Published in final edited form as:
J Neural Eng. 2008 June ; 5(2): 144–154. doi:10.1088/1741-2560/5/2/005.

IMPROVED BLADDER EMPTYING IN URINARY RETENTION BY ELECTRICAL STIMULATION OF PUDENDAL AFFERENTS

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Abstract

Urinary retention is the inability to empty the bladder completely, and may result from bladder hypocontractility, increases in outlet resistance, or both. Chronic urinary retention can lead to several urological complications and is often refractory to pharmacologic, behavioral, and surgical treatments. We sought to determine whether electrical stimulation of sensory fibers in the pudendal nerve could engage an augmenting reflex and thereby improve bladder emptying in an animal model of urinary retention. We measured the efficiency of bladder emptying with and without concomitant electrical stimulation of pudendal nerve afferents in urethane anesthetized rats. Voiding efficiency (VE=voided volume/initial volume) was reduced from 72±7% to 29±7% following unilateral transection of the sensory branch of the pudendal nerve (UST) and from 70±5% to 18±4% following bilateral transection (BST). Unilateral electrical stimulation of the proximal transected sensory pudendal nerve during distention-evoked voiding contractions significantly improved VE. Low intensity stimulation at frequencies of 1–50 Hz increased VE to 40–51% following UST and to 39–49% following BST, while high intensity stimulation was ineffective at increasing VE. The increase in VE was mediated by increases in the duration of distention-evoked voiding bladder contractions, rather than increases in contraction amplitude. These results are consistent with an essential role for pudendal sensory feedback in efficient bladder emptying, and raise the possibility that electrical activation of pudendal nerve afferents may provide a new approach to restore efficient bladder emptying in persons with urinary retention.

1. Introduction

The urinary bladder accumulates and stores urine (continence) and evacuates urine at an appropriately selected time and place (micturition or voiding). During voiding the bladder contracts and the external urethral sphincter (EUS) relaxes to allow urine flow. Urinary retention is the inability to empty the bladder completely, and can lead to significant complications. Non-obstructive retention occurs when bladder pressure is insufficient to overcome outlet resistance and may result from bladder muscle hypocontractility (Jonas *et al* 2001), increases in the outlet resistance (Goodwin *et al* 1998), or both. Chronic urinary retention can lead to several urological complications including reflux, upper urinary tract damage, urinary tract infection, and overflow incontinence. Urinary retention is often refractory to pharmacologic, behavioral, and surgical approaches (Aboseif *et al* 2002), and many persons must resort to intermittent self-catheterization to empty their bladder which

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itself is associated with frequent urinary tract infections (Shaker and Hassouna 1998). The purpose of this study was to investigate whether electrical stimulation of pudendal nerve afferents improved bladder emptying in an animal model of urinary retention.

Sensory fibers in the pudendal nerve (afferents) innervating the urethra sense urine flow and reflexly generate positive feedback to enhance bladder contraction strength and duration, to inhibit the external urethral sphincter, and to augment emptying. Fluid flow in the urethra evokes firing in pudendal afferents innervating the urethra in cats (Talaat 1937, Todd 1964) and rats (Le Feber *et al* 1998), and this sensory signal augments the amplitude of ongoing bladder contractions in cats (Barrington 1931, 1941, Garry *et al* 1959) and increases the frequency of isometric bladder contractions in rats (Jung *et al* 1999). Similarly, electrical stimulation of the urethral sensory branch of the pudendal nerve in cats leads to excitation of the bladder (Mazieres *et al* 1997, Jiang and Lindström 1999), inhibition of the external urethral sphincter, and voiding (Shefchyk and Buss 1998). Similar studies in humans have documented generation of bladder contractions by urethral fluid flow (Karlson 1953, Bump 2000, Shafik *et al* 2003a) or intraurethral electrical stimulation (Gustafson *et al* 2003, 2004). Conversely, disruption of the sensory input from the urethra to the augmenting reflex decreases voiding efficiency. Transection of the sensory branch(es) of the pudendal nerve leads to a reduced in voiding efficiency in rats (Cruz and Downie 2005, Peng *et al* 2008), and silencing urethral afferents with intraurethral anesthesia reduces voiding efficiency in rats (Peng *et al* 2008) and humans (Shafik *et al* 2003b).

In this study we sought to determine whether electrical stimulation of pudendal afferents could engage the augmenting reflex and thereby improve bladder emptying in an animal model of urinary retention. We measured the efficiency of bladder emptying with and without concomitant electrical stimulation of pudendal nerve afferents following acute unilateral or bilateral transection of the sensory branch of the pudendal nerve. Transection of the sensory branch(es) of the pudendal nerve led to a significant reduction in voiding efficiency, and voiding efficiency was significantly improved by electrical stimulation of pudendal nerve afferents. These results are consistent with an important role for pudendal sensory feedback in efficient bladder emptying, and raise the possibility that electrical activation of pudendal nerve afferents may provide a new approach to restore efficient bladder emptying in persons with non-obstructive urinary retention.

2. Materials and methods

2.1. Experimental preparation

All animal care and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Duke University. Female Sprague-Dawley rats (n=17) weighing between 270 and 320 g were anesthetized with urethane (1.2 g/kg, s.c.). The tail vein was catheterized for fluid and drug administration, and body temperature was maintained between 36–38° C with a recirculating water blanket.

In this study two types of preparations were used. A first series of experiments (n=7) was conducted with isovolumetric bladder pressure measurements. Bladder volume was maintained by catheterizing the bladder via the urethra with a polyethylene (PE) tube 50 (0.58 mm ID and 0.96 mm OD) tied in place with a ligature around the external urethral orifice. The second series of experiments (n=10) was performed with continuous transvesical infusion cystometry and an open urethra. The urinary bladder was exposed via a midline abdominal incision and a PE tube 50 was inserted into the bladder lumen for bladder pressure measurements. The bladder end of the PE tube was heated to form a collar, passed through a small incision at the apex of the bladder dome, and secured with a purse-string suture. The bladder was filled at 0.12 ml/min with physiological saline at room temperature,

and each trial included at least three voiding contractions. Two insulated silver wire electrodes (0.05 mm diameter) with exposed tips were inserted into the lateral aspects of the mid-urethra to record the electromyogram (EMG) from the EUS. Finally, the abdominal wall was closed with nylon suture.

