Title:
A Spinal Micturition Reflex Mediated by Afferents in the Deep Perineal Nerve

Authors:
Joseph W Boggs, Brian J Wenzel, Kenneth J Gustafson, and Warren M Grill

Affiliations:
1. Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio
2. Department of Biomedical Engineering, Duke University, Durham, North Carolina

Running head:
Micturition Reflex Mediated by the Deep Perineal Afferents

Correspondence:
Warren M. Grill, Ph.D.
Department of Biomedical Engineering
Duke University
Box 90281
Durham, NC 27708-0281
(919) 660 5276 (phone)
(919) 684 4488 (fax)
warren.grill@duke.edu
Abstract

Reflexes mediated by urethral sensory pathways are integral to urinary function. This study investigated the changes in bladder pressure and urethral sphincter activity resulting from electrical stimulation of afferents in the deep perineal nerve (DP), which innervates the urethra and surrounding muscles, before and after acute spinal cord transection (SCT) in cats anesthetized with α-chloralose monitored via blood pressure and heart rate. DP stimulation elicited bladder contractions before and after SCT but only if the bladder contained a sufficient volume of fluid (78% of the volume needed to cause distention-evoked reflex contractions). The volume dependence was mediated by a neuronal mechanism in the lumbosacral spinal cord and was not attributable to length-tension properties of the detrusor muscle. Stimulation at 2 – 40 Hz initiated bladder contractions, but 20 – 40 Hz was more effective than lower frequencies in evoking and sustaining bladder contractions for the duration of the stimulus train. Decreases in urethral sphincter activity occurred during sustained bladder contractions evoked by 20 – 40 Hz stimulation before and within 16 hours following SCT. After SCT, average bladder pressure increases evoked by DP stimulation were smaller than those evoked prior to SCT, but in some animals, bladder pressures elicited by DP stimulation continued to increase as time after SCT increased and reached pre-transection amplitudes at 8 – 16 hours post-transection. These data confirm the presence of a spinal circuit that can mediate coordinated bladder-sphincter responses and show that afferents from the DP can activate this circuit under appropriate conditions.
1. Introduction

The lower urinary tract has two functions, continence (referring to the storage phase in which the bladder holds urine) and micturition (denoting the process of urine evacuation by contraction of the bladder and concomitant relaxation of the external urethral sphincter (EUS)). In the spinally intact animal, the bladder stores urine until descending inputs from the brain command the bladder to contract and the urethral sphincter to relax (Holstege et al. 1986; Holstege et al. 1987). The traditional view of coordinated reflex micturition holds that it is mediated by a spinal cord - brainstem - spinal cord reflex loop initiated by input from pelvic nerve afferents signaling bladder distention (de Groat 1990). However, a recent study challenges this view by showing that stimulation of a rostral sensory branch of the pudendal nerve evoked weak micturition-like activity even after acute spinal transection (Shefchyk and Buss 1998). The present study investigated the ability of activation of afferents in the deep perineal nerve (DP), a caudal branch of the pudendal nerve (Martin et al. 1974), to evoke micturition-like activity with and without the brainstem. The results demonstrate that deep perineal afferents can evoke a spinal reflex capable of coordinated micturition-like activity and that the reflex was sensitive to bladder volume and the firing rate of deep perineal afferents.

Flow of urine through the urethra can evoke afferent firing in the deep perineal branch of the pudendal nerve (Todd 1964) and initiate bladder contraction through a spinobulbospinal reflex loop (Barrington 1931, 1941). Despite the implication that deep perineal receptors transduce fluid flow and the resulting afferent firing elicits the micturition response, the ability to induce a micturition-like response through direct stimulation of DP afferents has not been investigated. The established doctrine of the micturition cycle states that the brainstem is essential for coordinating the bladder-sphincter activity that enables micturition (Morrison 1987; Nitti and Combs 1998), but the necessity
of the brainstem in the micturition pathway mediated by DP afferents cannot be evaluated until the bladder-sphincter response to direct DP activation is investigated. This study determines if DP activation can elicit coordinated bladder-sphincter activity and if a spinal reflex exists to maintain bladder-sphincter synergy after spinal cord transection. The existence of a spinal micturition-like reflex initiated through cranial pudendal sensory nerve stimulation has been shown previously, but it was limited to 1 hour post spinalization and was conducted in animals that did not exhibit distention evoked reflex contractions (Shefchyk and Buss 1998). The present study extends the Shefchyk and Buss (1998) investigation by determining in animals responsive to bladder distention if a urethral sensory pathway can mediate a micturition-like reflex several hours after acute spinalization.

Urethral fluid flow produces bladder contractions when the bladder contains a sufficiently large volume but not when it contains a small volume (Barrington 1931, 1941). Increases in bladder pelvic nerve activity evoked in response to electrical stimulation of the cranial pudendal sensory nerve show a similar dependence on bladder volume in animals anesthetized with \( \alpha \)-chloralose (Mazieres et al. 1997), but bladder response to electrical stimulation of the sensory nerve is not documented in the same detail as the bladder response to fluid flow (Robain et al. 2001) to allow direct comparison of the volume thresholds. The present study quantifies the volume needed for DP stimulation to elicit a bladder contraction relative to the volume required to elicit a distention evoked reflex bladder contraction. This investigation also determines if the volume dependence arises from detrusor length-tension properties or is neural in origin and whether the volume dependence changes after spinal transection.

