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Bilateral pudendal afferent stimulation improves bladder emptying in rats with urinary retention

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Abstract

- To determine whether bilateral electrical stimulation (BiES) of the transected pudendal sensory nerves could further enhance the voiding efficiency beyond that produced by unilateral electrical stimulation (UniES) of transected pudendal afferents in rats with urinary retention.
- The efficiency of bladder emptying with either UniES or BiES of pudendal nerve afferents was measured after acute bilateral transection of the sensory branch of the pudendal nerve.
- The effects of UniES and BiES on voiding in a partially denervated bladder and acute spinal transection, respectively, were determined.
- The voiding efficiency (VE) was reduced from 69 to 22% after bilateral transection of the sensory branch of the pudendal nerve. UniES or BiES increased the VE to 49–62%.
- Although in most instances BiES consistently generated more efficient bladder emptying than did UniES, these differences were not significant.
- Both UniES and BiES increased VE after unilateral pelvic nerve transection, demonstrating efficacy in a partially denervated bladder.
- The enhancement of VE by either UniES or BiES was preserved after acute T₉–T₁₀, demonstrating the spinal origin of this augmenting reflex.
- The results of the present study are consistent with an essential role for pudendal sensory feedback in efficient bladder emptying, and unilateral and bilateral electrical activation of pudendal nerve afferents are equally efficient in improving bladder emptying in an animal model of urinary retention.

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- This could provide an approach to improve bladder emptying in patients with non-obstructive urinary retention.

Keywords

denervated; voiding efficiency; pelvic nerve

INTRODUCTION

The lower urinary tract has two main functions: storage of urine (continence) and periodic elimination of urine (micturition or voiding). During micturition, the bladder contracts and the external urethral sphincter (EUS) relaxes to allow urine flow. Non-obstructive chronic urinary retention is caused primarily by a hypocontractile detrusor (weak contraction), although the detailed pathological mechanisms are still unclear [1]. The inability to empty the bladder efficiently can result in several urological complications, including reflux, upper urinary tract damage, UTIs and overflow incontinence. Retention in many patients is refractory to standard therapies [2], and most patients must resort to self-catheterization, which results in frequent UTIs and reduced quality of life [3].

We demonstrated previously that unilateral electrical stimulation (UniES) of the pudendal sensory nerve improved bladder emptying in a rat model of urinary retention, created by acute bilateral transection of the pudendal sensory nerve [4]. This approach was premised on electrical activation of the 'augmenting reflex' where excitation of pudendal urethral afferents facilitated bladder contraction and emptying [5,6]. During micturition, fluid flow in the urethra evokes firing in pudendal afferents [7], and this sensory signal activates reflex augmentation of the amplitude and frequency of ongoing bladder contractions [5,6,8]. Conversely, transection of the pudendal nerves or intra-urethral anaesthesia reduces voiding efficiency in rats [9] and humans [10]. Thus, electrical stimulation provides a potential means to restore or amplify the augmenting reflex in cases in urinary retention.

In the present study, we sought to determine whether bilateral electrical stimulation (BiES) of the pudendal sensory nerve could further enhance the voiding efficiency (VE) beyond that produced by UniES. For example, bilateral stimulation of the sacral roots produced bladder inhibition with stimulation intensities that were sub-threshold for inhibition with unilateral stimulation [11]. We measured the VE with either UniES or BiES of pudendal nerve afferents after acute bilateral transection of the sensory branch of the pudendal nerve. Further, we extended our previous studies to determine the effects of UniES and BiES on voiding in a partially denervated bladder and after acute spinal transection.

MATERIALS AND METHODS

All animal care and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Taipei Medical University. Female Sprague–Dawley rats ($n = 23$) weighing 200–250 g were anaesthetized with urethane (1.2 g/kg, s.c.). The tail vein was catheterized for fluid and drug administration, and the body temperature was maintained at 36–38°C with a recirculating water blanket.

Two different preparations were used to quantify the effects of pudendal afferent stimulation on isovolumetric reflex bladder contractions and on bladder emptying. In the isometric experiments ($n = 8$) bladder volume was maintained by catheterizing the bladder via the urethra with a polyethylene (PE) tube 50 (0.58 mm internal diameter and 0.96 mm outside diameter) tied in place with a ligature around the external urethral orifice. The voiding experiments ($n = 15$) were performed with continuous transvesical infusion cystometry and

an open urethra. In all experiments, 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. 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 sutures.