The PE tube was connected via a 3-way stopcock to an infusion pump and to a pressure transducer (Deltran DPT-100, Utah Medical Products, Midvale, UT, USA) to measure intravesical pressure and control bladder volume. The intravesical pressure and EUS EMG were amplified, filtered, and sampled at 5 kHz (Dash 8Xe, Astro-Med, Inc., RI, USA).

Either unilateral (UST) or bilateral (BST) transection of the sensory branch of the pudendal nerve was performed in each rat. The sensory branches of the pudendal nerves were exposed and transected via a posterior approach by incising the distal portions of the gluteus major muscles (McKenna and Nadelhaft 1986, Pacheco *et al* 1989). The ilium and sacrum bones were separated and the sensory branches of pudendal nerves were isolated and transected. In all rats the proximal end of the transected pudendal sensory branch on one side was mounted in a bipolar cuff electrode for electrical stimulation. Regulated current cathodic, monophasic stimulus pulses were applied with pulse duration of 0.1 ms, amplitude between 0.025 – 1.0 mA, and frequency between 1 – 50 Hz (Pulsar 9bp, FHC Inc., Bowdoinham, ME). The current threshold to evoke the pudendal-pudendal reflex (i.e., an EUS EMG evoked by stimulation of the proximal transected pudendal sensory branch) was measured (n=8) with stimuli applied at 1 Hz.

2.2 Conditional electrical stimulation during isovolumetric bladder contractions

Electrical stimulation was applied during rhythmic isovolumetric bladder contractions present in preparation 1 after bilateral transection of the sensory branch of the pudendal nerves. Initially, the bladder was filled via the urethral catheter at a rate of 0.12 ml/min, and the infusion was stopped when the first isovolumetric bladder contraction appeared (usually with an amplitude larger than 20 cm-H_2O). Conditional electrical stimulation was started at the first peak of the rhythmic bladder contraction pressure and lasted 30 s (figure 2). Stimulation was applied during approximately every other contraction, and the order of presentation of the different stimulus frequencies and amplitudes was randomized. The bladder was emptied every 30–40 minutes with 10 min of equilibration before filling again. The area under the bladder pressure curve during a bladder contraction was measured to quantify the effects of electrical stimulation. For the sample size of 7 and alpha=0.05, the power (1-beta) to detect an effect size equal to one half of one standard deviation was estimated to be 0.41 and to detect an effect size equal to one standard deviation was estimated to be 0.98.

2.3 Conditional electrical stimulation during continuous infusion cystometry

Electrical stimulation was applied during voiding bladder contractions present in preparation 2 after either unilateral (n=4) or bilateral (n=4) transection of the pudendal sensory nerve. Conditional electrical stimulation was triggered at the first peak of the intravesical pressure during the micturition bladder contraction and stopped when the pressure returned to the baseline present before the contraction (figure 4). With the sample size of 4 and alpha=0.05, the power to detect an effect size equal to one half of one standard deviation was estimated to be 0.16 and to detect an effect size equal to one standard deviation was estimated to be 0.53.

2.4 Continuous electrical stimulation during continuous infusion cystometry

Electrical stimulation was applied continuously during bladder filling in preparation 2 after unilateral (n=2) transection of the pudendal sensory nerve (figure 6A) and stopped after the peak pressure during the first micturition contraction.

2.5 Data analysis

Cystometric parameters were measured to quantify the effects of electrical stimulation on voiding: (1) micturition volume threshold (VT), defined as the infused volume of saline sufficient to induce the first voiding contraction; (2) contraction amplitude (CA), the maximal pressure during voiding; (3) bladder contraction duration (CD) during voiding; (4) bladder contraction area, the area of bladder contraction under the bladder pressure curve; (5) intercontraction interval (ICI), the interval between two consecutive voiding contractions; and (6) voiding efficiency (VE), the ratio between voided volume (VV) and the VT. The VV was obtained from the value of VT minus residual volume (RV) of saline withdrawn through the intravesical catheter after the final voiding contraction.

All data are presented as mean ± standard deviation. Analysis of variance (ANOVA) followed by Tukey HSD *post hoc* paired comparisons (SigmaStat, SPSS, Chicago, IL, USA) was used to compare voiding parameters across different conditions, and $p< 0.05$ was considered significant for all analyses.

3. Results

3.1 Electrical stimulation threshold of the pudendal-pudendal reflex

The minimum current amplitude to elicit reflex activation of the EUS (figure 1) by stimulation of the proximal transection pudendal sensory nerve ranged from 0.01 to 0.07 mA (0.05±0.02 mA, n=8), and the latency of the EUS reflex response was 25±0.80 ms.

3.2 Effects of conditional electrical stimulation during isovolumetric bladder contractions

An example from one experiment of the effect of unilateral pudendal sensory nerve stimulation on isovolumetric bladder contractions following bilateral transection of the sensory branch of the pudendal nerves (BST) is shown in figure 2. The 30 s epochs of stimulation at 1 Hz, which started at the first peak of the contraction, had either excitatory or inhibitory effects depending on the stimulation intensity. Stimulation at lower amplitudes (0.025–0.1 mA) appeared to increase the duration of the contractions, and thus the contraction area, as compared to the contractions without stimulation, whereas higher stimulation amplitudes (0.4–1.0 mA) appeared to decrease the bladder contraction duration. The bladder pressure decreased sharply after the onset of higher amplitude stimulation, and thus the bladder contraction duration was reduced.

The apparent excitatory and inhibitory effects of pudendal afferent stimulation on bladder contraction area were similar at other stimulation frequencies and were observed consistently in all experiments. Figure 3 summarizes the effects on the bladder contraction area of unilateral electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve at different stimulation frequencies and amplitudes (n=503 trials across 7 rats). Two factor ANOVA indicated that the absolute contraction area was dependent on the stimulation amplitude ($p<0.001$), but our results did not demonstrate a significant effect of stimulus frequency ($p=0.097$). Further, post-hoc paired comparisons (Tukey HSD) indicated that the contraction area was larger than control with low intensity stimulation (I=0.025–0.02 mA, $p<0.05$) and smaller than control with higher intensity stimulation (I=0.4–1.0mA, $p<0.05$). Following BST, unilateral pudendal sensory nerve

stimulation at low amplitudes augmented isovolumetric bladder contractions, and stimulation at high amplitudes inhibited isovolumetric bladder contractions.

