Characterization of Bulbospongiosus Muscle Reflexes Activated by Urethral Distension in Male Rats

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Abstract

The urethrogenital reflex (UGR) is used as a surrogate model of the autonomic and somatic nerve and muscle activity that accompanies ejaculation. The UGR is evoked by distension of the urethra and activation of penile afferents. The current study compares two methods of elevating urethral intraluminal pressure in spinalized, anesthetized male Sprague Dawley rats (N=60). The first method, penile extension UGR, involves extracting the penis from the foreskin so that urethral pressure rises due to a natural anatomical flexure in the penis. The second method, Penile clamping UGR, involves penile extension UGR with the addition of clamping of the glans penis. Groups of animals were prepared that either received no additional treatment, surgical shams or received bilateral nerve cuts (4 nerve cut groups); either the pudendal sensory nerve branch (SbPN), the pelvic nerves, the hypogastric nerves or all 3 nerves. Penile clamping UGR was characterized by multiple bursts, monitored by electromyography (EMG) of the bulbospongiousus muscle (BSM) accompanied by elevations in urethral pressure. The penile clamping UGR activity declined across multiple trials and eventually resulted in only a single BSM burst, indicating desensitization. In contrast, the penile extension UGR, without penile clamping, evoked only a single BSM EMG burst that showed no desensitization. Thus, the UGR is composed of two BSM patterns: an initial single burst, termed urethrobulbospongiousus (UBS) reflex, and a subsequent multiple bursting pattern (termed ejaculation-like response, ELR) that was only induced with penile clamping urethral occlusion. Transection of the SbPN eliminated the ELR in the penile clamping model, but the single UBS reflex remained in both the clamping and extension models. Pelvic nerve (PelN) transection increased the threshold for inducing BSM activation with both methods of occlusion but actually unmasked an ELR in the penile extension method. Hypogastric nerve (HgN) cuts did not significantly alter any parameter. Transection of all 3 nerves eliminated BSM activation completely. In conclusion, penile clamping occlusion recruits penile and urethral primary afferent fibers that are necessary for an ELR. Urethral distension without significant penile afferent activation recruits urethral primary afferent fibers carried in either the pelvic or pudendal nerve that are necessary for the single burst UBS reflex.
Introduction

Sexual behavior in males is comprised of penile erection, emission, and ejaculation of seminal fluid. These components are regulated by supraspinal and spinal mechanisms and require coordination of sympathetic, parasympathetic, and somatic nerves. During the emission phase autonomic nerves (pelvic and hypogastric) regulate secretion of seminal fluids into the urethra. Expulsion of the seminal fluids is thought to occur in response to urethral afferent activation due to the presence of seminal fluids in the urethra (via stretch or chemoreceptors) and penile afferent activation due to mechanical stimulation of the penis (via cutaneous mechanoreceptors) and is characterized by rhythmic contractions of striated perineal muscles, primarily the bulbospongious muscle (BSM) (2, 14, 21, 42, 43, 56). In acutely spinalized (T8-10), urethane-anesthetized rats, clamping the tip of the penis, while infusing fluid into the urethra produces repeated, phasic contractions of the BSM (11, 30, 32). This pattern of BSM activity is similar to that recorded during ejaculation in conscious, behaving rats and was termed the urethrogenital reflex (UGR). The BSM rhythmic contractions recorded during the UGR in rats is also similar to the BSM activity in men during ejaculation (4, 5, 43). In addition, the BSM activity in both men and rats, involves a spinal reflex that can be triggered by stimulation of penile afferent fibers of the dorsal nerve of the penis (18, 23, 46). This reflex is thought to mimic the expulsion phase of ejaculation, since it is characterized by rhythmic contractions of the perineal muscles, which is necessary to expel seminal fluids (11, 18, 21, 24, 32, 40, 43, 44). Therefore, the UGR is considered a surrogate for the peripheral and spinal components of somatic and autonomic reflexes that control ejaculation and has been used to determine the anatomical, physiological and pharmacological characteristics of ejaculatory-like reflexes (ELR).

Both penile and urethral stimulation (distension and/or pressure) are considered to provide ejaculatory sensory input in human and rat (9, 32, 41, 43, 44). The sensory branch of the pudendal nerve (SbPN) carries both penile and urethral primary afferent fibers, while the pelvic nerve (PelN) and the hypogastric nerve (HgN) carry urethral primary afferent fibers (7, 13, 16, 17, 27). The role of the SbPN, PelN and HgN in afferent pathways of peripheral sexual stimuli evoking ELR have been reported but are not fully understood (7, 12, 29, 45). Penile clamping can activate various types of mechanoreceptors and nociceptors or even cause tissue damage depending on how the “clamp” is applied. In preliminary experiments, we attempted to minimize activation of glans penis mechanoreceptors by putting a thin ligature through the tip of the penis and gently pulling the penis from the sheath. Fortunately we were able to occlude the urethra and cause urethral distension that generated pressures equivalent to pressures produced by penile clamping. Using this penile extension method to elevate urethral pressure, we found significant differences in activation of the UGR. The present study characterizes the two urethral distension methods, the role of the SbPN and PelN in conveying sensory input to spinal ejaculation control centers, and establishes baseline data for further physiological characterization of the “adequate stimuli” for urethral and glans penis primary afferent fibers in triggering the UGR.

