Sexually dimorphic micturition in rats: relationship of perineal muscle activity to voiding pattern.

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ABSTRACT

In the present study we examined the possibility that striated muscle activity might underlie sexually dimorphic micturition in rats. Micturition dynamics, the gross anatomy of the external urethral sphincter and the participation of the striated perineal muscles in micturition were compared in urethane-anesthetized adult male and female rats. Bladder contraction characteristics and in particular the magnitude of bladder high frequency pressure waves during voiding differed between sexes. Dissections indicated that the sphincter was more extensive and thicker in males than in females. Electromyography showed that in both sexes the sphincter discharged in bursts that correlated with the rising phase of high frequency bladder pressure oscillations. Regional differences in discharge pattern were seen in the sphincters of males, with the proximal part of the sphincter showing components activated during bladder filling. Bulbospongiosus, ischiocavernosus and cremaster muscles were also activated during bladder contraction in males. In both sexes transection of the motor branch of the lumbosacral plexus eliminated the bladder high frequency oscillations and reduced voided volume. Neurectomy did not affect bladder pressure but reduced voiding efficiency by 45% in males. In females the bladder pressure was dramatically decreased but voiding efficiency only decreased by 24%. Our findings suggest that in rats, striated perineal muscles contribute to the sexually dimorphic micturition. Activity of the dimorphic perineal muscles may regulate genital and urinary urethra expulsive functions, helping to expel seminal plug and fluids through the long urethra in the male.
Key words: electromyography, bulbospongiosus muscle, cremaster muscle, motor nerve transection
INTRODUCTION

In mammals, lower urinary tract function consists of storage of urine in the bladder with brief periods of voiding of urine. These processes involve spinal and supraspinal reflexes (3, 4, 31). Storage and release of urine requires coordination between the urinary bladder and the external urethral sphincter (EUS). During urine storage the bladder is inactive but the sphincter shows tonic contraction (42) that helps to support urinary continence (1). During voiding in species such as human beings and cats, sphincter activity stops as the bladder contracts so that voiding is efficient (9, 40, 50). After spinal cord injury above the lumbosacral spinal segments, concurrent contraction of bladder and EUS (bladder-sphincter dyssynergia) appears during voiding, leading to a high urethral resistance and poor bladder emptying (2, 13, 18, 37).

In contrast to cats and humans, rats show simultaneous activation of the bladder and the EUS during voiding (1, 23, 24). However, in rats, co-contraction of the bladder and sphincter is not considered to be dyssynergic because bladder emptying occurs. During the sustained phase of bladder contraction, the EUS bursts at 6-10 Hz and the intraluminal bladder pressure shows high frequency oscillations (HFO) (24, 28, 47, 48). The HFOs correlate temporally with the burst activity of the EUS (14, 23) and are abolished when a ligature of the bladder neck separates the bladder pressure from the urethral pressure (5). Neuromuscular blockade or bilateral transection of the pudendal nerve (which is considered to carry the bulk of the innervation of the EUS) also eliminates the HFO and increases residual urine (1, 5, 23). These findings have led to the conclusion that
in rats the EUS activity causes the HFO and that this striated motor activity is necessary for efficient urine expulsion during micturition (5, 23). However, it has been observed that the amplitude of the pressure oscillations is greater in males than in females (27).

The possible mechanisms for and the significance of the sexual differences in the HFO are poorly understood. Based on previous work, we would conclude that the EUS muscle is sexually dimorphic (36, 38, 49). However, its motoneuron pool is said to be monomorphic (32). There has been no direct comparison of the gross anatomy of the EUS in male and female rats. Perineal muscles that are anatomically related to the urethra, such as bulbospongiosus (BS), ischiocavernosus (IC) and cremaster, are well developed in males but are vestigial or absent in adult female rats (17, 32). Whether they are also activated rhythmically during voiding in males has not been analyzed.

The aims of the present study was to examine the possibility that striated muscle activity might underlie sexually dimorphic micturition in rats. As a first step, the dynamics of micturition was characterized in detail in both sexes. The gross anatomy of the EUS was then examined and the EMG activity of perineal striated muscles closely related to the urethra was recorded during micturition. Finally, the participation of the perineal striated muscles to male and female urine expulsion was also analyzed in denervation studies.

A preliminary report of some of these findings has been presented (6).

MATERIALS AND METHODS
Eighteen male (325 – 400 g) and 18 female (280 - 320 g) Wistar rats were used. The rats were maintained on a 12/12 light/dark cycle with food (rodent pellets) and water provided *ad libitum*. The experiment protocol was approved by Dalhousie University Committee on Laboratory Animals, according to the guidelines of the Canadian Council on Animal Care and the American Physiological Society Guiding Principles in the Care and Use of Animals. Micturition dynamics in intact and neurectomized animals and the electromyographic activity of the perineal muscles during voiding were determined in rats anesthetized with urethane (ip, 1.2 g/kg) and placed on a heated pad.