3.3 Effects of conditional electrical stimulation following unilateral sensory nerve transection during continuous infusion cystometry

Subsequently stimulation was applied during open urethra cystometry to determine the effects on bladder emptying. Examples of the effects of conditional unilateral electrical stimulation of the proximal transected pudendal sensory nerve branch following unilateral transection of the sensory branch of the pudendal nerve (UST) are shown in figure 4A, and the cystometric parameters are summarized in Table 1. Acute UST reduced the bladder contraction amplitude, the bladder contraction duration, the bladder contraction area, and the voiding efficiency as compared to prior to UST (Table 1). The lower voiding efficiency appeared to decrease the intercontraction interval (figure 4A), since there was a larger residual volume after the impaired contractions and at a constant filling rate the bladder again reached threshold volume in less time, but the reductions in intercontraction interval were not significant (Table 1).

Conditional low amplitude (0.05 mA) stimulation following UST increased the contraction duration and the voiding efficiency, leading to an apparent increase in intercontraction interval (figure 4A), but again the latter was not significant. The bladder contraction area was reduced to 43 % of control following UST, but augmented significantly to 70–97 % of control by low amplitude conditional electrical stimulation (Table 1). Conditional higher amplitude (0.2 mA) stimulation appeared to cause immediate inhibition of the bladder, but the reductions in contraction area and voiding efficiency were not significant (figure 4A, Table 1).

A summary of the changes in voiding efficiency following UST and conditional unilateral stimulation of the proximal transected sensory branch of the pudendal nerve is shown in figure 5A. The voiding efficiency was reduced significantly from 72 ± 6.9 % in control conditions (C_0) to 29 ± 6.9% following UST. Conditional low amplitude stimulation increased the absolute voiding efficiency to ~ 50 %, and considering the parameters that caused significant changes in voiding efficiency, electrical stimulation increased the mean voiding efficiency by 71 %. Two factor ANOVA revealed that the voiding efficiency was dependent on the stimulation amplitude (p=0.038), but our results did not demonstrate a significant effect of stimulus frequency (p=0.321). Post-hoc paired comparisons indicated that the enhancement in bladder emptying was significant with both 1 Hz and 20 Hz stimuli at 0.05 mA. Changes in voiding efficiency with other combinations of parameters did not reach statistical significance. The lack of an effect of stimulation frequency may have reflected the sample size, yet this was appropriate for an initial feasibility study designed to detect robust effects of stimulation. In considering the effects of conditional stimulation following UST, the average effect of stimulation as compared to no stimulation was 0.65 standard deviations, and our sample size enabled detection of this effect size with a power of 0.49. Similarly, the average difference in voiding efficiencies across stimulation conditions was 0.75 standard deviations, and our sample size enabled detection of this effect with power of 0.63. Thus, conditional electrical stimulation of the pudendal sensory nerve with appropriate parameters augmented contraction area and bladder emptying following unilateral sensory pudendal nerve transection.

3.4 Effects of conditional electrical stimulation following bilateral sensory nerve transection during continuous infusion cystometry

Examples of the effects of conditional unilateral electrical stimulation of the proximal transected pudendal sensory nerve branch following BST are shown in figure 4B, and the

cystometric parameters are summarized in Table 2. Acute BST reduced the contraction amplitude, the bladder contraction area, and the voiding efficiency, and the lower voiding efficiency resulted in a significant reduction in the intercontraction interval.

Conditional low amplitude (0.05 mA) unilateral stimulation following BST increased the contraction area and the voiding efficiency. The bladder contraction area was reduced to 38 % of control following BST, but augmented significantly to 45–88 % of control by low amplitude conditional electrical stimulation (Table 2). Conditional stimulation at larger amplitudes also appeared to increase contraction area following BST, except at the largest amplitude and higher frequencies, but these changes were not significant.

A summary of changes in voiding efficiency following BST and conditional unilateral stimulation of the proximal transected pudendal sensory nerve branch is shown in figure 5B. Bilateral sensory branch transection led to an even larger reduction in voiding efficiency than UST (from 70 % in control to 18 % following BST). Conditional low amplitude stimulation again increased the absolute voiding efficiency to ~ 50 %, and considering the parameters that caused significant changes in voiding efficiency, electrical stimulation increased the mean voiding efficiency by 132%. As was the case following unilateral transection, low amplitude stimulation across frequencies increased voiding efficiency, and two-factor ANOVA revealed that low amplitude stimulation produced greater voiding efficiency at all stimulus frequencies (p=0.013 for stimulation amplitude and p=0.058 for stimulation frequency). Stimulation at 1 Hz at 0.05–0.2mA, at 20 Hz at 0.05–0.1 mA, or at 50 Hz at 0.05 mA all increased voiding efficiency (Table 2). Thus, unilateral conditional electrical stimulation of the pudendal sensory nerve augmented bladder emptying following bilateral sensory pudendal nerve transection and restored bladder emptying to levels similar to those generated by stimulation following UST.

3.5 Effects of continuous electrical stimulation following unilateral sensory nerve transection during continuous infusion cystometry

The results of the isovolumetric and conditional stimulation experiments demonstrated that the bladder response was determined primarily by the current intensity, with low current intensities most likely to augment bladder contractions and voiding and high current intensities most likely to inhibit bladder contractions. Therefore, stimulation amplitudes of 0.025 mA and 0.5 mA were selected to determine whether the same excitatory and inhibitory effects were also evoked by continuous electrical stimulation of pudendal afferents during continuous infusion cystometry. Examples of the changes in the cystometrogram following UST with and without continuous 20 Hz stimulation at either 0.025 mA or 0.5 mA are shown in figure 6. The volume threshold for evoking the bladder contraction was reduced to 80–87 % of the volume threshold following UST by continuous electrical stimulation at 0.025 mA, while the volume threshold was increased to 120–125% of those following UST by continuous electrical stimulation at 0.5 mA (figure 6B). Two-factor ANOVA indicated that the absolute volume threshold was dependent on the stimulation amplitude (p<0.001), but our results did not demonstrate a significant effect of stimulus frequency (p=0.411). Further, post-hoc paired comparisons indicated that the volume threshold was larger than control with higher intensity stimulation (I=0.5mA, p=0.022), but there was no apparent effect of low amplitude stimulation at any frequency (I=0.025 mA, p=0.19).