Material and Methods

Animals. Adult, male, Sprague-Dawley rats (250-400 g, N= 60 Charles River) were housed in cages (3/cage) with free access to water and food. The colony room was maintained on a 12 hr/12 hr light/dark cycle. All experiments were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by the Urogenix IACUC. All efforts were made to minimize animal stress and to reduce the number of animals used.

Surgery and data collection. Rats were initially anesthetized with 3.0% isoflurane (VEDCO, Inc., Saint Joseph, MO, USA) and subsequently injected subcutaneously with 1.2 g/kg urethane (Acros Organics, Geel, Belgium). The skin and muscle over the middle thoracic vertebrae were incised, and the spinal
cord was exposed by a laminectomy and transected at the T8-T10 level. The spinal cord transection was performed at least 90 minutes before the experiment started.

The abdomen was opened, and a saline-filled polyethylene tubing with a cuff at the tip (PE 50®, Becton Dickinson, Franklin Lakes, NJ, USA) was inserted through a small incision in the dome of the bladder and passed caudally into the urethra just past the bladder neck. The catheter was tied in place at the bladder neck. The other end of the urethral catheter was connected to a T-tube, allowing continuous urethral saline perfusion (5 µl / sec) by an infusion pump (PHD2000 infusion, Harvard Apparatus, Holliston, MA, USA) and measurement of urethral pressure (CDXIII Transducer, Argon Medical Devices, Athens, TX, USA). The signal was amplified using a bridge amplifier (Transbridge 4M, World Precision Instruments, Sarasota, FL, USA). The seminal vesicle ducts were tied bilaterally to prevent saline from flowing into the seminal vesicle during urethral occlusion. A suture was passed through the tip of the penis and a loose loop made that did not constrict the penis (6-0 silk suture with a 26 mm ½ circle, cutting needle). This suture remained loose and was used to gently extend the penis from the foreskin so that either the extension or clamping method could be performed (figure 1 and see below).

After a perineal skin incision to expose the BSM, a pair of Teflon coated stainless steel electrodes with tip exposed (0.05 mm in diameter, MT GIKEN, Japan) was placed in the muscle for recording BSM-electromyogram (BSM-EMG). The electrical signals were amplified (2K) and filtered (½ amp low pass at 10Hz and ½ amp high pass at 10 kHz) with a P511 AC amplifier (Grass Instruments Division, Astro-Med, Inc., West Warwick, RI, USA). Urethral pressure and BSM-EMG signals were acquired continuously on a personal computer using LabChart (Version 7, ADInstruments, Inc., Colorado Springs, CO, USA).

Urethral occlusion. Two methods were used to evoke BSM-EMG activity: penile extension and penile clamping (figure 1). For both methods the penis was gently pulled 1-1.5 cm from the foreskin using the loose ligature through the tip of the penis (as described above) until the dorsal penile vein was seen at the base of the penis. This extension produces an anatomical occlusion of the urethra by “kinking” the lumen and is responsible for the pressure increase associated with urethral saline perfusion. The penis remained extended until a large burst of BSM-EMG activity and a rapid rise in urethral pressure was noted (the threshold pressure), and the penile extension was immediately released allowing the penis to retract, with subsequent expulsion of urethral fluid and rapid return of urethral pressure to baseline (figure 2).

For penile clamping, immediately after extending the penis as above, the glans penis was clamped with forceps (1.3mm tip) whose tips were rubber-coated (figure 1). The force of the clamp was estimated to be 120-300g/mm². The clamp remained on the extended penis until the threshold pressure was reached, at which time the clamp was immediately removed allowing the penis to retract with release of urethral fluid and pressure.

The penile extension and occlusion (with or without clamping) was performed at least 30 min after the surgical procedures. Each occlusion was repeated five times with 15 min intervals between each occlusion in groups with intact peripheral nerves. The occlusion was applied at least three times in the neurectomized rats.

Acute neurectomies. Seven groups of animals were prepared: Group 1 had all nerves intact (N=12). Group 2 had bilateral transection of the SbPN (N=12). The SbPN was isolated in the ischiorectal fossa and dissected free from the internal pudendal vein and cut. Group 3 had bilateral PeIN transection (N=12). The PeIN was exposed through a ventral abdominal incision and cut central to the major pelvic ganglion at the point just distal to its separation from the levator ani nerve (7). Group 4 had bilateral transection of the HgN (N=12). Hypogastric nerves were identified between the inferior mesenteric
ganglion and major pelvic ganglion and cut just distal to the inferior mesenteric ganglion. Group 5 (N=6) received bilateral neurectomy of all 3 peripheral nerves (SbPN, PelN and HgN). These groups were randomly divided equally into penile extension or penile clamping experiments. In addition, 2 sham groups were examined: group 6 (N=2) bilateral isolation of the SbPN as in group 2, but the nerves were not cut; and group 7 (n=4) in which the PelN was isolated bilaterally as in group 3 but not cut. Both penile extension and penile clamping were performed in the sham groups.