The PE tube was connected via a three-way stopcock to an infusion pump and a pressure transducer (P23XL-1, Becton Dickinson, Franklin Lakes, NJ, USA) to measure the intravesical pressure and control the bladder volume. The intravesical pressure and EUS EMG were amplified, filtered and sampled at 5 kHz (Biopac MP 36, BIOPAC Systems, Santa Barbara, CA, USA).

The sensory branches of the pudendal nerves were transected bilaterally in each rat. The sensory branches of the pudendal nerves were exposed via a posterior approach by incising the distal portions of the gluteus major muscles. The ilium and sacrum were separated, the sensory branches of the pudendal nerves were identified through blunt dissection [12, 13], carefully isolated, marked with a silk suture and then transected with fine scissors. The proximal ends of the transected pudendal sensory branches were both mounted with bipolar cuff electrodes for electrical stimulation. Regulated-current, cathodic, monophasic pulses were applied with a pulse duration of 0.1 ms, an amplitude of 0.025–0.3 mA, and a frequency of 1–50 Hz via two stimulators (Model 2100, A-M Systems, Carlsborg, WA, USA). The stimulators were controlled by a LabView (National Instruments, Austin, TX, USA) program that sampled and analysed the bladder pressure signal and generated a voltage signal to each of the stimulators via a digital-to-analogue converter (USB-6212, National Instruments). The stimulator(s) was triggered when the cystometric pressure reached a preset threshold.

To establish the appropriate range of stimulation currents, the current threshold required 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 [4].

After bilateral transection of the sensory branch of the pudendal nerves, the bladder was filled via the urethral catheter, and the infusion was stopped when the first isovolumetric bladder contraction appeared. Electrical stimulation was automatically applied at the onset of every other isovolumetric bladder contraction, beginning when the bladder contraction pressure exceeded 50 cmH₂O and lasting for 30 s (Fig. 1). The laterality alternated between unilateral and bilateral stimulation, unilateral stimulation alternated between left and right sides, and the stimulation frequency and amplitude were randomized. The bladder was emptied every 30–40 min with 10 min of equilibration before filling again. The area under the curve of bladder pressure as a function of time during a bladder contraction was measured to quantify the effects of electrical stimulation.

After bilateral transection of the sensory branch of the pudendal nerves, the bladder was filled via the superpubic catheter until voiding occurred and continued such that each trial included at least three voiding contractions. Unilateral or bilateral electrical stimulation of the sensory branch of the pudendal nerve(s) was applied during voiding bladder contractions when the bladder pressure exceeded 20 cmH₂O and stopped when the pressure dropped below this threshold (Fig. 2).

Successive pelvic neurotomies were conducted ($n = 6$) to determine whether pudendal afferent stimulation augmented voiding in a partially denervated bladder. The pelvic nerves were exposed bilaterally via a midline abdominal incision, and loops of silk sutures were placed around each nerve. The overlying muscle and skin were sutured. Multiple trials of unilateral (contralateral to the side of the pelvic neurotomy) or bilateral pudendal afferent stimulation were conducted during continuous infusion cystometry 2–3 h after each acute pelvic nerve transection (unilateral and then bilateral). Electrical stimulation was applied during voiding bladder contractions when the amplitude bladder pressure exceeded 15 cmH₂O and stopped when the pressure dropped below this threshold (Fig. 3).

Spinal cord transection (SCT) at the T₉–T₁₀ vertebral level was conducted ($n = 3$) to determine the contributions of spinal vs supraspinal pathways to the augmentation of voiding by pudendal afferent stimulation. After a T₉–T₁₀ laminectomy, the dura matter and spinal cord were transected with fine scissors. The severed ends of the spinal cord typically retracted 1–2 mm and were inspected under a surgical microscope to ensure complete transection. The overlying muscle and skin were sutured closed after SCT, and trials of continuous infusion cystometry began 3–4 h after SCT. Unilateral or bilateral electrical stimulation was applied during voiding bladder contractions, triggered when the bladder pressure exceeded 20 cmH₂O and stopped when the pressure dropped below this threshold (Fig. 4).

