the floor grid were used to determine voided weight, voiding duration, and urine flow rate. Urine flow rate was calculated based on weight of urine passed from initiation of the void to the point at which weight no longer increased. Data stored in Xcel® files was used to perform these calculations. Records of changes in weight are continuously time-stamped, and urine flow rate is calculated by dividing the weight of urine passed by the duration of the void. Micturition frequency, average voided volume, and interval time between micturitions were measured regardless of whether the urine fell between or on the bars of the floor grid [6].

Statistical analysis

Data were expressed as mean ± SEM. Student's t-test was used to assess differences. A p value ≤ 0.05 was considered to be significant.

Results

The software we developed effectively linked the camera and balance to the Raspberry Pi 3 computer. Data was then stored in a desktop computer in Xcel® files. A detailed description of the parts required, assembly, and operation of the Void Sorcerer system is available at https://obrien.urology.wisc.edu/core-resources/uroflowmetry/. The components required are readily obtainable, and the protocol for using this system is provided in detail.

Daily urine output varies with the strain, size, age, and gender of mice with a reported range of less than 2 ml/day to over 10 ml/day and a voiding frequency of 10 or more times/day [17, 18]. When male SAMP6 mice were tested after receiving regular drinking water, only 1 to 3 voids were observed from each animal during a 2 hour period (2 ± 0.4, n = 6, **Figure 2A**) and interval time between micturitions was 2635.6 ± 722.3 s, n = 4. The average voided volume was 0.27 ± 0.03 g. Due to the limited number of micturitions, clean urinations were not consistently observed among these mice. Providing the same mice sweetened water for 48 hours prior to and during testing significantly increased micturition frequency to 10 ± 1 (n = 6,

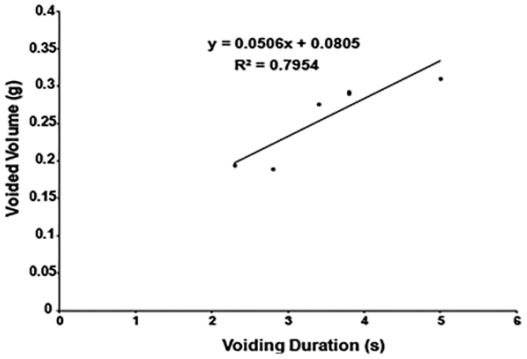

Figure 3. Linear regression analysis revealed a positive relationship between voided volume and voiding duration in mice receiving sweetened water. n = 6.

$P < 0.001$ compared to mice that received regular water, **Figure 2B**) during a 2 hour test period. The average voided volume (0.25 ± 0.02 g; n = 6, $P > 0.05$, **Figure 2**) was no different than that observed in mice that received regular water. The interval time between micturitions was 770.5 ± 83 s (n = 6, $P < 0.05$ compared to mice that received regular water). Increased micturition frequency also allowed observation of at least 3 clean urinations from each animal that were used to measure void duration and flow rate. The frequency of defecation and weight of fecal pellets varied among animals (**Figure 2**). Fecal pellets ranged in weight from 0.03 to 0.1 g. Linear regression analysis revealed that voided volume positively correlated with void duration ($R^2 = 0.795$, **Figure 3**). Average flow rate was 0.075 ± 0.004 g/s, n = 6, which was remarkably consistent among animals. Droplets, stream and mixed micturition patterns were all observed.

A video file illustrating a recorded void in which a drop of urine clings to a bar of the floor grid is included as Supplementary Video.

Discussion

We provide software and necessary instructions for building the Void Sorcerer uroflowmetry system. Detailed information on this, as well as data acquisition in mice and data analysis is available at (available at https://obrien.urology.wisc.edu/core-resources/uroflowmetry/).