**Measurements.** The measurements taken to generate tables 1 and 2 are diagrammed in figure 2. The BSM-EMG activity was measured as the root mean square (RMS: the square root of the sum of the squared amplitude values) over the designated time (1 or 30 sec, see figure 2).

**Statistical Analysis.** All data was presented as mean and standard error of the mean. All statistical analysis was performed with Prism 5 for Windows (Graphpad Software, Inc., San Diego, CA, USA). One way and Two way ANOVA with Dunnett’s multiple comparison, or Bonferroni’s multiple comparison post tests, respectively, were used to examine the effect of repeated trials, the effect of various nerve cuts, and to compare clamping versus extension methods. Individual tests are described in each respective table legend. p<0.05 was considered statistically significant.

**Results**

**General characteristics.** Baseline urethral pressure prior to infusion ranged between 18 and 24 mmHg and urethral compliance ranged from 2.9 to 4.6 μl / mm Hg across all trials in all experiments with no correlation to the method of occlusion (i.e. penile clamping or extension), trial number or neurectomy. At variable times after initiation of filling (about 20-40 sec), during the linear portion of the volume-pressure curve, 3-5 small BSM-EMG bursts might be recorded (see BSM-EMG activity marked by asterisk in figure 2). These small BSM-EMG bursts were never accompanied with a sharp elevation in urethral perfusion pressure (UPP, figure 2) and had small amplitude, short duration (usually < 1 sec), and moderate frequency (about 70 – 100 spikes/sec). This activity was sporadic and not reliably produced within or between individual animals and is not considered a component of the UGR, so it will not be discussed further.

**Penile clamping-evoked urethrogenital reflex (UGR).** In this study, the characteristics of the first trial of penile clamping-evoked UGR were very similar to previous reports (9, 30, 32). During occlusion and filling, urethral pressure increased in a linear fashion until it reached approximately 80 ± 10 mm Hg (figures 2-4, table 1). At that point (i.e. threshold pressure) a distinctive, large amplitude, high frequency (492 ± 48 spikes/sec), short duration (580 ± 110 msec) burst of BSM-EMG activity was recorded which was accompanied by a sharp inflection in UPP (figure 2). We have termed this response the urethrobulbospongiosus (UBS) reflex. At this point the penile clamp was released, fluid (and sometimes a seminal plug) was forcefully expelled from the urethra, and UPP returned toward baseline. While the UPP was falling and for a short time after its return to baseline, additional bursts of BSM-EMG activity (table 1) were recorded with an interburst interval of ~1-4 sec; these bursts were accompanied by transient elevations in intraurethral pressure. We have termed this response the ejaculatory-like response (ELR) (figure 2). Penile clamping alone (i.e. without infusion of the urethra to elevate urethral pressure) did not produce this pattern of BSM-EMG bursting.

When penile clamping was repeated in each rat, there was evidence of desensitization of the ELR (figure 3, table 1). For example, by the 4th or 5th occlusion the number of bursts and the RMS of BSM bursting (30sec) significantly decreased, and the threshold UPP significantly increased. In addition, the duration of the bursting appeared lower by the 5th trail. In contrast to the waning of the ELR, the initial
BSM-EMG burst (the UBS reflex) remained consistent across multiple trials. In summary, the multiple bursting BSM-EMG activity (i.e., ELR) after release diminished over multiple trials. By the 5th trial, the penile clamping-evoked BSM-EMG burst looked very similar to the penile extension-evoked initial BSM-EMG burst i.e. the UBS reflex (see below).

**Penile extension-evoked UGR.** Penile extension produced a linear increase in urethral pressure and small bursts of BSM-EMG activity that were similar to the penile clamping-evoked UGR (table 1). Also, at a similar UPP threshold, a single, large amplitude, high frequency (331 ± 60 spikes/sec), short duration (580 ± 40 msec) burst of BSM-EMG activity was associated with a sharp increase in UPP with forceful expulsion of the infused saline from the urethra (e.g., figures 3 and 4). Therefore, penile extension UGR evoked the UBS reflex. However, the ELR was not observed (figure 3 and table 1). The UBS reflex did not desensitize over multiple trials (table 1).

**Neurectomies.** In sham groups 6 and 7, both the extension and clamping UGR produced similar responses as the control group, confirming that surgical manipulations did not alter the evoked responses. Transection of the SbPN abolished the multiple bursting BSM-EMG activity (i.e., the ELR) (table 2, figure 4B) in clamping UGR, but did not affect the UBS reflex in either clamping or extension UGR. A non-significant increase in latency and threshold UPP (table 2, figure 4B) was also observed in the clamping UGR.