For data analysis, the following cystometric variables were measured to quantify the effects of pudendal afferent stimulation on voiding: micturition volume threshold (VT), defined as the infused volume of saline sufficient to induce the first voiding contraction; contraction amplitude (CA), defined as the maximum pressure during voiding; bladder contraction duration (CD) during voiding; bladder contraction area, the area of bladder contraction under the bladder pressure curve; inter-contraction interval (ICI), the interval between two consecutive voiding contractions; and (6) VE, the ratio between the voided volume (VV) and the VT. The VV was obtained from the value of VT minus the residual volume (RV) of saline withdrawn through the intravesical catheter after the final voiding contraction. In addition, the duration of bursting period (BP) EUS EMG (Fig. 2) was calculated to determine whether pudendal afferent stimulation produced effects on EUS activity [9].

All data are presented as means $\pm$ standard deviation. Analysis of variance (ANOVA) was used to identify factors that influenced cystometric variables and *post hoc* paired comparisons were made using Tukey's honest significant difference (HSD) (SigmaStat, SPSS, Chicago, IL, USA). Power analyses were also conducted to determine the probability of rejecting the null hypothesis when it was, indeed, false.

RESULTS

The pudendal–EUS reflex was evoked by 1 Hz UniES of the proximal end of the transected sensory branch of the pudendal nerve, and the minimum current amplitude to elicit an EMG response in the EUS reflex ranged from 0.01 to 0.05 mA (0.03 ± 0.02 mA, $n = 8$).

Examples of the effects of UniES and BiES of the proximal end of the transected sensory branch of the pudendal nerve(s) on ongoing isovolumetric bladder contractions are shown in Figure 1. The 30 s epochs of stimulation at 2 Hz were triggered when the bladder pressure exceeded 50 cmH₂O. Both UniES and BiES, with stimulation amplitudes ranging from 0.025 to 0.1 mA, appeared to increase the duration of the contractions, and thus the contraction area, as compared with contractions without stimulation.

The effects of UniES and BiES at different frequencies and amplitudes on the area of isovolumetric bladder contractions ($n = 396$ trials across eight rats) are summarized in Table

1. The mean normalized contraction area increased to 104–147% of the control area across all tested stimulation variables. A three-factor ANOVA indicated that the contraction area was dependent on both the stimulation laterality (i.e., control, UniES and BiES, $P = 0.04$) and the stimulation amplitude ($P = 0.006$), but the results of the present study did not demonstrate a significant effect of the stimulus frequency ($P = 0.42$), of the interaction term between site and frequency ($P = 0.77$), or of the interaction term between amplitude and frequency ($P = 0.420$). Therefore, all data were combined across frequency, and the two-factor ANOVA indicated that, again, the contraction area was dependent on both the stimulation laterality ($P = 0.004$) and the stimulation amplitude ($P = 0.042$). Subsequent, *post hoc* paired comparisons (Tukey's HSD) across laterality indicated that the contraction areas with both UniES ($P = 0.01$) and BiES ($P < 0.001$) were larger than those without stimulation, and that the contraction areas during BiES were larger than those during UniES ($P = 0.007$). However, *post hoc* paired comparisons between individual amplitudes did not identify specific amplitudes that produced larger contraction areas than any other amplitude.

The results of the isovolumetric experiments demonstrated that the effects of pudendal afferent stimulation were not dependent on the stimulation frequency. Therefore, to determine the effects of UniES and BiES on bladder emptying, different stimulation amplitudes (0.025–0.2 mA) were applied at a fixed stimulation frequency (20 Hz) during continuous-infusion cystometry with an open urethra. An example of the effects of UniES and BiES of the proximal transected pudendal sensory nerve after bilateral transection of the sensory branch of the pudendal nerve (BST) on the cystometrogram and EUS EMG activity is shown in Figure 2, and the cystometric and EUS EMG variables are summarized in Table 2.