Using this system, we determined several parameters associated with micturition and characterize micturition patterns in male SAMP6 mice. Uroflowmetry has been used to evaluate LUT function in various animal models [4, 9, 10, 19]. Using a system similar to the one described in this report, Nicholson et al. [15, 16] examined mice treated with testosterone and estradiol and demonstrated that evaluation of video recordings of voiding could be used to discriminate between dibbling voids in the treated mice from passage of a stream of urine in control untreated animals [15, 16]. While this cannot discriminate between impaired bladder function and urethral narrowing, characterization of the void pattern provides additional information regarding LUT function that cannot be obtained from other methods such as spontaneous void spot assay or anesthetized cystometry. Further, observation of voids provides information regarding whether or not the urine struck on the mesh flooring or passed unimpeded to the underneath plexiglass plate.

The pilot study was performed to determine whether sufficient voids were produced for analysis after normal water intake or whether increased water intake induced by offering sweetened water was required to generate enough voiding events for consistent assessment. We used 3 month old male SAMP6 mice, because preliminary studies in our laboratory suggested that these mice urinate more frequently than other strains (unreported data). Results confirm that data generated were consistent among mice. As previously discussed, these mice produced relatively few voids over the two hour study period when provided with regular water. While providing sweetened water increased urine output in this study, artificially increasing fluid intake prior to testing can significantly affect results, particularly in mice with diabetes insipidus, diabetes mellitus or other endocrinopathies. We are testing protocols that entail longer study periods, as well as investigating options for discriminating between voids and defecation in a dark environment, to address these issues.

The void spot assay (void spotting assay; voiding spot on paper assay) is another non-invasive test that has been used to assess lower urinary tract function in mice [12, 20, 21]. This test entails allowing individual unrestrained mice to urinate on filter paper for a period of time. The number and size of urine spots is then analyzed to assess void frequency and volume. As part of the O'Brien Center, our labora-

tories have described the Void Whizzard, a freely available software plugin for Fiji, an image processing package developed as a modification of ImageJ, that allows automated evaluation of urine spots on filter paper generated during void spot assays [12]. This program has significantly decreased time required for analysis of results of void spot assays. It is interesting to note that within our report of the application of this program, voiding during void spot assay occurred with much greater frequency than we observed with the Void Sorcerer system. We also observed that placing mice on mesh positioned above the filter paper significantly reduced the number of voiding events compared to mice placed directly on the filter paper [12]. This suggests that the presence of mesh flooring may reduce the frequency of voiding in mice. Both void spot assay and uroflowmetry are performed in the absence of significant environmental enrichment, and it remains unclear whether or not extended acclimation to housing on a mesh surface would result in an increase in voiding frequency beyond that observed in these studies, and we are investigating the effects of varying duration of acclimation on results.

Duration of uroflowmetry testing has ranged from two to 24 hours, and measurement of voiding typically relies on measuring output by weight recorded from a balance placed beneath a metabolic cage [7, 19, 22, 23]. Due to the small void volume produced by mice, it is essential to prevent elimination of feces from confounding recording urine output. Several methods have been developed to deal with this problem [8, 10, 14]. Typically, a funnel-like system made of polymers is used to separate feces from urine [10]. Despite the use of compounds to decrease adherence of urine to the surfaces that direct urine to the collection receptacle, some urine is retained within the collection system [10]. Other investigators have used screens to retain feces while allowing urine to fall onto the balance [8, 14]. However, urine can adhere to screens, as well as the mesh bottom of cages, decreasing registration of void frequency and volume [10]. Sidler et al. [10] applied a computer algorithm for distinguishing weight increase caused by urine from feces based on the relatively rapid decline in weight induced by urine striking the balance as a result of subsequent evaporation, while increased weight associated with feces remained stable. This approach has the advantage of allowing data collection during the dark cycle of the day. However, we have often observed a delay in decrease in weight subsequent to voiding urine, perhaps due to the plexiglass enclosures placed around the units. Urine can also be absorbed by fecal pellets, resulting in delayed evaporation. Leung et al. [6] used video cameras to monitor activity of mice, as well as excretion of urine and feces. When a weight increase was sensed by the balance, a synchronized video recording was generated. They identified micturition events by viewing the video, similar to the system described in this report. Unfortunately, the system used by these investigators is not generally available, and this is the primary impetus for this report.