Bladder contraction areas were increased by low amplitude stimulation at 20 Hz to 112–138% of those following UST, while higher amplitude stimulation reduced the bladder contraction areas to 59–72 % of those following UST across all stimulation frequencies (figure 6C). As with conditional stimulation, the absolute contraction area was dependent on the stimulation amplitude (p<0.001), but not the stimulation frequency (p=0.68). Further,

post-hoc comparisons indicated that the contraction area was larger than control (i.e., augmented) with low intensity stimulation (I=0.025mA, p=0.046) and smaller than control (i.e., inhibited) with higher intensity stimulation (I=0.5mA, p=0.032).

Similarly, the voiding efficiency was significantly increased from ~ 30 % following UST to 40–45 % by continuous low amplitude stimulation, while higher intensity stimulation reduced the average VEs to 19–24 %, but these changes were not significant (figure 6D). The voiding efficiency was dependent on the amplitude (p<0.001) of continuous electrical stimulation, but our results did not demonstrate a significant effect of frequency (p=0.605). Voiding efficiency with stimulation at 0.025 mA was larger than following UST (p=0.005), but the reductions in voiding efficiency with stimulation at 0.5 mA were not significant (p=0.225).

4. Discussion

The objective of this study was to determine whether electrical stimulation of pudendal nerve afferents improved voiding efficiency in rats following acute transection of the sensory branch of the pudendal nerve(s). Either unilateral or bilateral transection of the sensory branch of the pudendal nerve reduced voiding efficiency (Cruz and Downie 2005, Peng *et al* 2008), and subsequent unilateral stimulation of the proximal transected pudendal sensory nerve either excited or inhibited the bladder depending on the stimulation current amplitude. Smaller current amplitudes increased the area (pressure-time integral) of isovolumetric bladder contractions, whereas larger current amplitudes reduced the area of isovolumetric contractions. The amplitude-dependent excitation or inhibition of the bladder was also observed during voiding contractions with an open urethra, and conditional low amplitude stimulation significantly enhanced voiding efficiency by 71 % and 132 % following UST or BST, respectively. Further, these effects were observed both when stimulation was delivered conditionally during the contraction and when stimulation was delivered continuously during bladder filling.

The minimum stimulation current amplitude to elicit the pudendal-EUS reflex (0.05±0.02 mAwith 0.1 ms duration pulses) was similar to thresholds (0.003–0.025 mA with 0.2 ms duration pulses) reported previously (McKenna and Nadelhaft 1989), and the latency (25±0.80 ms) was also consistent with previously reported latencies of 25–30 ms (McKenna and Nadelhaft 1989). We did not detect an early latency EMG response observed previously (12±2.4 ms), presumably because transection of the sensory branch of the pudendal nerve eliminated any direct motor response (McKenna and Nadelhaft 1989). We transected the anatomically defined sensory branch(es) of the pudendal nerve (McKenna and Nadelhaft 1986, Pacheco *et al* 1989, 1997), and although there may be motor axons within the “sensory” branch of the nerve, EUS activity was still present during bladder filling and voiding (Peng *et al* 2008).

The use of monophasic stimulation can result in shifts in the electrode potential that enable potentially damaging electrochemical reactions (Merrill *et al* 2005) including changes in pH (Swiontek *et al* 1980). Since the present study employed monophasic stimulation, the products of these reactions could have impacted neuronal excitability, and increased the variance of the results. We randomized the delivery of different stimulation parameters, and apparently the differences in responses to different stimulus parameters were large enough to overcome any changes in nerve excitability due to monophasic stimulation.

Previous studies in rats have reported exclusively bladder inhibition by pudendal afferent stimulation, but this difference can be explained by the strong dependence of the polarity of the bladder response on the stimulation intensity. Excitation of the bladder was evoked at stimulus strengths of 0.5–4 times the threshold of the pudendal-EUS reflex, whereas

previous reports of inhibition in rat used stimuli (0.8 mA) that were sixteen times our measured threshold (Jiang and Lindstrom 1998). Similarly, large amplitude stimulation of pudendal afferents produced bladder inhibition in both cat (Lindstrom *et al* 1983, Mazieres *et al* 1998, Tai *et al* 2006) and human (Vodusek *et al* 1986, Ohlsson *et al* 1989), although both frequency and stimulus train duration also play an important role in determining the response polarity in cat (Boggs *et al* 2006).

The differential responses evoked at low and high stimulation intensities may arise from activation of different classes of pudendal afferents according to their diameters. Pudendal afferents comprise a wide range of fiber types, including myelinated Aβ and Aδ fibers, unmyelinated c-fibers, and possibly Aα fibers (Bradley *et al* 1973, Perl 1992, Yoshimura *et al* 2003). Larger diameter nerve fibers generally have lower thresholds for extracellular stimulation (Fang and Mortimer 1991), and the myelinated Aα or Aβ fibers should have lowest threshold while the unmyelinated c-fibers have the highest stimulation threshold (Li and Bak 1976). These results are consistent with bladder excitation resulting from activation of the larger myelinated A-type fibers at low stimulation amplitudes and inhibition resulting from the inhibitory urethro-vesical reflex mediated by urethral c-fibers (Thor and Muhlhauser 1999) activated by higher stimulation amplitudes.