Pelvic nerve transection resulted in a significant increase in the latency and threshold UPP to the first BSM-EMG burst during penile extension UGR, as well as a non-significant increase in threshold UPP and latency in clamping UGR (table 2 and figure 4C). Surprisingly, pelvic neurectomy “unmasked” an ELR following release of penile extension (table 2, figure 4C). This response was similar to the BSM-EMG activity seen with the clamping UGR (figure 4C) in control animals.

Transsection of the HgN did not change either the penile clamping or penile extension-induced UGRs (table 2, figure 4D). Transection of all 3 nerves completely eliminated both the penile clamping- and penile extension-induced BSM-EMG i.e. both UBS and ELR were absent (data not shown).

**Discussion**

The findings of the current study confirm and extend previous reports that have used urethral infusion with penile clamping to elevate urethral pressure and evoke the UGR (9, 29, 31). By comparing extension- and clamping-evoked UGR activity, we have been able to isolate two components, an initial BSM-EMG burst that involves urethral afferent fibers carried by the pelvic and pudendal nerves, which is termed the UBS reflex. The second component of the UGR comprises multiple bursts of BSM-EMG activity that follow release of penile clamping (or release of penile extension in animals with a pelvic neurectomy). These multiple bursts of EMG activity are remarkably similar to BSM-EMG patterns recorded during ejaculation in conscious rats and humans; thus we have termed this clustered pattern of BSM-EMG bursting an ELR (19, 22, 24, 28, 36, 39, 40).

The bursting BSM-EMG activity recorded during the UGR is quite different from the bursting urethral rhabdosphincter EMG activity and BSM EMG activity recorded during voiding in rats (15, 49, Karicheti V. unpublished observations). During voiding, the urethral rhabdosphincter (innervated by pudendal motor neurons like the BSM) shows a rhythmic EMG bursting pattern, but the frequency of the bursting is 6 Hz – much faster than the 0.25 - 0.5 Hz bursting of the BSM-EMG during the UGR (49). Furthermore, the 6 Hz bursting of the urethral rhabdosphincter and pelvic floor muscles only occurs at the peak of a micturition bladder contraction. Since the bladder remained completely empty during the current study, it is not reasonable to think that bladder contractions occurred at any time during our experiments. In addition, the BSM-EMG bursting was seen after bilateral transection of the pelvic
nerves, which eliminates afferent and efferent activity to and from the bladder, respectively. Finally, high frequency contractions of the urethral rhabdosphincter are only seen in rat, while low frequency rhythmic bursting of the BSM during ejaculation is seen in multiple species (6, 10, 43). Considering the above evidence, it is reasonable to speculate that 2 distinct “pattern generators” exist in the rat spinal cord, one activated during micturition and another activated during ejaculation. Future experiments recording EMG activity from both the BSM and urethral rhabdosphincter, simultaneously, while inducing micturition and UGR activation, would be useful to confirm this possibility.

Electrical stimulation of the dorsal nerve of the penis, (a branch of the SbPN) in multiple species including humans and rats, triggers rhythmic bursting of the BSM which mimics ejaculation, suggesting that penile activation alone might trigger ejaculation (38, 46, 48, 54, 55). However, studies conducted in humans have also shown that direct stimulation of the bulbous and prostatic urethra can trigger reflex BSM-EMG bursting which is similar to ELRs (42, 59). In addition, distension of the bulbous urethra produced rhythmic BSM-EMG activity which was reproducible and which disappeared after anesthetization of the bulbous urethra, further suggesting that urethral afferents may also play a role in ejaculation (42). Little direct information is known about the nerves involved in mediating this response. The BSM rhythmic contractions may occur via more than one mechanism, which may depend on other factors, such as the presence or absence of a full erection or the presence of seminal fluids in the urethra. The hypothesis that 3 factors are involved seems reasonable: seminal fluids released during emission act on urethra afferents (chemo sensitive and stretch receptors) to alter the sensitivity of penile (DPN) afferents; penile erection alters the sensitivity of the penile afferents; and that glans penis stimulation immediately prior to ejaculation facilities or triggers the strong muscle contractions to eject the semen.

The ELR, observed in the present study is also similar to BSM-EMG activity evoked by direct stimulation of the medial region of the L3/L4 spinal cord where the lumbar spinothalamic neurons (Lst) i.e. proposed spinal pattern generator neurons for ejaculation (SPGE) are located (1, 6, 14, 50, 51). In behavioral copulation experiments, the SPGE was shown to be essential for ejaculation, as lesions of the Lst neurons resulted in animals that showed normal mounts and intromissions but these animals failed to ejaculate (1, 50). The Lst neurons (SPGE) are activated with the UGR, by both urethral distension and stimulation of the dorsal penile nerve, and contractions of the BSM muscle via dorsal penile nerve stimulation can be prevented by inhibition of mitogen activated protein (MAP) kinase signaling in Lst neurons (29, 46). Thus, it is proposed that the ELR component of the UGR involves activation of the SPGE.

