J Exp Clin Med 2012;4(3):157–164

Contents lists available at SciVerse ScienceDirect

Journal of Experimental and Clinical Medicine

journal homepage: http://www.jecm-online.com

ORIGINAL ARTICLE

Sex Differences in the External Urethral Sphincter Activity of Rats

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ARTICLE INFO

Article history:
Received: Dec 8, 2011
Accepted: Mar 30, 2012

KEY WORDS:
electromyography;
external urethral sphincter;
intravesical pressure;
myosin heavy chain

Purpose: The external urethral sphincter (EUS) is regarded as an important structure involved in urinary continence and micturition, so a rodent animal model was employed to investigate these urinary functions. However, few studies have compared the physiological and histochemical characteristics of the EUS between male and female rats in detail. The aims of this study were to extensively examine the properties of the EUS in rats of both sexes via cystometric electromyography (EMG) and histochemical measurements.

Methods: EUS-EMG and intravesical pressure were simultaneously recorded using continuous cystometric monitoring in order to provide a quantitative evaluation of EUS activity and voiding function. A histochemical examination of the striated EUS muscle was also performed using immunolabeling techniques to study the myosin heavy chain isoforms.

Results: Cystometric measurements, including the bladder volume threshold, contraction amplitude, contraction duration, contraction area, intercontraction interval, and voided volume, were significantly larger in male rats in comparison with female rats. In addition, a longer EUS burst period, silent period, and total silent period and a larger number of silent periods, a higher frequency of burst discharges, and a shorter active period were found in male rats. Only type II fibers (100%) were seen in the striated urethral muscle of the upper segment of the urethra in male rats, whereas in females both type I and II striated muscle fibers were present in all segments of the urethra with proportions ranging from of 6–14% for type I and 86–94% for type II fibers.

Conclusion: This study investigated both the physiological and histochemical differences in the properties of the EUS that underlie sexually dimorphic EUS burst activities in rats. The present study provides useful information for future urological research.

1. Introduction

The lower urinary tract has two main functions: storage and periodic elimination of urine. These functions are regulated by a complex neural control system located in the brain and spinal cord that coordinates the activities of the two components of the lower urinary tract: the reservoir (urinary bladder) and the outlet (bladder neck, urethra, and urethral sphincter). Normally, these structures exhibit reciprocal activities. During urine storage, the reservoir is quiescent and the intravesical pressure (IVP) remains low, whereas activity in the outlet gradually increases during bladder filling in order to maintain continence. These organs are regulated by three sets of peripheral nerves: the sacral parasympathetic (pelvic nerves) and thoracolumbar sympathetic nerves, which innervate the bladder and proximal urethra, and that sacral somatic nerves, which innervate the external urethral sphincter (EUS).[1]

The rat has gained great popularity as the main species for use in animal models that investigate urine storage and micturition functions in various pharmacological experiments. However, several anatomical and functional differences in the lower urinary tract are different between male and female rats. Anatomically, some studies indicate that female rats generally have a shorter urethra and thinner EUS overlaying the urethra in comparison with male rats.[2–4] Other studies have reported that the bulbospongiosus (BS), ischiocavernosus (IC), and cremaster muscles are well developed in males but are vestigial or absent in adult female rats.[5,6] Bladder functions also demonstrate sexual differences in terms of the voiding patterns in rodents.[7] Furthermore, Kontani and Shiraoya indicate that the urethral activities in rats in response to pudendal electrical stimulation exhibit sexual differences that

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1878-3317/$ – see front matter
doi:10.1016/j.jecm.2012.04.006

result from the different neurotransmitters that control urethral smooth muscle activities.[8]

Although several studies demonstrate the importance of EUS burst activity in allowing efficient voiding in the rat,[7,9] few studies have compared the properties of EUS activity in detail between male and female rats. In our recent studies, we developed a novel experimental design in order to analyze EUS burst discharges in urethane-anesthetized rats after nerve damage.[10,11] In the present study, we continue to use this method to determine whether different sexes of the rat have distinct patterns of EUS activity during the micturition reflex. Both EUS electromyography (EMG) and IVP were measured during continuous cystometry in order to provide a quantitative evaluation of the interactions between EUS activity and urodynamics during micturition. In addition, striated muscle fibers in the urethra are classified into two major types—type I and II fibers—and different types of striated muscle most likely exhibit distinct characteristics in terms of muscle activity and function. To the best of our knowledge, no previous study has compared the striated fiber types that compose the urethra of male and female rats. Therefore, in order to obtain insights into sexual differences in the urethral contractile activity of the rat, histochemical examinations of the striated muscle in the urethra were also carried out in this study.

2. Materials and methods

2.1. General preparation

Eighteen male (276–300 g) and 18 age-matched female Sprague Dawley rats (201–225 g) were used in this study. All rats were anesthetized with urethane (1.2 g/kg, s.c.). The body temperature was maintained at 36–38°C with the use a heating lamp. In addition, the femoral vein was catheterized for fluid and drug administration. All experimental procedures were approved by the institutional animal care and use committee of Taipei Medical University.