The voiding efficiency in intact anesthetized rats before any nerve transections or electrical stimulation was only ~ 70%, which is lower than the ~98–99% voiding efficiency measured in conscious animals (Yaksh *et al* 1986, Walter *et al* 2005). Similar low voiding efficiencies were reported in other studies (Cheng and de Groat 2004, Cruz and Downie 2005, Peng *et al* 2006), and presumably resulted from the urethrane anesthesia inhibiting reflex bladder contractions and reducing the contraction pressure during micturition (Yaksh *et al* 1986). The physiology of the bladder and the EUS appear to be preserved under urethane, and urethane is "*the most suitable anesthetic for physiological experiments that require demonstration of reflex micturition*" (Matsuura and Downie 2000). Although anesthetic effects are a limitation of the present studies, decreases in voiding efficiency due to nerve transection and subsequent changes in voiding efficiency during sensory nerve stimulation were still readily detected.

The effects of pudendal sensory nerve stimulation are primarily transient, but short-term carryover effects do exist. Continuous stimulation of inhibitory genital afferents for 5 minutes produced protracted inhibition of bladder-to-bladder reflexes for 5–25 min in the cat (Jiang and Lindström 1999), and increased distention evoked reflex contraction volume thresholds for at up to 40 minutes in rats (Jiang and Lindström 1998). Bladder-to-bladder reflexes were enhanced for at least 60 min following 5 minutes of stimulation of either bladder afferents or urethral afferents in cats (Jiang and Lindström 1999), and similar effects were also observed in rats (Jiang and Lindström 1996). However, these effects are not likely to have influenced the present results as the order of stimulation was randomized across stimulation frequency and amplitude both within and across animals, and, except for the continuous stimulation experiments (figure 6), the duration of stimulation was limited to 30 s epochs. The mean difference in the area of control contractions ranged from 7% to 12% of the mean control contraction area across the 7 isometric experiments, and significant excitatory and inhibitory effects of stimulation on contraction area were readily detected with this level of variance.

There are important limitations of the acute nature of our model of retention. We used acute unilateral or bilateral transection of the sensory branch of the pudendal nerve to create a model of urinary retention. Nerve transection did indeed reduce voiding efficiency, and similar reductions in voiding efficiency were observed following urethral anesthesia (Peng *et al* 2008), thereby illustrating the importance of urethral sensory feedback in voiding

efficiency. However, this model does not include the long-term changes that may accompany chronic damage to the pudendal nerves or chronic urinary retention. Bilateral transection or crush of the pudendal nerve in rats produced changes in voiding behavior, short-term reductions in voided volume that recovered by two weeks after crush, and no changes in urinary frequency (Heidkamp *et al* 1998, Kerns *et al* 2000, Sakamoto *et al* 2000). Chronic transection of the pudendal nerves produced reductions in detrusor contraction amplitude accompanied by reductions in voiding efficiency that were attributed to the loss of pudendal sensory feedback as well as changes in the EMG burst pattern (Peng *et al* 2006). Further, plastic changes in reflexes may occur following chronic retention (Steers and de Groat 1988, Steers *et al* 1991, Vizzard 2006) and these changes were not reflected in our animal model. Finally, degeneration of transected sensory fibers in the chronic model may leave them unavailable for stimulation, and although studies show robust reinnervation of the external urethral sphincter by pudendal motor axons (Heidkamp *et al* 1998, Kerns *et al* 2000, Sakamoto *et al* 2000, Peng *et al* 2006), the fate of damaged or transected sensory neurons is not clear.

Another limitation is the use of the rat as our model of urinary retention. Although rats are widely used for physiological and pharmacological studies of the lower urinary tract (de Groat *et al* 1993), the rat (and dog) exhibits phasic patterns of EUS activity during voiding, in contrast to the (complete) relaxation of the sphincter observed in humans (and cats). The phasic EUS activation is essential to efficient voiding in the rat (Streng *et al* 2004) and is disrupted following transection of the sensory branch of the pudendal nerve or urethral anesthesia (Peng *et al* 2008). Therefore, it is not clear whether the present results will translate to human, where the neural control of EUS activity differs from that in the rat.

Unilateral electrical stimulation of pudendal nerve afferents improved voiding efficiency in the rat following acute pudendal sensory nerve transection. This is consistent with an important role for pudendal urethral afferents activating the augmenting reflex to produce efficient voiding in cats (Barrington 1931, 1941) and rats (Peng *et al* 2008). There is also evidence suggesting that the augmenting reflex operates in humans. Silencing urethral afferents with anesthesia reduces voiding efficiency (Shafik *et al* 2003b); conversely, activation of urethral afferents by urethral fluid flow (Karlson 1953, Bump 2000, Shafik *et al* 2003a) or intraurethral electrical stimulation (Gustafson *et al* 2003, 2004) generates bladder contractions in humans. Our concept is to use electrical stimulation to amplify the sensory feedback from pudendal urethral afferents to increase bladder contraction amplitude and/or duration – and this may provide a means to enhance bladder emptying in persons with retention.

Acknowledgments

This study was supported by the U.S. National Institutes of Health Grant R01 NS050514 to W.M. Grill and the National Science Council (095-2917-I-006-002), Taiwan, R.O.C. to C.W. Peng. The authors thank Gilda Mills for her outstanding technical assistance.

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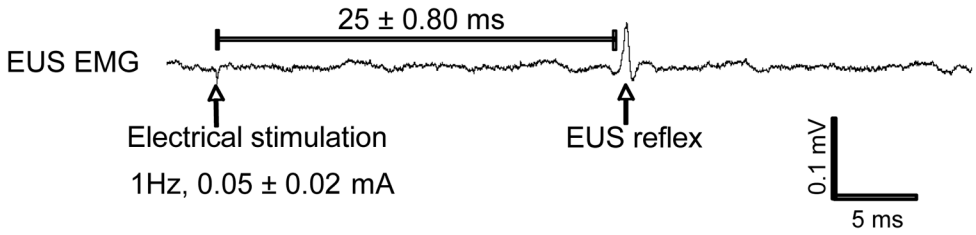

Figure 1.
Reflex electromyographic response in the external urethral sphincter evoked by electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve.