The current study demonstrated that the ELR evoked by penile clamping desensitizes upon repeated activation in an individual animal. However, the UBS remains and shows no desensitization. This remaining single burst of BSM-EMG activity is similar to the BSM-EMG burst produced by penile extension-induced elevations in urethral pressure, as well as the single burst produced by penile clamping-induced urethral pressure elevation when the SbPN is transected. The phenomenon of desensitization of the UGR has been previously suggested, using a different experimental protocol, and was termed ‘exhaustion’ of the response (9). Desensitization is unlikely due to activation of nociceptors or tissue damage as the same phenomenon was observed when either forceps or a plastic microvascular clip was used to clamp the urethral meatus (Karicheti V., Marson L., unpublished observations). In addition, desensitization of the ELR has been seen in our laboratory’s preliminary experiments using electrical stimulation of the dorsal nerve of the penis or the SbPN (31), as well as in behavioral studies of sexual exhaustion after prolonged sexual activity (57, 58). The existence of the UBS that does not desensitize, suggests that desensitization may involve the pattern generator rather than the primary afferents or BSM motor neurons.

The importance of penile afferent fibers in initiating the ELR was also shown by transecting the SbPN, which carries afferent fibers from the penis (dorsal penile nerve) and urethra to the spinal cord
This procedure resulted in an increase in the UPP threshold and a decrease in burst duration and RMS values of both the penile clamping-induced and penile extension-induced UGR. The UBS reflex (a single burst) was present after SbPN cuts, in both procedures, suggesting that this response is mediated by urethral afferents that do not travel through the SbPN (probably through the PelN, see below). The importance of the SbPN in mediating the afferent input of the ELR has previously been shown in the male and female rat in which bilateral transection of the SbPN abolished the UGR (7, 32). Moreover, electrical stimulation of the dorsal penile nerve evokes the ELR; while bilateral transections of the dorsal penile nerve, prevents ejaculation in copulating rats and eliminates the ELR (38).

The role of pelvic afferent fibers was also examined in the present study. Pelvic neurectomy produced increases in the UPP threshold for both the penile clamping and the penile extension evoked UBS, suggesting that some urethral afferent fibers mediating the UGR traverse the pelvic nerve. Surprisingly, pelvic neurectomy also “unmasked” a penile extension-evoked ELR, similar to that produced by the penile clamping-evoked UGR. This finding is important because it suggests that an ELR can be induced by urethral afferent fibers (presumably traversing the pudendal nerve) without substantial input from the glans penis afferent fibers. Since a significant increase in the threshold pressure to evoke the UGR was seen after pelvic neurectomy, we cannot rule out the possibility that this elevated pressure may be responsible for the ELR. An alternative explanation is that there are afferent fibers in the pelvic nerve that may inhibit the activation of the ELR. Thus, it may be that the pelvic nerve contains separate populations of urethral afferent fibers that can excite or inhibit BSM-EMG activation. However, our experimental protocol does not allow us to rule out involvement of pelvic efferent fibers that control urethral smooth muscle tone, and this possibility should be explored in future studies.

Transection of the HgNs did not alter either the penile clamping or penile extension-evoked UGR. While a combination transection of the pelvic, pudendal, and hypogastric nerves eliminated all BSM-EMG activity. These data suggest that the HgN, under normal circumstances, does not contribute to the UGR, while the SbPN and PelN carry all the urethral and penile afferent fibers capable of activating the UGR. This agrees with previous studies examining the UGR in the female rat (7).

Compiling all the results from our studies, we propose a model (figure 5) to describe the activation of BSM-EMG motor neurons resulting from distension of the urethra following its occlusion through clamping of the glans penis or through extension of the penis without clamping. Our model proposes that activation of urethral stretch receptors, whose primary afferent axons are carried in both the SbPN and PelN, initiates a single burst discharge of BSM motor neurons, i.e. the UBS reflex. When the urethral occlusion is released and stretch of the urethra is absent, the driving force for BSM-EMG activity is removed and the BSM motor neurons become quiet. When penile afferent fibers are additionally stimulated by clamping the glans penis to occlude the urethra, the combined input from both penile mechanoreceptors and urethral stretch receptors via thinly myelinated and unmyelinated fibers (A beta, A delta and C fibers) which mediate low threshold slowly adapting afferent responses as well as high threshold noxious stimuli (25, 47), converge on the SPGE located in the L3/L4 spinal cord, which subsequently produces a pattern of repeated activation of BSM motor neurons and results in a cluster of multiple BSM-EMG bursts that resemble ejaculation in behaving rats (i.e. the ELR). Since neither urethral distension alone (i.e. penile extension with urethral infusion) nor penile stimulation alone (i.e. penile clamping without urethral distension) activates the ELR when all innervation is intact, the model includes a convergence of urethral and penile afferent inputs onto a common pathway. The convergence could occur at a number of places in the spinal cord, but since penile and urethral afferent fibers overlap in the L5/L6 dorsal horn and dorsal gray commissure, we believe this is a reasonable proposal (3, 26, 27, 33-35, 52, 53).