_Cystometrogram (CMG)._ In anesthetized rats the bladder was exposed by a midline abdominal incision and a silastic cannula (1.5 mm outer diameter) was secured in the bladder dome. The abdominal incision was closed in two layers. The cannula was used for saline infusion (0.1 ml/min) using a syringe infusion pump and bladder pressure was monitored on a side-line. On continuous infusion (continuous CMGs) repeated voiding episodes were observed and the following parameters were determined (Figs. 1 and 2): intercontraction interval (ICI), pressure threshold (PT), peak pressure 1 (PP1), peak pressure 2 (PP2), contraction duration (CD), expulsion time (ET), pressure decrease during voiding (PDDV); number, amplitude and duration of HFO (N. HFO, A-HFO, D-HFO), and voided volume (VV). After 30 minutes of continuous saline infusion single CMGs were recorded. To obtain a single CMG the bladder was emptied and infusion of saline (0.1 ml/min) was begun. The infusion was stopped immediately after the
first bladder contraction and the bladder drained to obtain the post-voiding residual volume. From single CMGs maximum pressure of the bladder contraction (MP), residual volume (RV) and VV were determined. Bladder capacity and voiding efficiency were calculated as VV + RV and VV * 100% / (VV + RV), respectively.

*Electromyogram (EMG).* A skin incision was made at the level of the pubic symphysis and the right rostral half of the pubic bone removed. A pair of Teflon-coated silver wires (uncoated diameter 0.05 mm, bared for about 1 mm at the tip) were inserted into the target muscles. The skin was sutured closed to prevent drying of the tissue. Bladder pressure and EMG signals (bandpass 300 Hz – 3 KHz) were amplified and digitized at 200 Hz and 5KHz, respectively (Power1401, Cambridge Electronic Design) and displayed and stored on a Pentium 4 computer running Spike 2 software (version 5.01, Cambridge Electronic Design).

*Gross anatomy of the EUS.* Rats were euthanized with sodium phentobarbital (100 mg/kg, ip.). The lower urinary tract was dissected and digital photographs of were taken and managed in Corel PHOTO-PAINT (version 11.0). The gross features of the EUS were determined using a stereoscopic microscope at 16X, 25X and 40 X.

*Nerve transection.* After making a midline longitudinal incision in the back, the motor branch of the lumbosacral plexus was identified according to previous studies (7, 34) and bilaterally transected. Continuous, and at least two single CMGs were recorded before and 15 min after the nerve transection.
Statistical analysis was performed using GB-STAT Statistical Software (version 5.0, Dynamic Microsystems). The parameter values for each animal were the averages of the values obtained from three bladder contractions. No. HFOs and PDDV were normalized as No. HFOs / ET and PDDV / ET, respectively. Statistical comparisons between male and female CMG parameters were conducted by unpaired Student t-test. Urodynamic parameters before and after motor nerve transection were analyzed using paired Student t-test. P < 0.05 was taken to indicate a significant difference.

RESULTS

Dynamics of micturition. Micturition characteristics were determined in 10 males and 10 females during continuous low rate (0.1 ml/min) infusion of physiological saline at room temperature. Small (2-3 mm Hg) and large (20-30 mm Hg) bladder contractions were present during the first 15-25 min of continuous saline infusion. The small bladder contractions did not produce urine loss. In 90 per cent of the animals, the small bladder contractions disappeared by about 30 minutes after the start of saline infusion, and the trace during the filling phase was smooth. Significant volumes of urine were expelled during the large bladder contractions (see below). In males, three patterns of bladder contractions were observed. Type 1 was a bladder contraction consisting of a single peak of short duration (12-15 sec) containing no HFO and resulting in no urine expulsion (Fig. 1A, number 1). Type 2 was a long duration bladder contraction (19-40 sec), consisting of two or more peaks with no HFO (Figs. 1A and 2A, number 2). Only
droplets of fluid (0.04-0.06 ml) were released at the bladder pressure peak. Type 3 was characterized by short duration bladder contraction (12-15 sec), consisting of two bladder pressure peaks separated by a period of HFO and intermittent stream-like expulsion, amounting to 0.3-0.6 ml of fluid (Figs. 1A and 2B, number 3).

The first two types were observed principally during the first 25 minutes after the continuous saline infusion began and approximately two hours after administration of urethane. After 30 min of infusion the type 3 pattern predominated.

In female rats, contraction types 1 and 2 were rarely observed (2 rats) after 15 min of saline infusion and the type 3 pattern predominated (Figs. 1B, 2C).