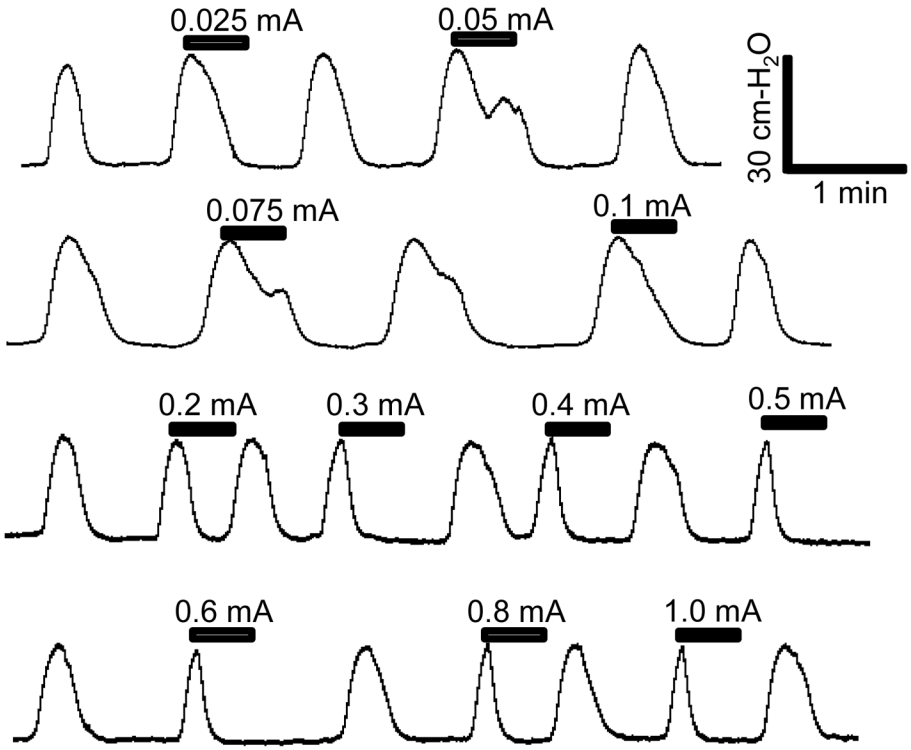

Figure 2.
Effects of unilateral electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve on isometric reflex bladder contractions. The horizontal bars indicate the duration of stimulation, which started from the peak of bladder contraction pressure and lasted for 30 s. The stimulus trains were all at 1 Hz with 0.1ms pulse widths.

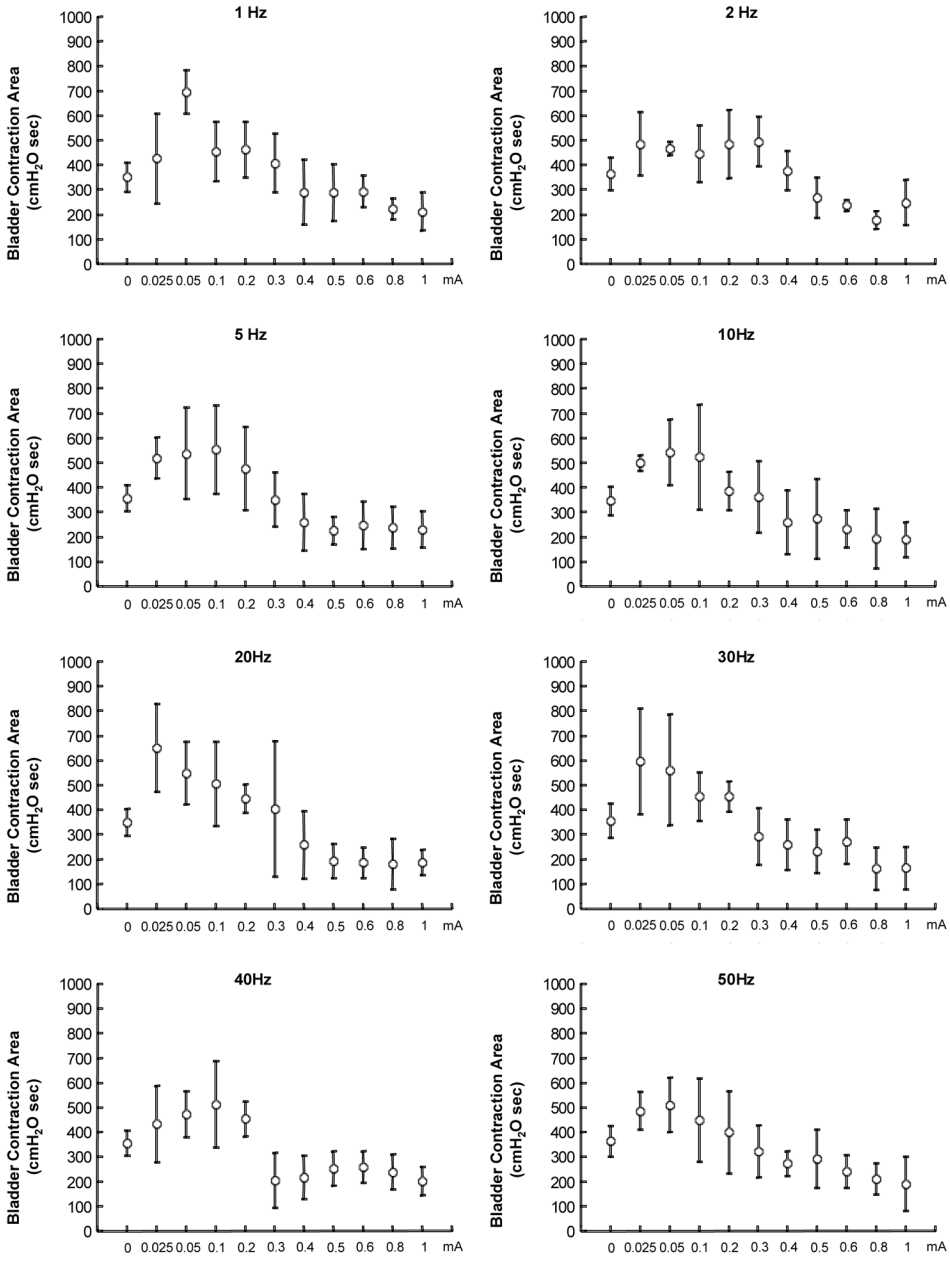

Figure 3.