The urinary bladder was exposed via a midline abdominal incision. Two insulated silver wire electrodes (0.05 mm in diameter) with exposed tips were inserted into the lateral sides of the midurethra, where the muscle fibers of the EUS were identified (Figure 1). The recorded EUS-EMG was similar to that recorded using electrodes implanted into the exposed EUS via an incision to the pubic symphysis and was completely blocked after administering the neuromuscular blocker, pancuronium bromide (1.0–1.5 mg/kg i.v. pancuronium; Organon, Istanbul, Turkey), which confirmed that the EMG activity originated from the striated sphincter muscles. A polyethylene (PE) tube (PE 60; 1.0 mm ID and 1.5 mm OD) was then inserted into the bladder lumen in order to obtain bladder pressure measurements. The bladder end of the PE tube was heated to form a collar and then passed through a small incision at the apex of the bladder dome. After the collar of the tube was tightened, the abdominal wall was closed using nylon sutures. The PE tube was in turn connected via a 3-way stopcock to an infusion pump so that it could be filled with physiological saline and to a pressure transducer that was used to monitor the bladder pressure (Figure 1). The rats underwent urodynamic and EUS-EMG examinations that usually began 3–4 hours after the induction of anesthesia. The bladder pressure and EUS-EMG were first amplified and sampled at 12-bit resolution using a biological signal acquisition system (Biopac MP 150; BIOPAC Systems, CA, USA).

2.2. Physiological investigations

The rats underwent urodynamic and EUS-EMG examinations that usually began 3–4 hours after the induction of anesthesia. After manually emptying the bladder, transvesical cystometry was performed at an infusion rate of 0.12 mL/minute with physiological saline at room temperature.[12] The urethra was then opened to allow the elimination of fluid during micturition. The infusion pump was turned off after two or three voiding contractions.

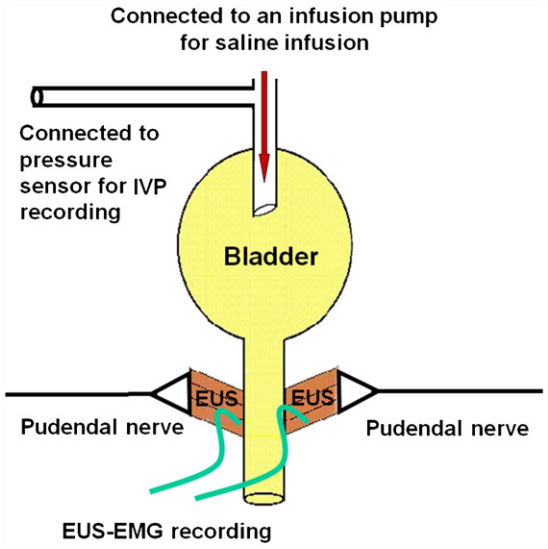

Figure 1 Schematic diagram of the experimental setup used to record bladder and external urethral sphincter (EUS)-EMG activities in rats. Two wire electrodes were inserted into the lateral sides of the midurethra to obtain EUS-EMG recordings. A polyethylene tube was inserted into the bladder lumen, which was in turn connected via a 3-way stopcock to an infusion pump (for filling with physiological saline) and a pressure transducer for monitoring the intravesical pressure (IVP).

Various cystometric parameters were measured: (1) the micturition volume threshold (VT), which is the volume of saline sufficient to induce the first voiding contraction; (2) the contraction amplitude (CA), which is the maximal pressure during voiding; and (3) the bladder contraction duration (CD) during voiding, as shown in Figure 2. Additional urodynamic parameters were also obtained: residual volume (RV), voided volume (VV), and voiding efficiency (VE). The RV is the volume of saline withdrawn through the intravesical catheter after micturition. The collection of fluid was facilitated by manually pressing the abdominal wall. VV is represented as VT minus RV, and the VV:VT ratio was used to compute VE.

Analysis of EMG activity was blinded to the status of the rats. Various EUS-EMG parameters were measured: (1) the burst period (BP), which was defined as the duration of the burst discharges; (2) the silent period (SP), which was defined as the duration of quiescence between two clusters of high-frequency spikes; and (3) the active period (AP), which was defined as the duration of the high-frequency EMG spikes separated by the periods of quiescence (Figure 3). The number of SPs in each bladder contraction was also calculated, and the SP:BP ratio represents the frequency of the burst discharges. The average urethral flow rate is presented as the ratio between VV and the total silent period (TSP) (i.e., VV:TSP). All data obtained from three micturition contractions were averaged for each animal with the aid of Acqknowledge software (BIOPAC Systems).

2.3. Histochemical examination

Several male and female rats ($n = 6$ for each gender) were used in histochemical examination of the striated muscle of the urethra. The animals were sacrificed by an overdose of urethane (4 g/kg, s.c.). The urethra was removed and immediately cut into three equal transverse segments: the upper, middle, and lower segments.