After 30 min of continuous saline infusion, the next 3 bladder contractions were analyzed. Statistical comparison between male and female CMGs indicated that seven of the eleven parameters differed between sexes (Table 1). ICI, PP1, ET, PDDV, No. HFO, A-HFO and VV were significantly greater in males than females (Table 1). Gender differences in No. HFOs and PDDV disappeared when normalized to ET; males 8.34 ± 1.46 vs females 8.65 ± 1.72 HFO/s and males 6.15 ± 0.56 vs females 6.82 ± 1.07 mmHg / s, p > 0.05, respectively.
Anatomical features of the EUS were determined in 4 males and 4 females, after the CMG analysis. Access to the EUS was achieved by carefully removing the pelvic bone. In males, striated muscle enveloped the prostatic (proximal portion) and membranous (distal portion) urethra behind the pelvic bone. This striated muscle (13.3 ± 1.3 mm long, 1.2 mm ± 0.1 thick) that can be observed even with naked eye was identified as the EUS because it was clearly differentiated from the pubococcygeous, BS or IC muscles, whose fibers also attach to the urethra wall. According to the anatomical localization the EUS was considered to have two sections. The proximal EUS was located rostral to the pelvic symphysis (Fig. 3A). It started at the base of the bladder and ended just before reaching the pubic ramus of the pelvic bone (4.5 ± 0.3 mm length). Under high magnification (25 X and 40 X) it was observed that its fibers originated in an aponeurosis located in the ventral wall of the urethra, 2-3 mm below the ureteric orifice, and ran laterally to insert in a tendinous structure in the dorsal wall of the urethra. Some of its fibers merged with the distal portion of the ejaculatory ducts (seminal glands, prostate, coagulant glands and deferent ducts) but did not surround the ejaculatory opening. Distal EUS was located behind the pelvic symphysis (Fig. 3C). Its fibers originated in the midline of the ventral wall of the urethra, ran caudolaterally to insert in the tendinous structure located in the midline of the dorsal wall of the urethra. The length of this portion of the EUS was 8 ± 0.5 mm.

-Insert Figure 3 about here-

In females, the dorsal wall of the urethra is extensively and firmly attached to the vaginal wall. The thin (hardly observed with naked eye) striated muscle
attached to the ventral wall of the urethra (5.1 ± 0.3 mm long, 0.2 ± 0.03 mm thick) was considered to be the EUS and was located only behind the middle portion of the pelvic symphysis (Fig. 3 B,D). There was no tendinous structure in the urethral midline where the EUS fibers could be anchored. Thin fibers originated in the urethra wall, and ran about 2-3 mm laterally to insert in the ventral wall of the vagina. They did not surround the dorsal wall of the urethra.

*Perineal striated muscle activity during micturition.* In males EMGs were recorded from proximal, or distal portions of the EUS as well as from the cremaster, IC and BS (ventromedial and ventrolateral portions) (32) muscles. In females, the wires were inserted in the EUS in the medial portion of the urethra, where the muscle was identified by gross anatomy. Each muscle was recorded in at least three animals. In EMG studies, a muscle was considered to be activated or inhibited when the amplitude of the EMG trace increased or decreased, respectively, by at least 30 per cent.

In both sexes, the EMG activity of each muscle during micturition was consistent between animals. In males, all perineal muscles (EUS, BS, IC and cremaster) discharged during the three types of bladder contraction. However, only the proximal EUS discharged during the filling period. The characteristics of the EMG activity pattern differed between muscles.

The EMG of the proximal EUS showed five components (Fig. 4A). During bladder filling after a type 3 bladder contraction, the first three EMG components appeared at low bladder pressure (2-4 mm Hg) (Fig. 4A, a,b,c). The amplitude of
these components was around 25 - 80 mV. Two larger components (300 - 500 mV) appeared at higher bladder pressure (10 - 25 mm Hg) (Fig. 4A, d,e). The amplitude and duration of activity of component e was greater in type 3 bladder contractions than in type 2 contractions (Figure 4A). As the bladder expelled urine during a type 3 contraction and pressure returned to basal, the EMG components disappeared in the reverse order of their appearance. However there was no obvious relationship between the cessation of EMG component activities and pressure events in the bladder. An expanded trace shows that the proximal EUS EMG discharge was continuous during type 2 bladder contraction (Fig. 5A). In contrast, the continuous activity was replaced with a larger amplitude intermittent discharge when HFO occurred during type 3 bladder contractions (Fig. 5B). This intermittent discharge occurred on the rising phase of individual HFO pressure peaks with clear silent periods on the falling phase where even the basal EMG activity was absent. The continuous EMG activity returned before the bladder reached its second peak. After a type 3 contraction there was a period of about 100 sec before basal EMG activity resumed (Fig. 4A). In type 2 contractions the highest amplitude component of the EMG was activated only at the initial highest pressure peak (Fig. 4A, e). The onset of component d was harder to assess but it remained activated only during the elevated bladder pressure in type 2 contractions. In type 3 contractions the activation of component d far outlasted the bladder contraction.