Changes in the absolute area of isometric reflex bladder contractions (mean±s.d) generated by unilateral electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve (n=503 trials across 7 rats, all with bilateral transection of the sensory branch of the pudendal nerves). The absolute contraction area was dependent on the stimulation amplitude ($p<0.001$), but not the stimulation frequency ($p=0.097$, ANOVA). The contraction area was larger than control with low intensity stimulation (I=0.025–0.02 mA, $p<0.05$) and smaller than control with higher intensity stimulation (I=0.4–1.0mA, $p<0.05$, Tukey HSD).

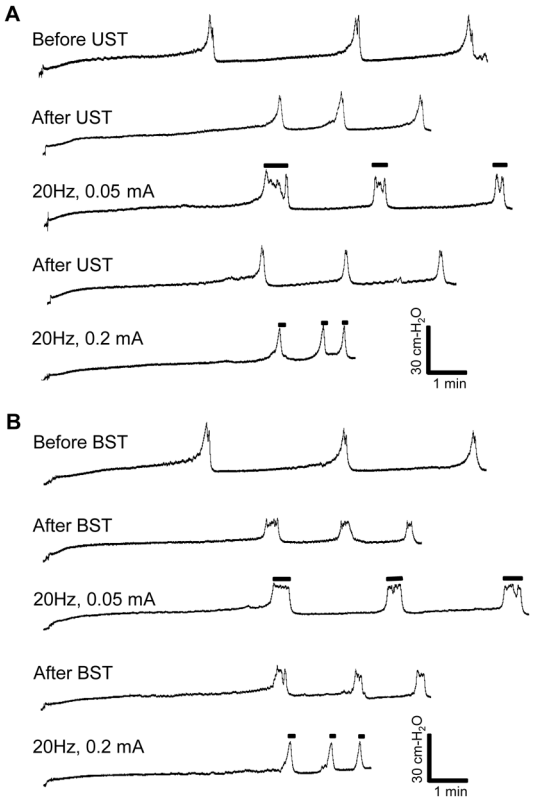

Figure 4.

Effects of conditional unilateral electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve on the cystometrogram (CMG) during continuous bladder infusion. (**A**) Effects of stimulation following unilateral transection of the sensory branch of the pudendal nerve. Control CMG before any nerve transection, CMG after unilateral transection of the sensory branch of the pudendal nerve (UST), CMG after UST with conditional electrical stimulation (20 Hz, 0.05 mA) applied during the reflex bladder contractions, intervening control CMG, and CMG with conditional electrical stimulation (20 Hz, 0.2 mA) applied during the reflex bladder contractions. (**B**) Effects of stimulation following bilateral transection of the sensory branch of the pudendal nerves. Control CMG before any nerve transection, CMG after bilateral transection of the sensory branch of the pudendal nerves (BST), CMG after BST with conditional electrical stimulation (20 Hz, 0.05 mA) applied during the reflex bladder contractions, intervening control CMG, and CMG with conditional electrical stimulation (20 Hz, 0.2 mA) applied during the reflex bladder contractions. The horizontal bars indicate the duration of stimulation, and all traces are from the same rat.

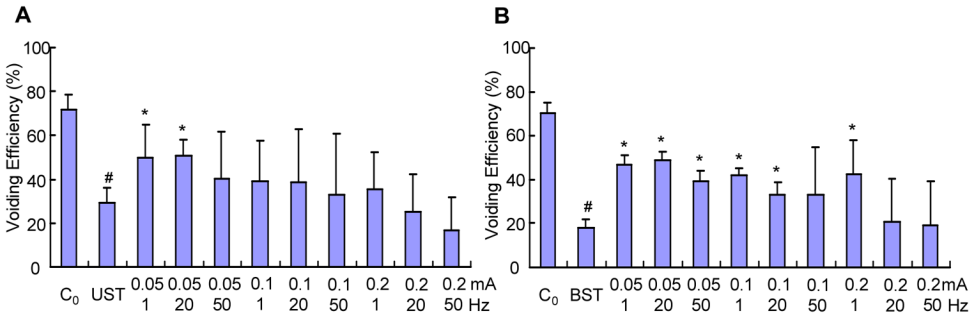

Figure 5.

The changes in voiding efficiency following (**A**) unilateral (UST) or (**B**) bilateral (BST) transection of the sensory branch of the pudendal nerve(s) and unilateral electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve with different amplitudes and frequencies. Each bar represents the mean ± standard deviation (n=4 rats in A, and 4 rats in B). # indicates a significant difference ($p< 0.05$) between before (C0) and after UST or BST, and * indicates a significant difference ($p< 0.05$) in after either UST or BST and trials on which stimulation was applied.

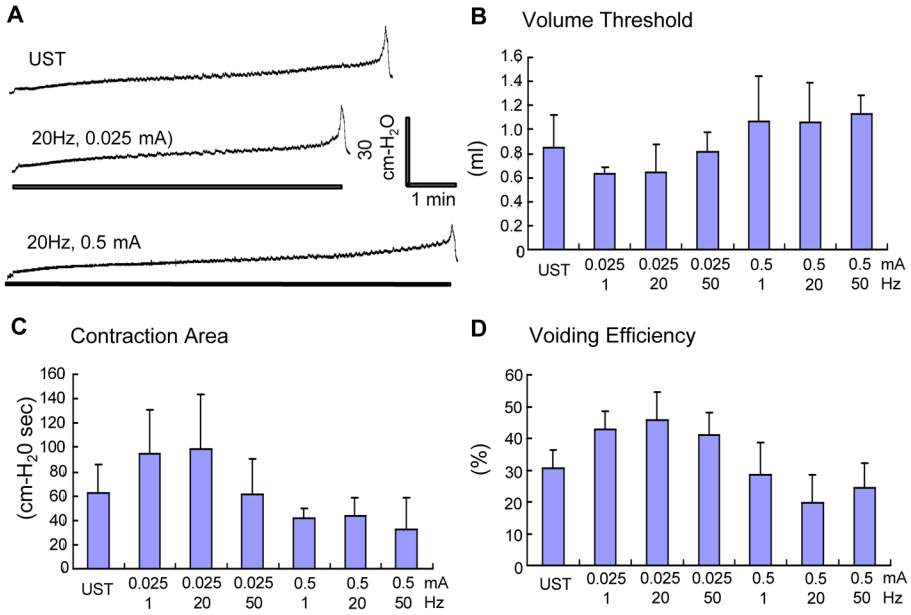

Figure 6.