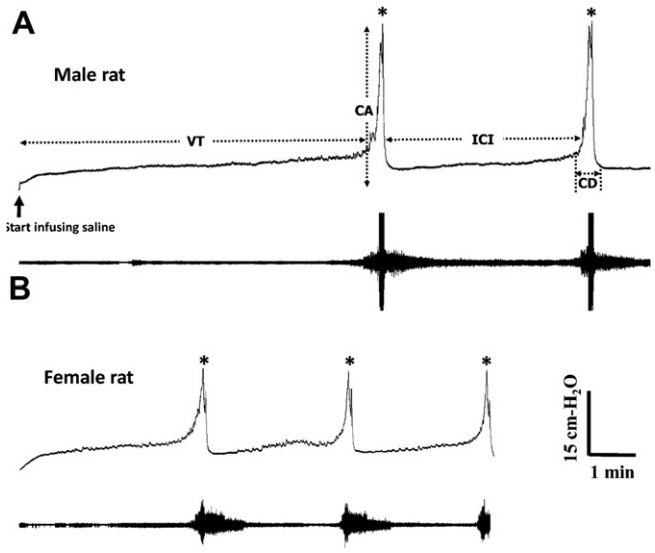

Figure 2 Typical pattern of intravesical pressure (IVP, top trace) and external urethral sphincter (EUS)-EMG activity (bottom trace) recorded during cystometric measurements of continuous transvesical infusion in anesthetized (A) male and (B) female rats. The asterisks indicate voiding. The cystometric parameters include the micturition volume threshold (VT), contraction amplitude (CA), intercontraction interval (ICI), and contraction duration (CD).

No fixation or pretreatment was performed before the immunostaining procedure. All urethral segments were embedded in OCT compound and snap-frozen in liquid nitrogen. The rostral site of each segment of the urethra was cut into 5-mm-thick sections using a cryostat at -20°C, then placed onto gelatinized slides for myosin heavy chain (MHC) isoform immunolabeling. Labeling of the MHC isoforms was performed using the monoclonal antibodies of the slow and fast MHC isoforms (Novocastra, Newcastle upon Tyne, UK). The antibody dilutions used were 1:20 for the slow and 1:10 for fast isoforms.[13] Negative-control experiments were also performed, in which the primary antibody incubation step was omitted. Fibers were typed according to the expression of the MHC isoform they displayed. Digital images of each section were captured, and Metamorph Image Processing Software (Molecular Devices, Downington, PA) was used to digitally measure the cross-sectional area of each fiber type in these images. Measurements of the upper, middle, and lower segments of the urethra were obtained from four sections of each urethral segment that were separated by approximately 200 μm in each animal.

2.4. Statistical analysis

All of the parameters obtained from the cystometric, EUS-EMG, and histochemical examinations are presented as the mean

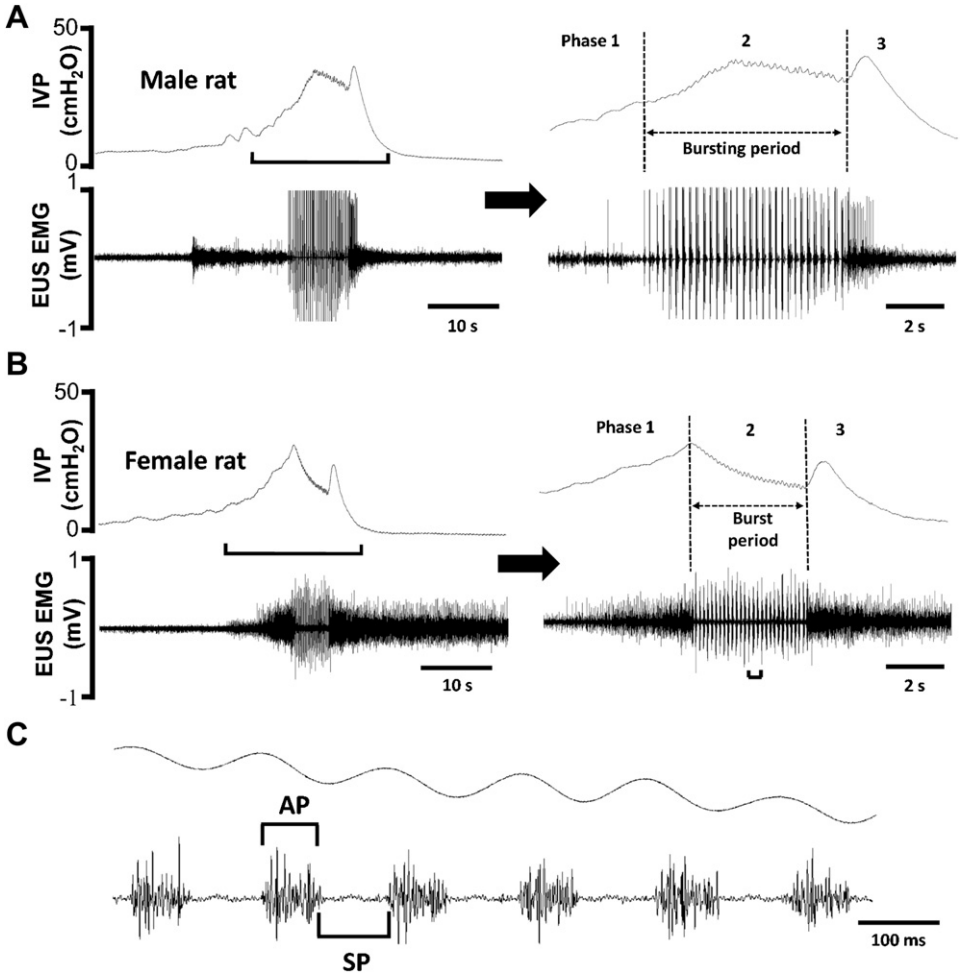

Figure 3 Patterns of intravesical pressure (IVP, top trace) and external urethral sphincter (EUS)-EMG activity (bottom trace) during one voiding event in anesthetized (A) male and (B) female rats. The traces on the right are expansions of the section of the traces on the left in brackets. The burst period (BP) can be clearly observed in both male and female rats in the expanded view of the voiding event. (C) Individual bursts are composed of active periods (APs) and silent periods (SPs), which are shown in the expanded BP portion (bracket) in (B). Oscillations in bladder pressures correlated with EUS-EMG bursts.