The EMG of the distal portion of the EUS showed no activity during bladder filling (Fig. 4B). The onset of EMG activity occurred at a bladder pressure
of around 9-10 mm Hg. A large-amplitude discharge occurred in association with HFO in type 3 bladder contractions, at pressures in the range of 20-25 mm Hg (Fig. 4B).

-Bursting activity was not present during type 2 bladder contractions (Fig. 5C). Although the EMG showed a rhythmic discharge pattern during HFO in type 3 contractions, the background activity was not completely eliminated during urine expulsion (Fig. 5D). Large-amplitude EMG activity lasted around 5-6 sec after bladder contraction returned to its basal level.

-The ventral BS (Fig. 6A), IC (Fig. 6B) and cremaster (Fig. 6C) muscles discharged during bladder contraction. The onset of EMG activity occurred at bladder pressures of 8-10 mm Hg and the largest amplitude components appeared at about 20 mm Hg. Some components of the EMG in BS and cremaster muscle continued to be active for 10-38 sec after bladder pressure returned to baseline (Fig. 6, A and C). Medial and lateral portions of the ventral BS muscle behaved similarly.

-The EMG discharge was continuous in BS (Fig. 7A) IC (Figure 7C) and cremaster (Fig. 7E) muscles during type 2 bladder contractions. Rhythmic EMG activity, similar to that seen in EUS, was recorded in BS (Fig. 7B) and IC (Fig.
7D) but not in cremaster (Fig. 7F) during the rising phase of HFO in type 3 bladder contractions. However, the EMG activity was not completely lost between rhythmic discharges (Fig. 7, B and D).

In females, there was little EUS EMG activity during the filling phase just before the bladder contraction. The EUS EMG discharge appeared when bladder pressure reached 4-7 mm Hg and reached its maximum when bladder pressure was about 19 mm Hg (Fig. 8A). The highest amplitude components of the EMG discharge showed a bursting pattern of activity during HFO then continued as a lower level tonic activity (Fig. 8B). Lower amplitude EMG components continued to be active 25-40 sec after the bladder pressure had returned to baseline (Fig. 8A).

Participation of the perineal muscles in micturition. In 2 males and 2 females, the motor branch of the lumbosacral plexus was transected bilaterally after establishing baseline CMG and EMG recordings. This surgery eliminated the activity of the EUS (in males and females), BS and IC (in males) elicited during bladder contractions.

The effect of sacral plexus neurectomy on parameters of micturition was analyzed in an additional 8 males and 8 females. In both sexes, neurectomy produced profound changes in the voiding pattern. In CMGs evoked by continuous infusion the ICI was reduced after neurectomy (Figs. 9A and 10A,
Table 2). Although HFOs were eliminated (Figs. 9B and 10B), drops of fluid were expelled during each bladder contraction. There were also gender differences in the contraction pattern changes. In males PT decreased significantly after neurectomy and CD increased but MP did not change (Figs. 9A and 9B, Table 2). By contrast PT did not change after the neurectomy in females but MP and CD decreased significantly (Figs. 10A and 10B, Table 2).

Motor nerve transection did not distort the filling phase of single CMGs (Fig. 11). Although bladder capacity increased in females (control 0.32 ± 0.05 ml, neurectomy 0.5 ± 0.1 ml, p<0.05), it did not in males (control 0.8 ± 0.15 ml, neurectomy 0.9 ± 0.2 ml). During filling there was no leakage of fluid. It was expelled only during the bladder contraction. Voided volumes were reduced by neurectomy in males but not in females (Table 3). MP decreased in females but not in males and CD increased in males but decreased in females (Table 3). Neurectomy also dramatically reduced voiding efficiency in both sexes although the effect was greater in males. In four males, after the neurectomy a plug of semen was found in the bladder.

**DISCUSSION**

This study provides both anatomical and functional bases for concluding that differences in activity of perineal striated muscle underlie the sexually dimorphic micturition observed in rats.
Anatomically, our dissections showed clear sexual dimorphism in the EUS of adult rats. In females the EUS is poorly developed whereas in males it is extensive and thick. These findings are supported by histological studies indicating that EUS is thick and complex in males and thin in females (35, 36, 38, 49) and lead to the expectation that males should have more EUS motoneurons in their spinal nuclei. However, it has been reported in a labeling experiment that the numbers of EUS motoneurons are equal in male and female rats (32). Perhaps not enough account was taken of the difference in bulk and extent of the EUS in this study.