Acute BST reduced bladder contraction amplitude, bladder contraction duration, ICI, bladder contraction area, and the VE compared with values before BST (Fig. 2, Table 2). Both UniES and BiES increased the CD and the contraction area, but had no effect on the contraction magnitude. VE was reduced dramatically after acute BST from 69 to 22%, but both UniES and BiES significantly increased VE to 50–62%. The larger VE with UniES and BiES also led to an increase in ICI. The duration of the EUS BP was reduced significantly by acute BST (Fig. 2, Table 2). Both UniES and BiES prolonged the BP, usually by evoking several fragments of bursting activity within a single bladder contraction (Fig. 2), and there was no significant difference between the effects of UniES and BiES on EUS BP (Table 2).

A two-factor ANOVA revealed that the VE was dependent on both the stimulation laterality ($P = 0.043$) and stimulation amplitude ($P = 0.018$). *Post hoc* paired comparisons (Tukey's HSD) indicated that the enhancement in bladder emptying was significant for both UniES and BiES, and although BiES consistently generated a 4–5% larger increase in the VE than UniES, there was no significant difference between the VEs with UniES and BiES ($P = 0.57$). The mean difference in effect on VE between UniES and BiES across stimulation conditions was 0.28 SD, and our sample size enabled detection of this effect size with a power ($1 - \beta$) of only 0.25. Further, *post hoc* paired comparisons between amplitudes did not identify specific amplitudes that produced larger VEs than any other amplitude.

After BST, the pelvic nerves ($n = 6$ rats) were successively transected to determine whether enhancement of bladder emptying by pudendal afferent stimulation was preserved in a partially denervated bladder. Examples of cystometrograms after unilateral and bilateral pelvic nerve transection, with and without concomitant electrical stimulation of pudendal afferents, are shown in Figure 3(A), and voiding efficiencies are summarized in Figure 3(B).

Unilateral pelvic nerve transection, conducted after BST, further reduced the bladder CA and the bladder CD, and the VE decreased from 18 to 8% ($P < 0.05$). Both UniES and BiES

increased the VE significantly, to ≈ 22 and $\approx 31\%$, respectively (Fig. 3B), but there was no significant difference between unilateral and bilateral stimulation ($P = 0.084$, $1 - \beta = 0.7$).

After subsequent contralateral (i.e. bilateral) pelvic nerve transection, all micturition contractions were eliminated and replaced by continuous small contractions with overflow urination occurring in a drip-like fashion, and VE was only $\approx 2\%$. Neither UniES nor BiES augmented bladder contractions or improved bladder emptying after bilateral pelvic nerve transection.

After BST, the spinal cord ($n = 3$ rats) was transected at T₉–T₁₀ (SCT) to investigate the reflex pathway mediating the enhancement of bladder emptying by pudendal sensory nerve stimulation. Examples of cystometrograms after SCT, with and without concomitant electrical stimulation, are shown in Figure 4(A), and voiding efficiencies are summarized in Figure 4(B). SCT caused impaired micturition with smaller contraction areas and shorter ICIs, and VE was significantly reduced, from ≈ 23 to $\approx 6\%$ after SCT. Subsequently, VE was increased significantly by either UniES or BiES, to ≈ 16 and $\approx 18\%$, respectively, and there was no significant difference between unilateral and bilateral stimulation ($P = 0.752$, $1 - \beta = 0.22$).

DISCUSSION

Both UniES and BiES of the transected pudendal sensory nerve(s) increased the duration (area) of isovolumetric bladder contractions and increased VE during cystometry with an open urethra from $\approx 22\%$ after bilateral sensory nerve transection to 46–62%, as compared with 69% under control conditions. Further, the improved bladder emptying by pudendal afferent electrical stimulation was preserved after acute unilateral (but not bilateral) pelvic nerve transection or thoracic spinal transection.

The VE in intact anaesthetized rats before any nerve transections or electrical stimulation was 69%, which was lower than the ≈ 98 –99% VE measured in conscious animals [14], and VE was reduced to only 22% after bilateral sensory nerve transection, supporting the pivotal role of pudendal sensory feedback in efficient voiding [9]. Unilateral pudendal afferent stimulation dramatically improved the VE and bilateral stimulation produced an additional 4–5% increase in the VE, thereby restoring VE to the level measured before any nerve transections, but to values less than the VE measured in conscious animals. This limitation presumably resulted from anaesthesia limiting bladder emptying both in control conditions and with stimulation. This assumption was supported by other studies [15,16] where VE restored using electrical stimulation in anaesthetized animals was generally no better than 75%. Although anaesthetic effects were a limitation of the present studies, urethane is ‘the most suitable anaesthetic for physiological experiments that require demonstration of reflex micturition’ [17].