value ± standard deviation (SD). Those parameters were statistically compared using Student's t test, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Typical bladder activity patterns and EUS-EMG cystometric measurements

The typical IVP and EUS-EMG measurements obtained in the male and female rats are depicted in Figure 2. Rats of both sexes exhibited similar micturition contraction patterns during the continuous intravesical infusion of saline. EUS-EMG exhibited low-amplitude tonic activity during the initial filling phase of cystometry and between micturition contractions, but this activity markedly increased in amplitude during bladder contractions. According to Maggi et al,[14] a single micturition contraction of the bladder can be divided into three phases of IVP: phase 1, rising phase; phase 2, high-frequency oscillation (HFO) phase; and phase 3, rebound and falling phase, as shown in Figure 3A and 3B. During micturition contractions, the EUS-EMG clearly showed a long BP, which lasted 4–6 seconds and was accompanied by IVP and superimposed with a series of HFOs (Figure 3c). Burst discharges in the BP were characterized by clusters of high-frequency spikes (AP) that were separated by periods of quiescence (SP), as shown in Figure 3C.

3.2. Measurements of the bladder activity parameters

Although the basic IVP pattern was similar in both male and female rats, there were marked quantized differences. Figure 4 summarizes all of the cystometric parameters obtained from the male and female rats. During cystometry, the average volume threshold for stimulating voiding in male rats was 2-fold higher than that measured in female rats (Figures 2 and 4A). The larger volume threshold appeared to increase the workload of the bladder activity when expelling urine; therefore, the average bladder contraction amplitude, duration, and area during voiding in male rats were significantly larger than those measured in female rats (Figure 4B–D). Similarly, because there was no significant difference in the residual volume or voiding efficiency between sexes (Figure 4G and H), male rats exhibited a larger intercontraction interval and voided volume than female rats (Figure 4E and F).

3.3. Measurements of EUS-EMG parameters

EUS-EMG measurements of the male and female rats are shown in Table 1. The average duration of BP in male rats (5.51 ± 0.94 seconds) was much longer than that measured in female rats (4.04 ± 0.40 seconds; $p < 0.05$). In addition, the silent period in males (108.0 ± 5.5 ms) was much longer than that measured in female rats (99.5 ± 3.2 ms), but the active period in male rats (38.7 ± 3.4 ms) was much shorter than that measured in female rats (65.7 ± 2.5 ms; Figure 5). Note that the average frequency of the burst discharge, i.e., the ratio of the number of silent periods to BP, in male rats (6.84 ± 0.35 Hz) was significantly higher than that measured in female rats (6.02 ± 0.13 Hz). The average urethral flow rate (VV:TSP) in male rats was significantly higher than that measured in female rats.

3.4. Histochemical examination

The proportions of the different types of EUS striated muscle fibers were further determined in male and female rats. Figure 6 shows an example of cross-sectional microphotographs of MHC isoform immunolabeling in the upper segment of a female rat. The results reveal that the two types of striated muscle fibers—slow (type I fiber) and fast MHC (type II fiber)—are both positively stained in the female urethral striated sphincter. A summary of the proportions of these fiber types in the upper, middle, and lower urethra in male and female rats is shown in Table 2. In female rats, both type I and II striated muscle fibers existed in all segments of the urethra, whereas type II striated fibers were the predominant fiber type across those segments of the urethra, with 86–94% all of the positively stained striated fibers being identified as type II (Table 2). In contrast, striated muscle in male rat was only positively stained in the upper segment of the urethra, and 100% of fiber types in this segment were type II. No type I or II striated muscle fibers were detected in the middle or lower segments of the urethra in male rats.

4. Discussion

The present study reveals that although the basic IVP and EUS-EMG patterns were similar between both sexes, there were marked quantized differences. The differences that were detected in male rats, in comparison with female rats, included: (1) a larger average volume threshold, bladder contraction amplitude, contraction duration, contraction area, intercontraction interval, and voided volume on cystometric measurements; and (2) a longer BP, silent period, and total silent period, larger number of silent periods, higher frequency of burst discharges, and a shorter active period. On the histochemical examination, the fiber type composition of male striated urethral muscle was only expressed in the upper segment of the urethra in 100% of type II fibers, whereas both type I and II fibers were present in all segments of the female urethra with proportions ranging between 6–14% for type I and 86–94% for type II fibers.

In this study, there were no significant differences in terms of the residual volume or voiding efficiency between the male and female rats (Figure 4G and H), but the average volume threshold for stimulating voiding in male rats was 2-fold higher than that of female rats (Figure 4A). One question that emerges from these results is: What is the mechanism that accelerates the large amount of saline that was evacuated from the bladder of male rats? Two mechanisms could account for this phenomenon. First, previous electrophysiological studies have shown that EUS-EMG in rats exhibits a 4–8-Hz burst pattern during voiding.[14,15] This EUS-EMG burst and, in particular, the duration of the silent period, which represent the relaxation and opening of the outlet, respectively, are essential for achieving efficient voiding.[16] This assumption is also demonstrated by our results, namely that the male rats exhibited a higher frequency of burst discharges than female rats and the total silent period in male rates was 1.68-fold longer than that measured in female rats (Table 1). Thus, the duration of the silent period (i.e., urethral relaxation) is an important factor that contributes to efficient bladder voiding. Second, the contractile ability of the detrusor muscle is another factor that influences voiding function. In this study, the bladder contraction amplitude and duration in male rats were 1.72-fold and 1.5-fold higher those measured in female rats, respectively. This result implies that the bladder contractile ability of male rats is better than that of female rats.