For simplicity, the model shows pelvic and pudendal urethral afferent fibers converging on a single neuronal pathway from the SPGE to subsequently produce a single burst of activity from BSM
motor neurons (i.e. the UBS reflex), but the 2 groups of afferent fibers could have separate pathways. Furthermore, the model shows convergence of penile and urethral fibers that is required to produce multiple bursting (i.e. ELR) occurring in the SPGE, but the convergence could occur at various points in the pathway. In addition, the model shows a single pudendal urethral afferent providing input to both the single burst UBS reflex, and multiple burst ELR pathways; and a single pelvic afferent fiber providing both excitatory input to the UBS reflex pathway and an inhibitory input to the pudendal urethral activation of the ELR pathway. However, it is likely that the excitatory and inhibitory inputs arise from separate populations of neurons, and the inhibition could occur at various points in the reflex pathway. Possibly pelvic afferent inhibition of ELR could play a role in coordinating sexual and excretory functions of the urethra.

**Perspectives and significance**

The UGR as originally described by McKenna and colleagues (11, 32) provided a starting point for the subsequent study of various aspects of ejaculatory control, such as identification of L₃/₄ ejaculatory interneurons, supraspinal regulation, and pharmacological studies. It is expected that it will continue to provide physiological and pharmacological information critical to understanding ejaculation in men. The current study provides critical baseline information regarding the “adequate stimulus” to induce ejaculation and the relative importance of primary afferent fibers carried in the sympathetic, parasym pathetic, and somatic nerves innervating the penis and urethra.

The “desensitization” of the UGR after repeated activation, may provide a model applicable to study of the “refractory period” observed during natural copulation. Importantly, the initial BSM-EMG burst did not show desensitization, suggesting that the site of desensitization is the pattern generator as opposed to primary afferent or bulbospongious motor neurons. Characterization of the adequate stimuli to trigger activation of the SPGE, the output of this pattern generator, and the refractory nature of the pattern generator are likely to provide useful insight regarding other spinal pattern generators that control respiration and locomotion. The surprising finding that pelvic nerve afferent fibers can provide an inhibitory signal to ejaculation opens an avenue to future research aimed at understanding coordination of urethral function during micturition and copulation. Obviously it would be disruptive to micturition if urine flow into the urethra produced an ejaculation-like response. It is hoped that our physiological characterization of the UGR provides a useful foundation for continued exploration of a function that is critical for animal husbandry and survival of all vertebrate species, as well as exploration of spinal reflexes and pattern generators in general.


Figure legends

Figure 1. Photographs showing the procedures for extension and clamping UGR. Under the resting conditions the penis remains within the foreskin, the tie through the penile glans is accessible for extending the penis from its foreskin during the UGR procedure. Extension UGR shows the penis extended from the foreskin and the normal kink of the penis (obstructive flexure) that is present which allows urethral pressure to rise during saline infusion. Clamping UGR shows the tip of the penis clamped to provide a closed system during the clamping UGR.

Figure 2. Example of penile clamping-induced urethral occlusion and resulting urethrogenital reflex (UGR) in a control rat. (A) Urethral pressure (top trace) and BSM-EMG (bottom trace). Start of urethral occlusion by penile clamping is indicated by the left, vertical dotted line, and the UPP gradually increases. When the UPP reaches ~ 80 mm Hg, a brief, large amplitude, BSM-EMG burst is recorded that is accompanied by a sharp increase in UPP. Immediately upon detecting the BSM-EMG burst, the clamp is released (noted by the right, vertical dotted line), fluid drains from the urethra and pressure drops. This pressure is recorded as the “threshold pressure”, and the time from start of occlusion to this first BSM-EMG burst is the “latency to first BSM burst”. As UPP drops, additional bursts of brief, large amplitude, BSM-EMG activity associated with sharp increases in UPP are recorded. The duration of bursting activity and the number of bursts are measured. Panel B shows an expanded trace of the BSM-EMG activity. Since the first burst could be reliably induced and exhibited consistent parameters under multiple conditions, it is termed the “urethrobulbospongiosus (UBS) reflex”. Its action potential duration, and RMS value (see tables 1 and 2) were measured. Since the later bursting resembles BSM-EMG activity recorded during ejaculation, it is termed the “ejaculation-like response” (ELR). Panel C is a further expansion of the BSM-EMG trace to show an example of the first burst, UBS reflex. When urethral occlusion is induced by penile extension, only the UBS reflex (i.e. first BSM-EMG burst) is seen.