Functionally, we found gender differences in several CMG parameters and for the first time we described that in males there is a concurrent activation of EUS, cremaster, IC and BS during bladder contraction. According to our EMG and denervation studies, the activity of the sexually dimorphic muscles surrounding the urethra helps to explain most of the gender differences in the dynamics of micturition of the rat. By contrast, gender differences in parameters such as IC, VV and bladder capacity seem not to be related to sphincter activity and more likely to be related to differences in autonomic control.

In males, the activity of all perineal muscles was found to be tonic during PP1 and PP2. On the other hand, with exception of the cremaster muscle, EMG activity was rhythmic during the period of HFOs. In agreement with previous reports (22, 23, 46), rhythmic EUS activity during HFOs was associated with brief increases in bladder pressure superimposed on a tonic bladder contraction. This pattern is interpreted as arising from rhythmic increases in urethral resistance
due to the EUS contraction (5, 28). Urine thus flows in the urethra only periodically, when the EUS is silent and the detrusor is still contracting (46). Because some urine is lost in these EUS silent periods, there is a gradual reduction in the basal pressure from which the HFOs arise leading to the stepped character of the PDDV.

The magnitude of PP1 and A-HFOs likely depend not only on detrusor smooth muscle activity but also on the effectiveness of urethral closure that maintains the isovolumic state of the bladder. The bulkier EUS in males may prevent proximal urethra funneling and may be more effective in tightly closing the urethra. Thus, its tonic activity may generate a higher PP1 and its rhythmic discharge larger amplitude HFOs. Conversely, in females the tonic and bursting activity of the poorly developed EUS may not completely close the urethra, leading to loss of detrusor pressure and thus smaller PP1 and HFOs.

Our denervation study indicated that EUS activity is required during voiding in both sexes but that the loss of EUS activity is expressed differently depending on sex. In females VE depends on a good bladder contraction that is related to factors like the magnitude of MP and CD. It is unlikely that lumbosacral motor neuréctomy directly influences the autonomic drive to the bladder. Therefore, the fact that lumbosacral motor branch denervation decreased these voiding parameters (MP, CD, VE) implies that they depend on EUS activity. These findings seem to counter the suggestion that the motor nerve in female rats does not contribute to urine expulsion (21). Possibly, the short and almost straight urethra of females requires EUS activity to occlude and provide
resistance in order to maintain an increased bladder pressure to support a positive bladder afferent feedback to the bladder contraction to empty the bladder efficiently. In males, the large MP was not reduced by motor neurectomy and CD almost doubled yet emptying was impaired, as indicated by decreased VE. These findings imply that the EUS activity during bladder contraction is not necessary to maintain the high bladder pressure necessary to sustain the bladder afferent activation. Perhaps the pronounced curvatures of the long male urethra provides enough resistance without EUS activation. However, striated muscle activity may be needed to assist fluid propulsion through the long curved male urethra, as has been suggested by others (21, 28). The lack of a decrease in MP after motor neurectomy in males agrees with results obtained after bilateral transection of the pudendal nerve or d-tubocurarine injection (21, 28).

In both sexes deficient bladder emptying, as reflected by reduced VE, is sufficient explanation for the increased RV and reduced ICI after motor neurectomy. The urinary continence was maintained after the motor nerve transection and there was no fluid escape when the bladder was not contracting. Previous studies have reported that transection or injury to the pudendal nerve in awake female rats causes a reduction in the size of the EUS and an increased number of small urine marks suggestive of urinary incontinence (15, 19). However, given our findings, it would be necessary to distinguish whether the change in the behavioral pattern represents stress urinary incontinence or frequency related to inefficient voiding.
Three CMG variables, No. HFO, PDDV and ET, are bigger in males than in females. However, after normalization to ET, No. HFOs and PDDV showed no gender dependence, implying that the original differences are related simply to the difference in ET. We suggest that the gender difference in ET is associated with different patterns of urine expulsion. In females the urethra may never be closed completely during voiding, allowing urine to flow during both silent periods and EUS bursting. This pattern of almost continuous urine expulsion, coupled with a lower bladder capacity, likely shortens ET in females. In contrast, males demonstrate a more spurting pattern of expulsion implying that fluid flow is interrupted during each EUS burst. This interrupted pattern likely leads to increased ET. We cannot rule out the possibility that this parameter, and probably PDDV, could be also influenced by the large male VV and a greater resistance to flow in the male urethra. Previous authors found gender differences in the urine flow rate of rats and suggested that females void more efficiently presumably due to a longer flow duration (45).