The voiding efficiency in both sexes of anesthetized rats was only 70–75%, which is lower than the 98–99% voiding efficiency measured in conscious animals.[17,18] This limitation presumably resulted from the aesthesia, which inhibited bladder emptying in all rats. Similar low voiding efficiencies have been reported in other studies.[7,10,16] The physiological functions of the bladder and EUS appear to be preserved under urethane, and urethane is "the most

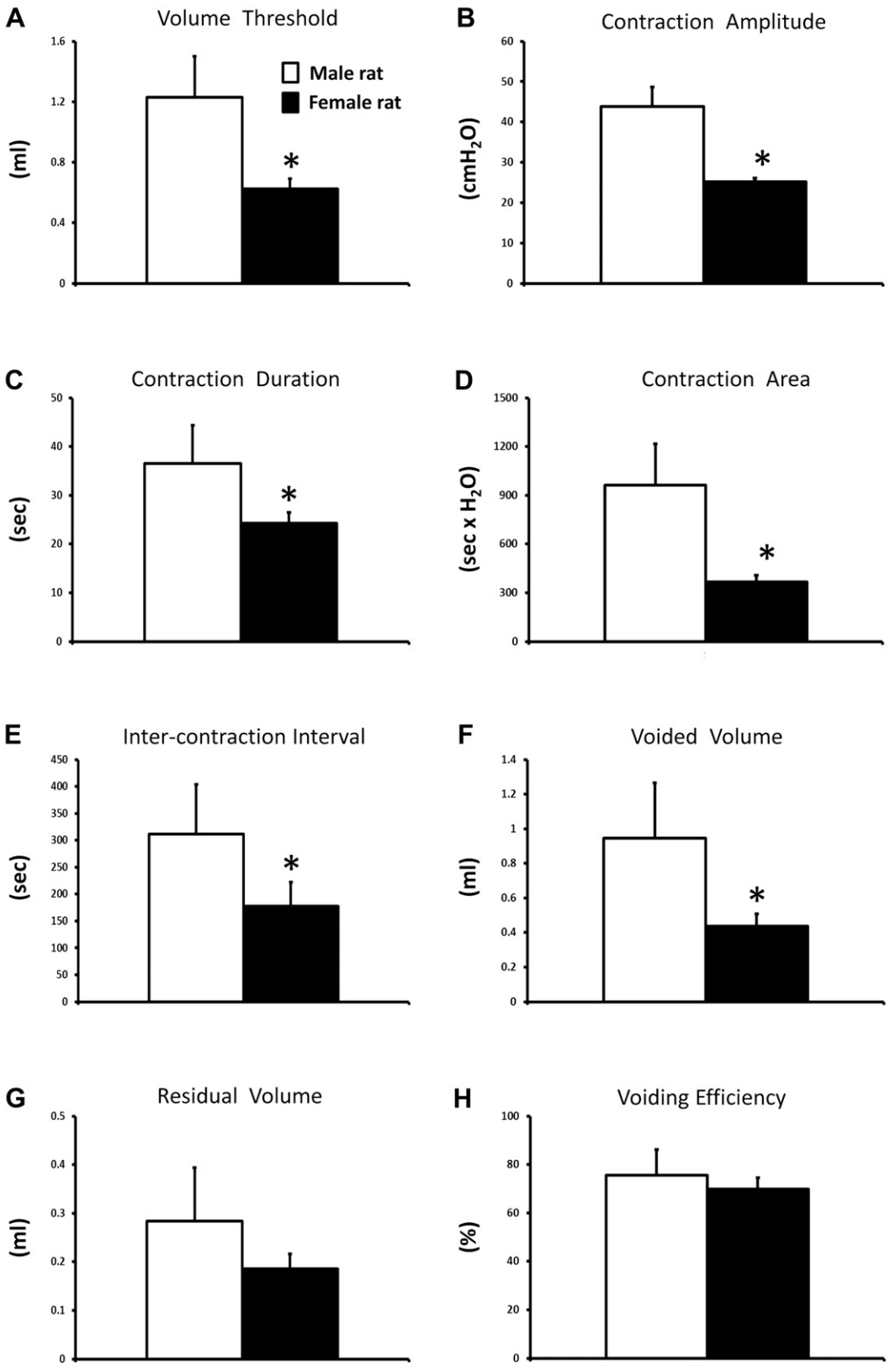

Figure 4 Summary of the parameters related to bladder activity including (A) the volume threshold, (B) maximal bladder contraction amplitude, (C) bladder contraction duration, (D) bladder contraction area, (E) intercontraction interval, (F) voided volume, (G) residual volume, and (H) voiding efficiency in male and female rats ($n = 12$ for each group). An asterisk (*) indicates a significant difference ($p < 0.05$) between male and female rats.