There are multiple opportunities to further refine and expand the utility of the Void Sorcerer system. Mice are nocturnal animals, and a majority of urinations occurs in the dark cycle [14, 18]. The Raspberry Pi camera module V2 is not cable of capturing micturitions in the dark. Therefore, testing must be done in a lighted room. In addition, viewing videos for characterizing micturition patterns and identifying clean voidings for subsequent analysis can be labor intensive. However, with this system, the need to only review a 30 second video movie triggered by a change in weight detected by the balance in combination with a graph of the change in mass over time generated from the data generated by the software in the Excel file, greatly streamline analysis and saved time. We are continuing to explore options to expand the use of this system to capture void events in the dark cycle, when mice are most active, including the use of infrared sensitive cameras, as well as automating image analysis using machine-learning technology.

Conclusion

In conclusion, our findings confirm that uroflowmetry can be useful for assessment of LUT function in mice. LUT function is assessed non-invasively in conscious, unstrained mice. Additionally, it permits investigating physiological and pathophysiological processes involved in aging or disease progression longitudinally in the same animals. More importantly, we have generated an open-access platform for researchers to access and to develop uroflowmetry systems in their own laboratories in a

cost effective manner. This will allow for ease of access, reproducibility across different laboratories and aide in standardizing testing procedures. Increased utilization of this platform will expand its utility and application to improve analysis of LUT function in mice.

Acknowledgements

Supported by NIH grants U54 DK104310 (DEB, CMV, WAR) and K99 ES029537 (KPK). We also want to express our sincere appreciation to Ronald W. Wood for invaluable support in development of this system and Jason Brenner for IT support.

Disclosure of conflict of interest

None.

Address correspondence to: Dale E Bjorling, Department of Surgical Sciences, DVM, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, WI 53706, USA. Tel: 608-263-4808; E-mail: dale.bjorling@wisc.edu

References

[1] Jarvis TR, Chan L and Tse V. Practical uroflowmetry. BJU Int 2012; 110 Suppl 4: 28-29.

[2] Lenherr SM and Clemens JQ. Urodynamics: with a focus on appropriate indications. Urol Clin North Am 2013; 40: 545-557.

[3] Haylen BT, Parys BT, Anyaegbunam WI, Ashby D and West CR. Urine flow rates in male and female urodynamic patients compared with the Liverpool nomograms. Br J Urol 1990; 65: 483-487.

[4] Andersson M, Aronsson P, Giglio D, Wilhelmson A, Jerabek P and Tobin G. Pharmacological modulation of the micturition pattern in normal and cyclophosphamide pre-treated conscious rats. Auton Neurosci 2011; 159: 77-83.

[5] Ito H, Pickering AE, Igawa Y, Kanai AJ, Fry CH and Drake MJ. Muro-neuro-urodynamics; a review of the functional assessment of mouse lower urinary tract function. Front Physiol 2017; 8: 49.

[6] Leung YY, Schwarz EM, Silvers CR, Messing EM and Wood RW. Uroflow in murine urethritis. Urology 2004; 64: 378-382.

[7] Schneider MP, Sartori AM, Tampe J, Moors S, Engmann AK, Ineichen BV, Hofer AS, Schwab ME and Kessler TM. Urodynamic measurements reflect physiological bladder function in rats. Neurourol Urodyn 2018; 37: 1266-1271.

[8] Yoshiyama M, Mochizuki T, Nakagomi H, Miyamoto T, Kira S, Mizumachi R, Sokabe T, Takayama Y, Tominaga M and Takeda M. Functional roles of TRPV1 and TRPV4 in control of lower urinary tract activity: dual analysis of behavior and reflex during the micturition cycle. Am J Physiol Renal Physiol 2015; 308: F1128-1134.