Figure 3. Examples of urethral perfusion pressure (UPP) and BSM-EMG activity recorded during the 1st (panels A and C) and 5th trials (B and D) of urethral occlusion when induced with penile clamping (A and B) or penile extension (C and D). The first trial with penile clamping-induced occlusion produces a robust ejaculation like response composed of multiple BSM-EMG bursts while the 5th trial results in only a single UBS reflex. In contrast the 1st trial of penile-extension-induced urethral occlusion produces only a single burst,(the UBS reflex) which remained consistent through the 5th trial. The black bar under the UPP indicates the time of urethral occlusion.

Figure 4. Effects of selective neurectomies on the UGR induced by penile clamping (left column) and penile extension (right column) -induced urethral occlusion and UPP and BSM-EMG activity. A. Control responses showing an ejaculation like response (ELR) composed of multiple BSM-EMG bursts. B. Following bilateral transection of the sensory branch of the pudendal nerve (SbPN). Note the loss of the ELR from the penile clamping-induced UGR but preservation of the urethrobulbospongiosus (UBS) reflex in both the penile clamping- and penile extension-induced UGR. C. Following bilateral transection of the pelvic nerve (PelN). Note in extension-induced UGR there is an increase in latency and threshold pressure, and the presence of an ELR (i.e. multiple bursts). D. Following bilateral transection of the hypogastric nerves (HgN) no changes were observed.

Figure 5. A proposed model for UGR pathways based on the results of the current study. Afferent terminals in the urethra are activated by urethral distension during slow filling of the urethra and occlusion of the urethra through either penile clamping or penile extension. These urethral afferents,
carried by both the pelvic nerve (dark blue) and pudendal nerve (green), excite dorsal horn interneurons which in turn relay through the SPGE to produce a single burst of firing in bulbospongiosus muscle (BSM) motor neurons and a single contraction of the BSM (pathway A and inset A in blue). Activation of urethral afferent fibers alone is only sufficient to activate a single burst from BSM motor neurons (UBS reflex). Mechanoreceptive afferent terminals in the glans penis (brown) are activated during penile clamping (but not during penile extension). These afferent fibers cannot activate a single burst from BSM motor neurons when stimulated by penile clamping alone. However, when urethral afferent and glans penis afferent fibers are activated simultaneously by a combination of urethral distension and penile clamping, this convergence of inputs relays through the SPGE to subsequently activate a multiple burst ELR from BSM motor neurons (pathway B and inset B in red), which then produces multiple rhythmic contractions of the BSM and expulsion of urethral fluids (i.e. ejaculation). Since transection of the pelvic nerve “unmasked” an ELR in the absence of penile clamping, an excitatory projection (dashed green line) from pudendal urethral afferent fibers to the ELR pathway is proposed, along with an inhibitory pathway (dashed dark blue line) through an inhibitory interneuron (denoted by the small white circle with the black letter “I”) from the pelvic nerve afferent fibers onto the ELR pathway. Spinal cord transection rostral to the L3 level is required to see this ELR due to descending inhibition from supraspinal centers (e.g. the lateral paragigantocellularis nucleus of the medulla denoted by the large white circle with the black letter “I”), and the model includes this supraspinal inhibitory input to the SPGE. Abbreviations: nucleus of the lateral paragigantocellularis (LPGC), dorsal root ganglion (DRG), nucleus tractus solitarius (NTS) nucleus raphe magnus (NRM), motor nucleus of the 7th cranial nerve (7N), nucleus raphe magnus (NRM), pyramidal tract (py), urethrobulbospongiosus (UBS), and ejaculation-like response (ELR).
A

![Graph A](image)

B

![Graph B](image)