In our CMG study the males displayed a greater variety of bladder contraction patterns than did females. The type 3 bladder contraction pattern has been called oscillatory micturition (45) and is presumed to represent the normal pattern of voiding in rats. The type 2 bladder contraction pattern has been referred to as incomplete micturition (29) or non-oscillatory micturition (45) and attributed to the anesthetic level (30). Preliminary studies confirmed an increased incidence of type 2 contractions when additional small doses of urethane were administered (Cruz, unpublished). However, the finding of type 2 contractions
occurring along with type 3 contractions hours after the injection of anesthetic implies that factors other than anesthesia may influence contraction pattern. Furthermore, the high incidence of type 2 contractions in male but not in female rats agrees with previous studies (29) and also implicates other factors. One possibility is that type 2 contractions are a part of the repertoire of bladder contraction patterns in the male.

Analysis of the relationship between bladder pressure and components of EMG activity implies that the muscle activities are elicited by reflexes activated by mechanoreceptors with different thresholds (around 3-5 mm Hg, 10 mmHg and 20 mm Hg). There is some evidence that low threshold mechanoreceptors are more prevalent in the bladder body and high threshold mechanoreceptor in the bladder base (41).

In males the EUS may have functionally differentiated regions. Only the proximal EUS began discharging during bladder filling and this would be consistent with a role of this region in maintaining high intrasphincteric pressure to facilitate urinary continence. Previous recordings with suction electrodes in this region did not record activity during bladder filling (44) and we can only suggest that the low level activity that we found was not recorded by the other design of electrode.

The rhythmic activity of the proximal and distal EUS fibers during voiding and the loss of VE that occurs when this activity is lost implies that both EUS regions may be important for fluid expulsion. The activity of different components of the EMG discharge in the sphincter may reflect a heterogeneity of control of
different motor units in the EUS. The rat EUS has fast and slow muscle fibers (36) that may partly underlie this heterogeneity of response.

Usually, the activity of cremaster, IC and BS has been related to reproductive function (16, 39). The motoneurons of these sexual dimorphic muscles are located at the lumbar spinal cord in three sexual dimorphic nuclei: cremasteric, dorsolateral and dorsomedial. The last two nuclei are the rat homologue of the sexually dimorphic Onuf’s nucleus in other species such as the human being (12). The motor nerves from these two nuclei exit the spinal cord in the motor branch of the lumbosacral plexus (34). The cremaster muscle is innervated by a distinctly different route, the genitofemoral nerve (52) arising from another dimorphic nucleus localized in the upper lumbar cord. Also its pattern of activity differs from the others in being continuous throughout the HFO period. The functional significance of the cremaster in rat micturition is unknown. On the other hand, the activity pattern of IC and BS resembles that of the EUS during bladder contraction. These muscles attach to the bulbar urethra and thus their contraction may be necessary to milk the bulbar urethra to expel urine. Damage to IC and BS has been speculated to be a factor connecting sexual and urinary dysfunctions with postmicturition dribbling in men (11).

In conclusion, sexually dimorphic micturition in adult rats can be explained in terms of the activity of dimorphic perineal muscles in urethral expulsive functions. In males, urinary and genital expulsive processes require coordinated activity of EUS, IC and BS. In females the activity of the EUS is necessary to sustain bladder contraction. Although by different mechanisms, the striated
muscles activities are necessary for efficient emptying of the bladder. In addition, strong striated muscle activity in male rats may be important for ensuring expulsion of the seminal pug through the long male urethra, thus avoiding urinary obstruction.

_Perspectives._ The work reported here contributes to our understanding of the potential role in sexually dimorphic lower urinary tract function of some striated perineal muscles surrounding the urethra. We have found that the adult female rat does not have a well-developed striated muscle surrounding the urethra, including the EUS. Perhaps pelvic striated muscle is less significant to maintaining continence in rats than in human because the former have a quadripedal posture. Nevertheless, rats are used for modeling female stress incontinence by vaginal distension (25, 43) or motor nerve damage (19, 20). However, EUS activity is also important to voiding function in the female rat. Therefore, it is important in these investigations to assess incontinence by provocation (8, 43) rather than by depending on voiding pattern (15, 19).

On the other hand, human beings are also troubled with a high prevalence of disorders of micturition. Although it is generally accepted that striated sphincter muscles are necessary to facilitate continence, whether the EUS is sexually dimorphic in the human being is controversial (10, 26, 51). The dimorphism is not likely to be as dramatic as in rats, because IC and BS that are vestigial in female rats, are smaller than in men but still developed in women (33). More studies are necessary to elucidate the role of EUS, BS and IC in the physiology of micturition in women and men.
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GRANTS

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Table 1. Urodynamic parameters in intact rats