Both UniES and BiES increased the durations of both the bladder contraction and EUS bursting, implying that the sensory feedback generated by pudendal afferent stimulation not only amplified bladder activation but also contributed to patterning of the EUS bursting activity, and this is consistent with the physiological roles of pudendal afferent feedback during voiding [9]. The burst activity reflects the rhythmic opening and closing of the outlet to produce the pulsatile flow of urine [18,19], and is essential to achieve efficient voiding in rodents [20]. The increased duration of EUS EMG bursts indicated that the urethra was open for a longer time and this could have contributed to the increased VE produced by UniES and BiES. In addition, the increased bladder CD generated by pudendal afferent stimulation corroborated the existence of a urethra-to-bladder reflex [21,22] where urine flow activates urethral afferents that, in turn, augment bladder contraction.

Activation of the augmenting reflex by UniES significantly increased VE after an acute unilateral pelvic neurotomy, and BiES only modestly increased the voiding efficiency (Fig. 3B). These results indicated that unilateral sensory activity generated by UniES was probably transmitted bilaterally to parasympathetic efferents innervating the bladder. This assertion is supported by previous histological and electrophysiological studies. Primary afferent axons from the pudendal nerve project bilaterally but with ipsilateral dominance [12], and neurons and/or axons passing through the dorsal commissure make monosynaptic and polysynaptic excitatory connections with contralateral parasympathetic preganglionic neurons [23,24]. As expected, the micturition reflex was totally abolished in rats after bilateral transection of the pelvic nerve, and there were no effects of pudendal afferent stimulation.

After acute spinal transection, bladder voiding was still improved by either UniES or BiES, and while overall VE was lower, the effect sizes of stimulation were similar to those measured with an intact spinal cord. These results imply that the neuronal circuitry recruited by electrical stimulation to augment the bladder emptying reflex resides within the lumbo-sacral spinal segments, and are consistent with previous results in cats after acute [15] or chronic [25] spinal transection. Spinal studies in humans have also demonstrated that intra-urethral electrical stimulation can generate reflex contraction of the bladder [26,27].

One limitation of the present study was the use of rats to investigate lower urinary tract function. Although rats are widely used for physiological and pharmacological studies of the lower urinary tract [28], they (and dogs) exhibit phasic patterns of EUS activity during voiding, by contrast to the (complete) relaxation of the sphincter observed in humans (and cats). Therefore, it is not clear whether the present results will translate to humans, where neural control of EUS activity differs from that in the rat.

Conservative treatment often fails in patients with lower urinary tract dysfunction. This work shows the feasibility of improving bladder emptying by either unilateral or bilateral electrical stimulation of pudendal afferents. This is an exciting prospect for neuro-rehabilitation as the pudendal nerve is readily accessed [29], and a sufficient branch-free length of nerve is available for implantation of a cuff electrode [30]. Amplifying sensory feedback from pudendal urethral afferents to improve bladder emptying could provide an alternative method for restoring bladder function in patients with chronic non-obstructive urinary retention.

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REFERENCES

1. Jonas U, Fowler CJ, Chancellor MB, et al. Efficacy of sacral nerve stimulation for urinary retention: results 18 months after implantation. *J Urol.* 2001; 165:15–19. [PubMed: 11125353]
2. Aboseif S, Tamaddon K, Chalfin S, et al. Sacral neuromodulation in functional urinary retention: an effective way to restore voiding. *BJU Int.* 2002; 90:662–665. [PubMed: 12410743]
3. Shaker HS, Hassouna M. Sacral root neuromodulation in idiopathic nonobstructive chronic urinary retention. *J Urol.* 1998; 159:1476–1478. [PubMed: 9554336]
4. Peng CW, Chen JJ, Cheng CL, Grill WM. Improved bladder emptying in urinary retention by electrical stimulation of pudendal afferents. *J Neural Eng.* 2008; 5:144–154. [PubMed: 18430976]
5. Barrington FJF. The component reflexes of micturition in the cat, parts I and II. *Brain.* 1931; 54:177–188.