[9] Kamei J, Ito H, Aizawa N, Hotta H, Kojima T, Fujita Y, Ito M, Homma Y and Igawa Y. Age-related changes in function and gene expression of the male and female mouse bladder. Sci Rep 2018; 8: 2089.

[10] Sidler M, Aitken KJ, Forward S, Vitkin A and Bagli DJ. Non-invasive voiding assessment in conscious mice. Bladder 2018; 5: e33.

[11] Wood RW, Baggs RB, Schwarz EM and Messing EM. Initial observations of reduced uroflow in transgenic adenocarcinoma of murine prostate. Urology 2006; 67: 1324-1328.

[12] Wegner KA, Abler LL, Oakes SR, Mehta GS, Ritter KE, Hill WG, Zwaans BM, Lamb LE, Wang Z, Bjorling DE, Ricke WA, Macoska J, Marker PC, Southard-Smith EM, Eliceiri KW and Vezina CM. Void spot assay procedural optimization and software for rapid and objective quantification of rodent voiding function, including overlapping urine spots. Am J Physiol Renal Physiol 2018; 315: F1067-F1080.

[13] Macoska JA, Wang Z, Virta J, Zacharias N and Bjorling DE. Inhibition of the CXCL12/CXCR4 axis prevents peri-urethral collagen accumulation and lower urinary tract dysfunction in vivo. Prostate 2019; 79: 757-767.

[14] Wood R, Eichel L, Messing EM and Schwarz E. Automated noninvasive measurement of cyclophosphamide-induced changes in murine voiding frequency and volume. J Urol 2001; 165: 653-659.

[15] Nicholson TM, Ricke EA, Marker PC, Miano JM, Mayer RD, Timms BG, vom Saal FS, Wood RW and Ricke WA. Testosterone and 17beta-estradiol induce glandular prostatic growth, bladder outlet obstruction, and voiding dysfunction in male mice. Endocrinology 2012; 153: 5556-5565.

[16] Nicholson TM, Moses MA, Uchtmann KS, Keil KP, Bjorling DE, Vezina CM, Wood RW and Ricke WA. Estrogen receptor-alpha is a key mediator and therapeutic target for bladder complications of benign prostatic hyperplasia. J Urol 2015; 193: 722-729.

[17] Drickamer LC. Odors in traps: does most recent occupant influence capture rates for house mice? J Chem Ecol 1995; 21: 541-555.

[18] Aizawa N, Homma Y and Igawa Y. Influence of high fat diet feeding for 20 weeks on lower urinary tract function in mice. Low Urin Tract Symptoms 2013; 5: 101-108.

[19] Giglio D, Wasen C, Molne J, Suchy D, Swanpalmer J, Jabonero Valbuena J, Tobin G

and Ny L. Downregulation of toll-like receptor 4 and IL-6 following irradiation of the rat urinary bladder. Clin Exp Pharmacol Physiol 2016; 43: 698-705.

[20] Bjorling DE, Wang Z, Vezina CM, Ricke WA, Keil KP, Yu W, Guo L, Zeidel ML and Hill WG. Evaluation of voiding assays in mice: impact of genetic strains and sex. Am J Physiol Renal Physiol 2015; 308: F1369-1378.

[21] Hill WG, Zeidel ML, Bjorling DE and Vezina CM. Void spot assay: recommendations on the use of a simple micturition assay for mice. Am J Physiol Renal Physiol 2018; 315: F1422-F1429.

[22] Hubscher CH, Montgomery LR, Fell JD, Armstrong JE, Poudyal P, Herrity AN and Harkema SJ. Effects of exercise training on urinary tract function after spinal cord injury. Am J Physiol Renal Physiol 2016; 310: F1258-1268.

[23] Sugiyama R, Aizawa N, Ito H, Fujimura T, Suzuki M, Nakagawa T, Fukuhara H, Kume H, Homma Y and Igawa Y. Synergic suppressive effect of silodosin and imidafenacin on non-voiding bladder contractions in male rats with subacute bladder outlet obstruction. Low Urin Tract Symptoms 2017; 9: 94-101.