<table>
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<th>Animal</th>
<th>ICI (sec)</th>
<th>PT (mm Hg)</th>
<th>PP1 (mm Hg)</th>
<th>PP2 (mm Hg)</th>
<th>ET (sec)</th>
<th>PDDV (mm Hg)</th>
<th>CD (sec)</th>
<th>No.</th>
<th>A-HFO (mm Hg)</th>
<th>D-HFO (sec)</th>
<th>VV (ml)</th>
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<td>346*</td>
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<td>20.4</td>
<td>2.7**</td>
<td>17.8**</td>
<td>16.7</td>
<td>20**</td>
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<td>N = 10</td>
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<td></td>
<td>28</td>
<td>0.5</td>
<td>1.3</td>
<td>1.7</td>
<td>0.20</td>
<td>1.62</td>
<td>1.2</td>
<td>2.0</td>
<td>0.1</td>
<td>0.01</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values are Means ± SEM; * = P<0.05, ** = p<0.01, *** = p < 0.001, ICI = intercontraction interval, PT = pressure threshold, PP1 = peak pressure 1, PP2 = peak pressure 2, ET = expulsion time, PDDV = pressure decrease during voiding, CD = contraction duration, A-HFO = amplitude of HFO, D = Duration of HFO, VV = voided volume.
Table 2. Urodynamic parameters in continuous CMGs in intact and neurectomized rats

<table>
<thead>
<tr>
<th>Animal</th>
<th>ICI (sec)</th>
<th>PT (mm Hg)</th>
<th>MP (mm Hg)</th>
<th>CD (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In</td>
<td>Nx</td>
<td>In</td>
<td>Nx</td>
</tr>
<tr>
<td>Males</td>
<td>318±48</td>
<td>108***±48</td>
<td>4.9±0.5</td>
<td>2.0**±0.2</td>
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<td>N = 8</td>
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<tr>
<td>Females</td>
<td>200±29</td>
<td>154**±29</td>
<td>4.2±0.6</td>
<td>3.9±0.4</td>
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<tr>
<td>N = 8</td>
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</tbody>
</table>

Values are Means ± SEM; * indicates significant difference between intact (In) and neurectomized (Nx) condition. * = P<0.05, ** = p<0.01, *** = p < 0.001, ICI = intercontraction interval, PT = pressure threshold, MP = maximum pressure, CD = contraction duration.
Table 3. Urodynamic parameters in single CMGs in intact and neurectomized rats

<table>
<thead>
<tr>
<th>Animal</th>
<th>PT (mm Hg)</th>
<th>MP (mm Hg)</th>
<th>CD (sec)</th>
<th>VE (%)</th>
<th>RV (ml)</th>
<th>VV (ml)</th>
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</thead>
<tbody>
<tr>
<td>Males</td>
<td>In</td>
<td>Nx</td>
<td>In</td>
<td>Nx</td>
<td>In</td>
<td>Nx</td>
</tr>
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<tr>
<td>N = 8</td>
<td>5.6 ± 0.4</td>
<td>5.7 ± 0.8</td>
<td>26.2 ± 2.0</td>
<td>24 ± 1.6</td>
<td>14 ± 2.5</td>
<td>25 ± 2.1</td>
</tr>
<tr>
<td>Female</td>
<td>In</td>
<td>Nx</td>
<td>In</td>
<td>Nx</td>
<td>In</td>
<td>Nx</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N = 8</td>
<td>6.7 ± 0.6</td>
<td>7.0 ± 0.5</td>
<td>18 ± 2.1</td>
<td>0.5 ± 0.5</td>
<td>5 ± 0.5</td>
<td>5.2 ± 0.2</td>
</tr>
</tbody>
</table>

Values are Means ± SEM; * indicates significant difference between intact (In) and neurectomized (Nx) condition. * = P<0.05, ** = p<0.01, *** = p < 0.001, PT = pressure threshold, MP = maximum pressure, CD = contraction duration, VE = voiding efficiency, RV = residual volume, VV = voided volume.
Figure 1 Cruz and Downie
Figure 2 Cruz and Downie
Figure 3 Cruz and Downie
Figure 5 Cruz and Downie
Figura 7 Cruz and Downie
Figure 10 Cruz and Downie
FIGURE LEGENDS

Fig. 1. Continuous cystometry in urethane-anesthetized male (A) and female (B) adult rats. Continuous saline infusion (0.1 ml/min) for about 15 minutes provoked micturition-like bladder contractions of 3 types (designated by numbers in traces): non-voiding contraction (1), long-lasting contractions that resulted in loss of drops or dribbling fluid (2) and voiding contractions that resulted in expulsion of a stream of fluid (3). Some parameters of the CMG are illustrated in Panel A: ICI = intercontraction interval, PT = threshold pressure for inducing bladder contraction, CD = bladder contraction duration. Time and pressure scales apply to both panels.