6. Barrington FJF. The component reflexes of micturition in the cat, part III. *Brain*. 1941; 64:239–243.
7. Feber JL, van Asselt E, van Mastrigt R. Neurophysiological modeling of voiding in rats: urethral nerve response to urethral pressure and flow. *Am J Physiol*. 1998; 274:R1473–R1481. [PubMed: 9612416]
8. Jung SY, Fraser MO, Ozawa H, et al. Urethral afferent nerve activity affects the micturition reflex; implication for the relationship between stress incontinence and detrusor instability. *J Urol*. 1999; 162:204–212. [PubMed: 10379788]
9. Peng CW, Chen JJ, Cheng CL, Grill WM. Role of pudendal afferents in voiding efficiency in the rat. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294:R660–R672. [PubMed: 18077513]
10. Shafik A, el-Sibai O, Ahmed I. Effect of urethral dilation on vesical motor activity: identification of the urethrovesical reflex and its role in voiding. *J Urol*. 2003; 169:1017–1019. [PubMed: 12576835]
11. Schultz-Lampel D, Jiang C, Lindstrom S, Thuroff JW. Experimental results on mechanisms of action of electrical neuromodulation in chronic urinary retention. *World J Urol*. 1998; 16:301–304. [PubMed: 9833307]
12. McKenna KE, Nadelhaft I. The organization of the pudendal nerve in the male and female rat. *J Comp Neurol*. 1986; 248:532–549. [PubMed: 3722467]
13. Pacheco P, Martinez-Gomez M, Whipple B, Beyer C, Komisaruk BR. Somato-motor components of the pelvic and pudendal nerves of the female rat. *Brain Res*. 1989; 490:85–94. [PubMed: 2758331]
14. Yaksh TL, Durant PA, Brent CR. Micturition in rats: a chronic model for study of bladder function and effect of anesthetics. *Am J Physiol*. 1986; 251:R1177–R1185. [PubMed: 3789199]
15. Boggs JW, Wenzel BJ, Gustafson KJ, Grill WM. Frequency-dependent selection of reflexes by pudendal afferents in the cat. *J Physiol*. 2006; 577:115–126. [PubMed: 16945977]
16. Yoo PB, Woock JP, Grill WM. Bladder activation by selective stimulation of pudendal nerve afferents in the cat. *Exp Neurol*. 2008; 212:218–225. [PubMed: 18502417]
17. Matsuura S, Downie JW. Effect of anesthetics on reflex micturition in the chronic cannula-implanted rat. *Neurourol Urodyn*. 2000; 19:87–99. [PubMed: 10602250]
18. Kruse MN, de Groat WC. Spinal pathways mediate coordinated bladder/urethral sphincter activity during reflex micturition in decerebrate and spinalized neonatal rats. *Neurosci Lett*. 1993; 152:141–144. [PubMed: 8515867]
19. Maggi CA, Giuliani S, Santicioli P, Meli A. Analysis of factors involved in determining urinary bladder voiding cycle in urethane-anesthetized rats. *Am J Physiol*. 1986; 251:R250–R257. [PubMed: 3740305]
20. Streng T, Santti R, Andersson KE, Talo A. The role of the rhabdosphincter in female rat voiding. *BJU Int*. 2004; 94:138–142. [PubMed: 15217449]
21. Barrington FJF. The component reflex of micturition in the cat. Parts I and II. *Brain*. 1931; 54:177–188.
22. Barrington FJF. The component reflex of micturition in the cat. Part III. *Brain*. 1941; 64:239–243.
23. Morgan C, Nadelhaft I, de Groat WC. The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. *J Comp Neurol*. 1981; 201:415–440. [PubMed: 7276258]
24. de Groat WC, Fraser MO, Yoshiyama M, et al. Neural control of the urethra. *Scand J Urol Nephrol Suppl*. 2001:35–43. discussion 106–25. [PubMed: 11409613]
25. Tai C, Wang J, Wang X, de Groat WC, Roppolo JR. Bladder inhibition or voiding induced by pudendal nerve stimulation in chronic spinal cord injured cats. *Neurourol Urodyn*. 2007; 26:570–577. [PubMed: 17304521]
26. Gustafson KJ, Creasey GH, Grill WM. A urethral afferent mediated excitatory bladder reflex exists in humans. *Neurosci Lett*. 2004; 360:9–12. [PubMed: 15082166]
27. Kennelly MJ, Arena KC, Shaffer N, et al. Electrical stimulation of the urethra evokes bladder contractions in a woman with spinal cord injury. *J Spinal Cord Med*. 2010; 33:261–265. [PubMed: 20737800]