C

![Graph C](image)
Table 1. UGR parameters measured repeatedly across 5 trials

<table>
<thead>
<tr>
<th>Occlusion</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold UPP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clamping</td>
<td>77.9 ± 5.6</td>
<td>70.6 ± 5.4</td>
<td>77.8 ± 5.6</td>
<td>82.9 ± 6.7</td>
<td>88.5 ± 6.3*</td>
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<tr>
<td>Extension</td>
<td>74.8 ± 6.1</td>
<td>74.0 ± 6.1</td>
<td>82.2 ± 7.3</td>
<td>83.0 ± 7.3</td>
<td>80.1 ± 6.5</td>
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<tr>
<td>Latency to 1st BSM burst (sec)</td>
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<td></td>
<td></td>
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<tr>
<td>Clamping</td>
<td>37.0 ± 3.2</td>
<td>27.3 ± 2.0</td>
<td>33.3 ± 4.2</td>
<td>34.6 ± 3.5</td>
<td>36.1 ± 4.4</td>
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<tr>
<td>Extension</td>
<td>38.1 ± 3.1</td>
<td>35.4 ± 2.0</td>
<td>41.8 ± 4.0</td>
<td>38.1 ± 3.9</td>
<td>39.3 ± 3.4</td>
</tr>
<tr>
<td>Duration of BSM bursting (sec)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clamping</td>
<td>15.6 ± 2.3*</td>
<td>19.4 ± 7.8*</td>
<td>17.7 ± 6.4*</td>
<td>14.1 ± 8.6</td>
<td>6.2 ± 3.7</td>
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<tr>
<td>Extension</td>
<td>0.8 ± 0.3</td>
<td>2.1 ± 0.5</td>
<td>0.6 ± 0.0</td>
<td>1.7 ± 0.7</td>
<td>0.5 ± 0.0</td>
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<tr>
<td>BSM bursts (number)</td>
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<td></td>
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<tr>
<td>Clamping</td>
<td>5.3 ± 0.6**</td>
<td>5.3 ± 0.4**</td>
<td>3.7 ± 1.2**</td>
<td>2.8 ± 0.7&quot;</td>
<td>2.2 ± 0.8&quot;**</td>
</tr>
<tr>
<td>Extension</td>
<td>1.2 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.0 ± 0.0</td>
<td>1.3 ± 0.2</td>
<td>1.0 ± 0.0</td>
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<tr>
<td>RMS of BSM bursting (30 sec) (µV)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Clamping</td>
<td>43.4 ± 2.9**</td>
<td>39.2 ± 5.8*</td>
<td>26.2 ± 8.5&quot;</td>
<td>22.9 ± 7.0&quot;&quot;</td>
<td>13.3 ± 5.6&quot;&quot;</td>
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<tr>
<td>Extension</td>
<td>9.4 ± 4.7</td>
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<td>5.0 ± 1.3</td>
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<td>RMS of 1st BSM burst (1 sec) (µV)</td>
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<tr>
<td>Clamping</td>
<td>162.3 ±35.3</td>
<td>154.6 ±38.2</td>
<td>165.2 ±43.1</td>
<td>155.4 ±40.8</td>
<td>160.1±42.0</td>
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<tr>
<td>Extension</td>
<td>194.7±18.4</td>
<td>201.7±18.6</td>
<td>182.1±29.8</td>
<td>189.9±23.1</td>
<td>186.0±26.5</td>
</tr>
</tbody>
</table>

The name of each parameter corresponds to the description in figure 1. Statistically significant differences are in bold type. Values are means ± SE. (n=6 / group). Repeated measures one-way ANOVA was done for each parameter to compare the trials. # p < 0.05; ## p < 0.01; compared with 1st occlusion in respective group (paired Dunnett’s multiple comparison test). Repeated measures two-way ANOVA with Bonferroni’s multiple comparison post-hoc test was done to compare each parameter between clamping and extension groups. * p < 0.05; ** p < 0.01 compared with extension group.
Table 2. UGR parameters after various selective neurectomies

<table>
<thead>
<tr>
<th>Neurectomy</th>
<th>Threshold UPP (mmHg)</th>
<th>Latency to 1st BSM burst (sec)</th>
<th>Duration of BSM bursting (sec)</th>
<th>BSM bursts (Number)</th>
<th>RMS of BSM bursting (30sec) (µV)</th>
<th>RMS of 1st BSM burst (1sec) (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Clamping</td>
<td>Extension</td>
<td>Clamping</td>
<td>Extension</td>
<td>Clamping</td>
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<tr>
<td></td>
<td></td>
<td>Clamping</td>
<td>Extension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clamping</td>
<td>77.9 ± 5.6</td>
<td>74.8 ± 6.1</td>
<td>37.0 ± 3.2</td>
<td>5.3 ± 0.6**</td>
<td>43.4 ± 2.9**</td>
<td>162.3 ± 35.3</td>
</tr>
<tr>
<td>Extension</td>
<td>90.5 ± 9.1</td>
<td>88.5 ± 4.5</td>
<td>62.0 ± 8.1</td>
<td>1.0 ± 0.0**</td>
<td>5.5 ± 0.7***</td>
<td>175.0 ± 18.7</td>
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<tr>
<td></td>
<td>104.1 ± 20.6</td>
<td>138.8 ± 15.7**</td>
<td>66.3 ± 16.7</td>
<td>3.3 ± 0.8</td>
<td>34.5 ± 6.6</td>
<td>112.2 ± 16.1</td>
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<tr>
<td></td>
<td>73.5 ± 2.6</td>
<td>87.0 ± 6.6</td>
<td>31.8 ± 2.5</td>
<td>5.8 ± 0.9**</td>
<td>47.6 ± 10.2**</td>
<td>125.0 ± 27.4</td>
</tr>
</tbody>
</table>

The name of each parameter corresponds to the name of each parameter in figure 1. Statistically significant differences are in **bold** type. Values are means ± SE. (n=6 / group). One-way ANOVA with Dunnett’s multiple comparison test was done for each parameter to compare the neurectomy models. * p < 0.05; ** p < 0.01; compared with control in respective parameter. Two-way ANOVA with Bonferroni’s multiple comparison post-hoc test was done to compare each parameter between clamping and extension. * p < 0.05; ** p<0.01 compared with extension group. Only the 1st UGR is presented in the table, subsequent UGRs showed similar responses.