Fig. 2. Detailed characteristics of bladder contractions seen on continuous cystometry (0.1 ml/min) in anesthetized rats. Type 2 contractions (A, shown in male) at expanded time base appeared as 2 partially merged pressure peaks. Type 3 contractions were characterized by the presence of high frequency pressure oscillations (HFO) of larger amplitude in males (B) than in females (C). Some CMG parameters analyzed: PDDV = pressure decrease during voiding, ET = expulsion time, PP1 = peak pressure 1, PP2 = peak pressure 2, A-HFO = amplitude of each high frequency oscillations, D-HFO = duration of each high frequency oscillations. Time and pressure scales apply to both panels.

Fig. 3. Digital images of the ventral exposure of the lower urinary tract in male (A,C) and female (B,D) adult rats. Panels A and B show the urinary bladder and
the proximal portion of the urethra that can be seen rostral to the intact pelvic bone. A proximal component of the EUS is seen only in males (A, arrow). After the pubic symphysis was removed the distal component of the EUS in male and the EUS in females could be seen (C, D). In males, the urethra behind the pelvic bone was completely surrounded by the medial and distal portions of the EUS (C, arrows). In females the striated muscle (EUS) is present only in the medial portion of the urethra (D, arrow).

Fig. 4. EMG activity in the proximal (A) and medial (B) EUS during continuous saline infusion (0.1 mm/min) in anesthetized male rats. Numbers on the pressure trace indicate the type of bladder contraction. Letters indicate different components of the EMG discharge (A) and * an artifact. The proximal EUS was activated during both bladder filling and bladder contraction whereas the distal EUS was activated only during bladder contraction. The EMG returns to the basal level only after a type 3 bladder contraction, when the bladder is empty. Time scale refers to both panels. Note the 10-fold difference in EMG voltage scale between panels.

Fig. 5. EMG activity of the proximal EUS (A,B) and medial EUS (C,D) during type 2 (A,C) and type 3 (B,D) bladder contractions in an anesthetized male rat. Note that in both locations low-amplitude continuous EMG activity was recorded during type 2 bladder contractions (A,C). During type 3 contractions, large-amplitude rhythmic EMG discharges correlated with HFO appeared (B,D). In the proximal
EUS, there was almost no EMG activity between the rhythmic discharges (B) but some low-amplitude EMG activity persisted between the HFO peaks in the distal EUS (D). Time scale refers to all panels. Note the 10-fold difference in EMG voltage scale between proximal (A,B) and distal (C,D) records.

Fig. 6. EMG activity of the BS (A), IC (B) and cremaster (C) muscles during continuous saline infusion (0.1 ml/min) in anesthetized male rats. Numbers in the pressure trace indicate the type of bladder contraction. EMG activity of all muscles increased during all types of bladder contraction. Some EMG components in the BS and cremaster muscles outlasted the bladder contraction. Time, voltage and pressure scales apply to all panels.

Fig. 7. EMG activity of the BS (A,B), IC (C,D) and cremaster (C,D) muscles during type 2 (A,C,E) and type 3 (B,D,F) bladder contractions elicited in the same male rat by continuous saline infusion (0.1 ml/min) under anesthesia. All muscles showed continuous EMG activity through the peak of a type 2 bladder contraction (A,C,E). In BS and IC muscles, a moderate degree of rhythmic activity appeared to be superimposed on tonic EMG activity during HFO in type 3 bladder contractions (B,D). However, the EMG discharge pattern in cremaster muscle did not differ appreciably between type 2 and type 3 contractions (E,F). Time and voltage scales apply to all panels.
Fig. 8. EMG activity of the EUS during type 3 bladder contractions elicited by continuous saline infusion (0.1 ml/min) in anesthetized female rat. High-amplitude EMG activity occurred during each bladder contraction and some components of the EMG discharge outlasted the bladder contraction by up to 30 sec (A). At an expanded time scale, rhythmic activity during HFO in the second micturition episode in panel A was evident (B). The rhythmicity in EUS discharge began before the onset of HFO but terminated at the end of HFO.

Fig. 9. Bladder contractions elicited by continuous saline infusion (0.1 ml/min) in anesthetized male rat before (A, B) and after (C, D) bilateral transection of the motor nerve of the lumbosacral plexus. B (intact) and D (neurectomized) show expanded bladder contraction traces. Neurectomy did not change the amplitude of the bladder contraction but ICI decreased, CD increased and HFO during voiding disappeared (D). Time and pressure scales apply to both panels.

Fig. 10. Bladder contractions elicited by continuous saline infusion (0.1 ml/min) in anesthetized female rat before (A, B) and after (C, D) bilateral transection of the motor nerve of the lumbosacral plexus. B (intact) and D (neurectomized) show expanded bladder contraction traces. Neurectomy eliminated HFO, and decreased the amplitude of bladder contraction as well as ICI and CD (D). Time and pressure scales apply to both panels.