28. de Groat, WC.; Booth, AM.; Yoshimura, N. Neurophysiology of micturition and its modification in animal models of human disease. Maggi, CA., editor. Vol. Vol 3. London: Harwood Academic; 1993. ed Maggi CA
29. O'Bichere A, Green C, Phillips RK. New, simple approach for maximal pudendal nerve exposure: anomalies and prospects for functional reconstruction. *Dis Colon Rectum*. 2000; 43:956–960. [PubMed: 10910242]
30. Gustafson KJ, Zelkovic PF, Feng AH, Draper CE, Bodner DR, Grill WM. Fascicular anatomy and surgical access of the human pudendal nerve. *World J Urol*. 2005; 23:411–418. [PubMed: 16333625]

Abbreviations

BiES	bilateral electrical stimulation
BP	bursting period
BST	bilateral transection of the sensory branch of the pudendal nerve
CA	contraction amplitude
CD	contraction duration
EMG	Electromyogram
EUS	external urethral sphincter
HSD	honest significant difference
ICI	inter-contraction interval
PE	Polyethylene
RV	residual volume
SCT	spinal cord transaction
UniES	unilateral electrical stimulation
VE	voiding efficiency
VT	volume threshold
VV	voided volume

FIG. 1.

Examples of the effects of UniES or BiES of the proximal end of the transected sensory branch of the pudendal nerves on isovolumetric reflex bladder contractions. (A) Isovolumetric bladder contractions without concomitant stimulation. (B)–(D) Either UniES or BiES was delivered during every other bladder contraction. The horizontal bars indicate 30 s of UniES or BiES, which began when the bladder pressure exceeded 50 cmH₂O. The stimulus trains were all at 2 Hz with various current amplitudes and a 0.05 ms pulse width.

FIG. 2.

Effects of UniES and BiES of the proximal end of the transected sensory branch of the pudendal nerve on bladder pressure and EUS EMG during continuous bladder infusion. Traces on the right are expansions of the section of the traces on the left in brackets. The BP is the duration of EUS EMG bursting period. (A) Bladder pressure and EUS EMG before any nerve transection; (B) bladder pressure and EUS EMG after BST; (C) bladder pressure and EUS EMG after BST with UniES (20 Hz, 0.05 mA); (D) bladder pressure and EUS EMG after BST with BiES (20 Hz, 0.05 mA). UniES or BiES was delivered when the bladder pressure exceeded 20 cmH₂O and stopped when the pressure returned to below this threshold, as indicated by the horizontal bars.

FIG. 3.

Effects of UniES and BiES of the proximal end of the transected sensory branch of the pudendal nerve on bladder pressure (A) and VE (B) after successive pelvic nerve transections (Nx). UniES or BiES (20 Hz, 0.05 mA) was delivered when the bladder pressure exceeded 15 cmH₂O and stopped when the pressure returned to below this threshold, as indicated by the horizontal bars in A. Each bar in B represents the mean $\pm$ SD ($n = 6$ rats). #, significant difference ($P < 0.05$) compared with after BST; *, significant difference ($P < 0.05$) compared with after unilateral pelvic Nx. BiNx, bilateral pelvic nerve transection.

FIG. 4.