Table 1 Parameters of external urethral sphincter (EUS)-EMG activity in male and female rats

	Males	Females
Burst period (s)	5.51 ± 0.94	4.04 ± 0.40*
Silent period (s)	108.0 ± 5.5	99.5 ± 3.21*
Active period (s)	38.7 ± 3.4	66.7 ± 2.45*
Number of silent periods (no.)	37.6 ± 6.7	24.33 ± 2.3*
Frequency of burst discharge (Hz)	6.84 ± 0.35	6.02 ± 0.13*
Total silent period (s)	4.06 ± 0.74	2.42 ± 0.26*
Flow rate (mL/s)	0.23 ± 0.04	0.18 ± 0.01*

The frequency of the burst discharge is represented as the ratio between the number of silent periods and the burst period. The flow rate is represented as the ratio between the voided volume and its total silent period. Values are the mean ± SD, $n = 12$.

*Statistically significant difference from the value of the male rat, $p < 0.05$.

suitable anesthetic for physiological experiments that require demonstration of reflex micturition".[19]

In this study the average urethral flow calculated by the VV:TSP ratio might not exactly reflect the actual flow velocity because the actual urethral flow in rats should demonstrate a nonlinear transformation pattern due to intermittent EUS activity. However, this experimental design was useful for determining the relationship between urodynamics and EUS-EMG. The urethral flow rate in male rats was significantly higher than that measured in female rats, which is mediated by several mechanisms, including: (1) the higher frequency of EUS burst discharge in male rats would produce higher pulsatile forces that would promote the evacuation of saline, (Table 1); and (2) the larger bladder contraction duration and amplitude in male rats reflects the fact that the detrusor muscle generates a larger contraction force to accelerate the release of saline (Figure 4).

The results of the histochemical experiments indicate that the striated urethral muscle in female rats is composed of both type I and II fibers, whereas in male rats it is entirely composed of type II fibers. These results imply that the striated urethral muscle in male rats is more susceptible to fatigue than that in female rats because type I muscle fibers are functionally slow-twitch fibers that use oxidative phosphorylation to obtain energy and are fatigue-resistant muscles, whereas type II fibers are fast fibers that may be less oxidative and more susceptible to fatigue.[20] Although we did not assess the fatigue properties of the striated urethral muscle in this study, some of the differences detected in the EUS-EMG measurements may be associated with the discrepancies of the urethral histochemical results. For example, the patterns in the EUS burst activities in male compared with female rats exhibited (1) a shorter duration of the active period, (2) EMG spikes with larger amplitudes, and (3) a higher frequency of bursts (Figure 5, Table 1). These findings are consistent with the fact that the contractile properties of fast-twitch fibers are characterized by a greater amplitude with a shorter duration of muscle contraction.[21]

On the other hand, our results reveal that the striated muscle of male rats is only present in the upper segment of the urethra, but in female rats the striated muscle was present along the entire urethra. The distribution of striated urethral muscle agrees with a previous gross anatomical study, which indicated that striated muscle is only present in the proximal portion of the urethra in male rats compared with female rats where striated muscle that is extensively attached to the entire urethral wall.[7]

A previous analysis of the fiber composition of striated urethral muscle documented contradictory results among different animal species. For example, in the rabbit, the proportions were 13% type I and 87% type II fibers,[22] whereas in the dog percentages were 24%

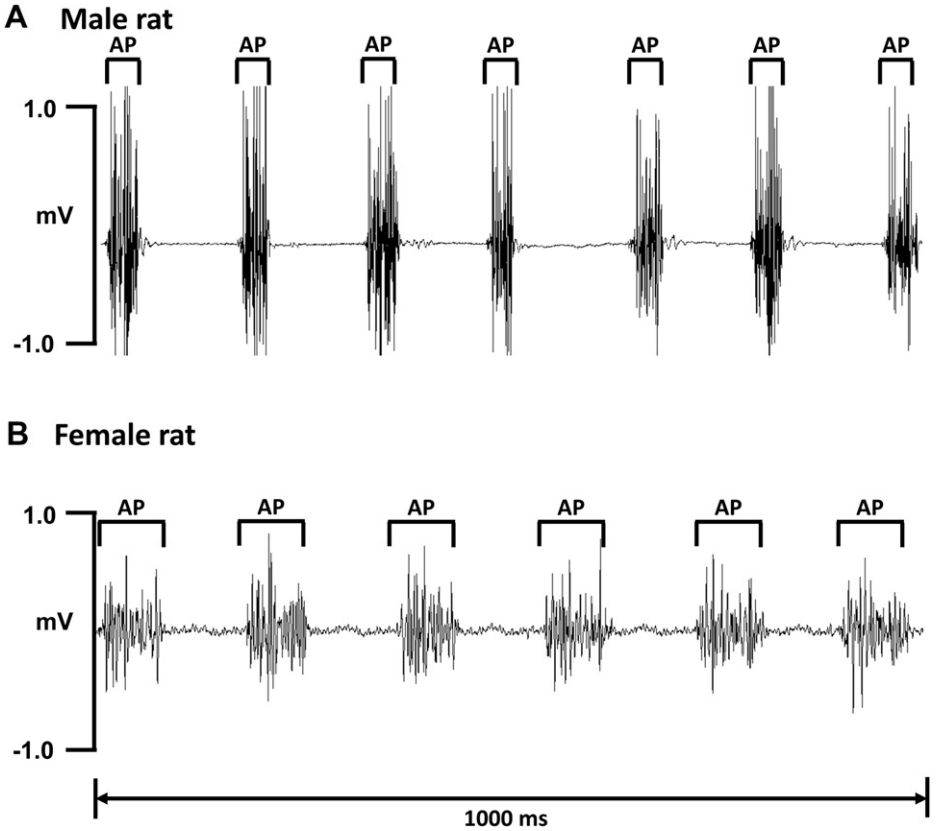

Figure 5 Comparison of the burst activity patterns on external urethral sphincter (EUS)-EMG between (A) male and (B) female rats. The male rat demonstratess significantly shorter active periods (APs) with a greater amplitude and a higher frequency of bursts compared with the female rat.