Effects of continuous unilateral electrical stimulation of the proximal end of the transected sensory branch of the pudendal nerve on the cystometrogram (CMG) during continuous bladder infusion following unilateral sensory branch transection (UST). (**A**) Examples of CMGs following UST, during excitatory stimulation (20Hz, 0.025 mA), and during inhibitory stimulation (20Hz, 0.5 mA). (**B**) Changes in the volume threshold with continuous electrical stimulation at different frequencies and amplitudes. The volume threshold was dependent on the stimulation amplitude ($p<0.001$), but not the stimulation frequency ($p=0.411$, ANOVA). The volume threshold with 0.5 mA stimulation was larger than control ($p=0.022$) and larger than with 0.025 mA stimulation ($p<0.001$), but the volume threshold with 0.025 mA stimulation was not different than control ($p=0.19$, Tukey HSD), (**C**) Changes in the absolute bladder contraction area with continuous electrical stimulation at different frequencies and amplitudes. The contraction area was dependent on the stimulation amplitude ($p<0.001$), but not the stimulation frequency ($p=0.68$, ANOVA). The contraction area was larger than control with 0.025 mA stimulation ($p=0.046$) and smaller than control with 0.5 mA stimulation ($p=0.032$, Tukey HSD) (**D**) Changes in voiding efficiency with continuous electrical stimulation at different frequencies and amplitudes. The voiding efficiency was dependent on stimulation amplitude ($p<0.001$) but not frequency ($p=0.605$). Voiding efficiency was larger than control with 0.025 mA stimulation ($p=0.005$), but not different than control with 0.5 mA stimulation ($p=0.225$, Tukey HSD). Each bar represents the mean ± standard deviation ($n=6$ trials across two rats).

Table 1

Effects of Conditional Unilateral Electrical Stimulation on Cystometric Parameters following Unilateral Transection of the Sensory Branch of the Pudendal Nerve (UST)

	Contraction amplitude (cm-H_2O)	Contraction duration (sec)	Intercontraction interval (min)	Contraction area (cm-H_2O x sec)	Voiding efficiency (%)
Before UST	39.4±1.0	30.9±4.2	3.56±0.80	274.0±93.8	71.7±6.9
After UST	30.3±2.4#	21.2±1.7#	2.66±0.86	118.4±18.7#	29.4±6.9#
1Hz, 0.05mA	32.5±3.7	30.2±6.6*	4.9±3.12	191.1±53.2*	49.7±15.2*
20Hz, 0.05mA	33.5±2.2	35.0±4.1*	5.19±2.60	266.7±67.8*	50.8±7.5*
50Hz, 0.05mA	32.7±4.1	32.6±9.2*	4.61±3.31	240.1±67.4*	40.2±21.4
1Hz, 0.10mA	30.8±5.9	28.4±10.4	4.85±3.26	172.4±69.2	39.5±18.3
20Hz, 0.10mA	30.7±5.9	24.0±9.1	4.91±3.85	134.0±64.2	38.9±24.2
50Hz, 0.10mA	30.2±5.2	21.8±10.4	4.08±3.79	121.0±38.4	33.0±27.7
1Hz, 0.2mA	28.3±4.2	24.0±6.9	4.36±2.51	132.1±26.3	35.7±16.7
20Hz, 0.20mA	30.4±5.0	19.6±7.6	2.28±1.33	96.1±41.0	24.9±17.4
50Hz, 0.20mA	29.7±5.6	19.0±5.6	2.59±2.51	95.1±47.3	16.5±15.7

Values are mean±SD, n=4.

# $p < 0.05$ indicates a significant difference in rats between before and after UST.

* $p < 0.05$ indicates a significant difference in UST rats between before and after conditional ES.

Table 2

Effects of Conditional Unilateral Electrical Stimulation on Cystometric Parameters following Bilateral Transection of the Sensory Branch of the Pudendal Nerve (BST)

	Contraction amplitude (cm-H_2O)	Contraction duration (sec)	Intercontraction interval (min)	Contraction area (cm-H_2O x sec)	Voiding efficiency (%)
Before BST	37.5±4.8	34.8±7.8	3.98±0.72	339.3±181.0	70.1±4.9
After BST	25.6±6.5#	37.3±10.7	2.56±1.12#	130.0±60.2#	18.2±3.5#
1Hz, 0.05mA	23.2±7.5	51.8±23.1	3.00±1.12	152.0±63.3*	46.7±4.6*
20Hz, 0.05mA	27.1±5.6	57.1±21.2	4.58±1.58	296.7±153.0*	49.1±3.5*
50Hz, 0.05mA	26.1±5.2	60.4±28.3	4.82±1.74	280.8±100.1*	39.1±5.2*
1Hz, 0.10mA	29.5±4.4	46.9±8.4	4.39±2.39	357.2±208.0	42.0±3.4*
20Hz, 0.10mA	25.2±5.4	34.3±7.1	2.57±1.31	230.7±104.0	33.2±5.4*
50Hz, 0.10mA	26.8±5.5	36.6±11.1	5.08±3.39	256.6±151.5	33.1±21.8
1Hz, 0.2mA	28.7±5.7	39.2±8.6	4.46±1.62	339.9±239.1	42.6±15.6*
20Hz, 0.20mA	26.4±6.9	18.7±8.1	2.16±2.63	109.6±83.9	20.8±19.5
50Hz, 0.20mA	21.6±5.5	22.4±15.7*	2.22±2.00	55.9±33.8	18.9±20.3

Values are mean±SD, n=4.

# $p < 0.05$ indicates a significant difference in rats between before and after BST.

* $p < 0.05$ indicates a significant difference in BST rats between before and after conditional ES.