Effects of UniES and BiES of the proximal end of the transected sensory branch of the pudendal nerve on bladder pressure (A) and VE (B) after acute T₉–T₁₀ SCT. UniES or BiES (20 Hz, 0.05 mA) was delivered when the bladder pressure exceeded 20 cmH₂O and stopped when the pressure returned to below this threshold, as indicated by the horizontal bars in A. Each bar in B represents the mean $\pm$ SD ($n = 6$ trials across three rats). #, significant difference ($P < 0.05$) compared with after BST; *, significant difference ($P < 0.05$) between post-acute SCT and trials in which stimulation was applied.

TABLE 1

Effects of unilateral (UniES) and bilateral (BiES) electrical stimulation on normalized isovolumetric bladder contraction area (%).

	UniES	BiES
Control	100.0%±3.4%	100.0%±3.4%
2 Hz, 0.025 mA	128.7%±45.8%	144.1%±54.2%
2 Hz, 0.050 mA	123.8%±30.8%	133.8%±43.7%
2 Hz, 0.100 mA	125.5%±38.6%	136.5%±41.3%
2 Hz, 0.200 mA	129.0%±33.3%	130.5%±31.4%
2 Hz, 0.300 mA	117.9%±26.4%	128.5%±27.2%
20 Hz, 0.025 mA	131.8%±31.8%	144.4%±37.8%
20 Hz, 0.050 mA	130.2%±50.1%	140.6%±39.0%
20 Hz, 0.100 mA	123.2%±23.5%	139.0%±42.5%
20 Hz, 0.200 mA	122.3%±44.4%	134.5%±39.1%
20 Hz, 0.300 mA	115.3%±36.0%	129.3%±25.7%
50 Hz, 0.025 mA	122.0%±31.2%	147.2%±55.8%
50 Hz, 0.050 mA	121.1%±34.7%	131.1%±37.9%
50 Hz, 0.100 mA	122.6%±30.9%	135.8%±38.8%
50 Hz, 0.200 mA	126.3%±28.4%	139.8%±42.6%
50 Hz, 0.300 mA	114.0%±37.3%	104.9%±29.3%

Control: without concomitant electrical stimulation.

Values are means±SD, $n = 8$ rats.

Effects of unilateral (UniES) and bilateral (BiES) electrical stimulation on cystometric parameters and EUS bursting period following bilateral transection of the sensory branch of the pudendal nerve (BST).

TABLE 2

	Contraction amplitude (cmH ₂ O)	Contraction duration (s)	Intercontraction interval (s)	Contraction area (cmH ₂ O × s)	Voiding efficiency (s)	Bursting period (s)
Before BST	37.9±2.8	33.2±3.1	145.9±21.7	409.5±134.2	69.0±4.2	4.09±0.56
After BST	30.9±3.0 #	27.4±4.4 #	79.5±36.8 #	194.0±75.6 #	22.2±8.3 #	2.77±0.46 #
20 Hz, 0.025mA, UniES	32.1±4.5	37.0±9.0	98.0±12.8	343.0±47.1 *	49.7±10.3 *	3.79±0.63
20 Hz, 0.025mA, BiES	32.6±2.5	35.3±10.6	86.9±19.4	391.9±63.8 *	53.0±7.7 *	3.81±0.62
20 Hz, 0.050mA, UniES	32.2±2.5	38.6±11.3 *	97.3±31.3	366.9±107.4 *	58.6±12.5 *	3.90±0.60 *
20 Hz, 0.050mA, BiES	33.9±3.4	39.0±9.0 *	99.0±25.9	410.2±52.8 *	62.2±12.5 *	4.29±0.51 *
20 Hz, 0.100mA, UniES	33.2±4.1	35.8±12.7	87.8±23.4	334.0±108.6 *	53.6±11.6 *	4.16±0.59 *
20 Hz, 0.100mA, BiES	32.0±2.8	40.0±9.7 *	93.5±40.9	408.6±86.4 *	57.8±4.9 *	4.49±0.65 *
20 Hz, 0.200mA, UniES	32.8±4.2	39.6±9.4 *	93.5±20.7	401.5±82.1 *	54.0±10.8 *	4.41±0.53 *
20 Hz, 0.200mA, BiES	31.1±1.3	35.4±12.1	88.5±36.0	344.2±118.1 *	54.4±6.4 *	3.86±0.73

Values are mean±SD, n =6 rats.

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 UniES or BiES.