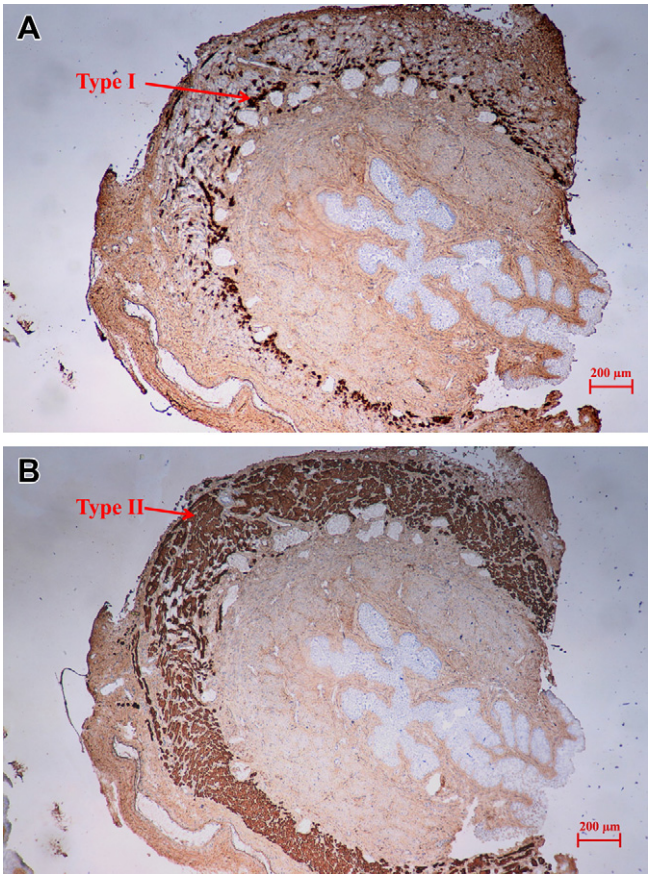

Figure 6 Cross-sectional microphotographs of the upper segment of the urethra obtained from a female rat. The serial sections show the immunohistochemical labeling of the (A) slow myosin heavy chain (MHC) (type I striated fiber) and (B) fast MHC (type II striated fiber) isoforms, where a larger amount of type II fibers was expressed in comparison with type I.

type I and 76% type II fibers.[23] In contrast to the predominance of type II fibers in the rabbit and dog, humans exhibit different proportions of fiber types in the urethra, with 66% type I and 34% type II.[24,25] Our results reveal that type II fibers are the predominant striated urethral fiber in both male (100%) and female (86–94%) rats, which is the same measured in rabbits and dogs but opposite to humans. It is still unclear why fiber compositions differ between humans and other animal species, but one possibility might be related to the patterns of EUS activity during voiding. The EUS in humans exhibits complete relaxation during voiding, while in rats and dogs a rapid burst pattern of EUS activity has been observed.[26] Therefore, it is reasonable that the composition of the striated fiber types in the urethra differ between animal species.

In summary, the present results indicate that there were significant differences in the IVP and EUS-EMG measurements. The voiding efficiencies in both sexes of rats were similar, but the volume threshold in males was 2-fold larger than that of females. In order to achieve efficient bladder voiding, the voiding pattern in male rats is compensated by a larger amplitude/duration of bladder contractions (contractile force) that coincides with a longer duration of the silent period (urethral relaxation). In addition, the distribution and composition of striated urethral muscle differed between sexes, and this finding is associated with different patterns of EUS burst activity. Because the rat is the most extensively investigated animal used in experiments on the lower urinary tract, this study provides a more-detailed understanding of the physiological and anatomical properties of the EUS for use in future research.

Table 2 Percentage of type I and II fibers in different segments of the urethra in male and female rats

Segment of urethra	Muscle fiber type	Males	Females
Upper	Type I	None	14 ± 5%
	Type II	100 ± 0%	86 ± 5%
Middle	Type I	None	11 ± 3%
	Type II	None	89 ± 3%
Lower	Type I	None	6 ± 3%
	Type II	None	94 ± 3%

Values are the mean ± SD, $n = 6$.

Acknowledgments

This study was supported by grants from the National Science Council, Taiwan (grant nos. NSC98-2221-E-038-005-MY2 and NSC100-2320-B-038-003-MY3) to C.-W. Peng.

References

1. Pacheco P, Martinez-Gomez M, Whipple B, Beyer C, Komisaruk BR. Somatomotor components of the pelvic and pudendal nerves of the female rat. *Brain Res* 1989;**490**:85–94.
2. Brading AF. The physiology of the mammalian urinary outflow tract. *Exp Physiol* 1999;**84**:215–21.
3. Praud C, Sebe P, Mondet F, Sebille A. The striated urethral sphincter in female rats. *Anat Embryol (Berl)* 2003;**207**:169–75.
4. Russell B, Baumann M, Heidkamp MC, Svanborg A. Morphometry of the aging female rat urethra. *Int Urogynecol J Pelvic Floor Dysfunct* 1996;**7**:30–6.
5. Jordan C. Androgen receptor (AR) immunoreactivity in rat pudendal motoneurons: implications for accessory proteins. *Horm Behav* 1997;**32**:1–10.
6. McKenna KE, Nadelhaft I. The organization of the pudendal nerve in the male and female rat. *J Comp Neurol* 1986;**248**:532–49.
7. Cruz Y, Downie JW. Sexually dimorphic micturition in rats: relationship of perineal muscle activity to voiding pattern. *Am J Physiol Regul Integr Comp Physiol* 2005;**289**:R1307–18.
8. Kontani H, Shiraoya C. Sex difference in urethral response to electrical stimulation of efferent nerves in the pudendal sensory branch of rats. *Int J Urol* 2002;**9**:586–95. discussion, 96.
9. Streng T, Santti R, Andersson KE, Talo A. The role of the rhabdosphincter in female rat voiding. *BJU Int* 2004;**94**:138–42.
10. Peng CW, Chen JJ, Chang HY, de Groat WC, Cheng CL. External urethral sphincter activity in a rat model of pudendal nerve injury. *Neurourol Urodyn* 2006;**25**:388–96.
11. Chen SC, Cheng CL, Fan WJ, Chen JJ, Lai CH, Peng CW. Effect of a 5-HT1A receptor agonist (8-OH-DPAT) on external urethral sphincter activity in a rat model of pudendal nerve injury. *Am J Physiol Regul Integr Comp Physiol* 2011;**301**:R225–35.
12. Peng CW, Chen JJ, Cheng CL, Grill WM. Role of pudendal afferents in voiding efficiency in the rat. *Am J Physiol Regul Integr Comp Physiol* 2008;**294**:R660–72.
13. Snow LM, Sanchez OA, McLoon LK, Serfass RC, Thompson LV. Myosin heavy chain isoform immunolabelling in diabetic rats with peripheral neuropathy. *Acta Histochem* 2005;**107**:221–9.
14. Maggi CA, Giuliani S, Santicioli P, Meli A. Analysis of factors involved in determining urinary bladder voiding cycle in urethan-anesthetized rats. *Am J Physiol* 1986;**251**:R250–7.
15. Kruse MN, Belton AL, de Groat WC. Changes in bladder and external urethral sphincter function after spinal cord injury in the rat. *Am J Physiol* 1993;**264**:R1157–63.
16. Cheng CL, de Groat WC. The role of capsaicin-sensitive afferent fibers in the lower urinary tract dysfunction induced by chronic spinal cord injury in rats. *Exp Neurol* 2004;**187**:445–54.
17. Yaksh TL, Durant PA, Brent CR. Micturition in rats: a chronic model for study of bladder function and effect of anesthetics. *Am J Physiol* 1986;**251**:R1177–85.
18. Walter JS, Fitzgerald MP, Wheeler JS, Orris B, McDonnell A, Wurster RD. Bladder-wall and pelvic-plexus stimulation with model microstimulators: preliminary observations. *J Rehabil Res Dev* 2005;**42**:251–60.
19. Matsuura S, Downie JW. Effect of anesthetics on reflex micturition in the chronic cannula-implanted rat. *Neurourol Urodyn* 2000;**19**:87–99.
20. Rodriguez-Veiga E, Mestre-Nieto L, Martinez-Sainz P, Garcia-Pascual A, Martin-Palacios S, Marin-Garcia P, Gonzalez-Soriano J. Stereological study of the external urethral sphincter in the female urethra of the lamb: a new model for studies on urinary continence. *Anat Histol Embryol* 2005;**34**:85–92.
21. Buffini M, O'Halloran KD, O'Herlihy C, O'Connell R, Jones JF. Comparison of the contractile properties, oxidative capacities and fibre type profiles of the voluntary sphincters of continence in the rat. *J Anat* 2010;**217**:187–95.

22. Okamura K, Tokunaka S, Fujii H, Miyata M, Mizunaga M, Hashimoto H, Yachiku S. Histochemical study of external urethral sphincter in the rabbit. Analysis with construction of histograms. *Nihon Hinyokika Gakkai Zasshi* 1989;**80**:1127–33.
23. Augsburger HR, Cruz-Orive LM. Influence of ovariectomy on the canine striated external urethral sphincter (M. urethralis): a stereological analysis of slow and fast twitch fibres. *Urol Res* 1998;**26**:417–22.
24. Okamura K, Tokunaka S, Yachiku S. Histochemical study of human external urethral sphincter. *Nihon Hinyokika Gakkai Zasshi* 1991;**82**:1487–93.
25. Ho KM, McMurray G, Brading AF, Noble JG, Ny L, Andersson KE. Nitric oxide synthase in the heterogeneous population of intramural striated muscle fibres of the human membranous urethral sphincter. *J Urol* 1998;**159**:1091–6.
26. de Groat WC. Integrative control of the lower urinary tract: preclinical perspective. *Br J Pharmacol* 2006;**147**(Suppl. 2):S25–40.