Afferent fiber firing rates between 2 and 40 Hz have been recorded from the deep perineal nerve during urethral fluid flow with rates from 20 to 40 Hz predominating during extended periods
of flow (Todd 1964). The preponderance of the higher frequency firing rates may suggest that the reflex is more responsive to specific inter-spike intervals. The bladder and pelvic nerve responses to cranial pudendal sensory nerve activation have been investigated over a range of continuous stimulation frequencies (1 – 50 Hz) in decerebrate animals and animals anesthetized with α-chloralose (Mazieres et al. 1997; Shefchyk and Buss 1998; Jiang and Lindstrom 1999), but the relative effect of stimulation frequency on either bladder or urethral sphincter response before or after spinalization has not been reported. The present study determines if the afferent firing rate influences the reflex response by stimulating the DP over the range of frequencies recorded during urethral fluid flow. This report also compares the capacity of different firing rates to evoke micturition-like activity after acute spinal transection.

The overall goal of this study is to investigate the existence of a spinal micturition-like reflex mediated by activation of the deep perineal nerve and to quantify the dependence of the reflex on bladder volume and the firing rate of DP fibers. The results challenge the traditional view that the brainstem is required to evoke a micturition-like response by demonstrating that the spinal circuitry can coordinate bladder-sphincter activity in response to electrical stimulation of the deep perineal nerve after the brainstem has been removed from the reflex loop.

2. Materials and methods

2.1 Experimental preparation

Acute experiments were conducted on 14 adult, sexually intact male cats 3.5–5.7 kg. All animal care and experimental procedures were followed according to NIH guidelines and were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Anesthesia was induced with ketamine HCl (Ketaset, 35 mg/kg, i.m., supplemented as required
during surgical preparation). A venous catheter was inserted in the cephalic vein, and anesthesia was maintained with \(\alpha\)-chloralose (Sigma, 65 mg/kg, i.v., supplemented at 15 mg/kg). A catheter was placed in the carotid artery to measure blood pressure. A surgical plane of anesthesia was maintained by ensuring the blood pressure was less than 140 mmHg and that blink and withdrawal reflexes were absent. A midline abdominal incision was made to expose the bladder; the ureters were ligated, transected proximal to the ligation, and drained externally. The bladder was cannulated with a 3.5 Fr (1.17 mm) catheter introduced with a hypodermic needle through the bladder dome, secured with a purse string suture, and the abdominal incision was closed in layers. The urethra was occluded with a 5 Fr (1.67 mm) catheter advanced through the urethra to lie within the bladder. Body temperature was maintained between 37\(^\circ\) C and 39\(^\circ\) C with a heating blanket; 0.9% saline with 8.4 mg/ml sodium bicarbonate and 5% dextrose added was administered i.v. (10-15 ml/kg/h), and end tidal CO\(_2\) was maintained between 3-4% with artificial respiration. In 8 animals the spinal cord was transected after collection of data with the spinal cord intact. A laminectomy was made to expose the thoracic spinal cord at T12; lidocaine was administered on the surface of the spinal cord, and the spinal cord was elevated and transected using a scalpel. A piece of Gelfoam was inserted between ends of the spinal cord and the exposure was closed.

2.2 Recording and stimulation

A solid-state pressure transducer (Deltran DPT-100, Utah Medical Products, Midvale, UT) connected to the suprapubic catheter was used to measure bladder pressure. The pressure signal was amplified, low pass filtered (cut-off frequency = 300 Hz), continuously recorded on a strip chart recorder (TA11, Gould Instruments), sampled at 24 kHz, and stored on digital tape (CDAT 16 Data Recorder, Cygnus Technology, Inc., Delaware Water Gap, PA). Bladder volume was
increased in 1 ml increments to determine the threshold volume ($V_{\text{thr}}$) for distension evoked contractions and the threshold volume for bladder contractions elicited by stimulation of the DP. Accuracy of bladder volumes was determined by emptying the bladder every 1-2 hr; and the bladder volumes reported were accurate to within ± 2 ml.

The gluteus superficialis and gluteofemoralis muscles were partially transected and reflected laterally to expose the pudendal nerve. The ipsilateral deep perineal nerve branch was isolated and stimulated with platinum bipolar hook electrodes and a constant current stimulator (Pulsar 9bp, FHC Inc., Bowdoinham, ME). Stimuli were cathodic monophasic pulses with amplitude of 100 $\mu$A-900 $\mu$A and duration of 100 $\mu$s applied as 5 s to 25 s trains with a frequency between 2 Hz and 40 Hz. The minimum current amplitude necessary to elicit bladder contractions was called threshold (DP$_{thr}$) and ranged from 150 $\mu$A to 450 $\mu$A (n = 10 cats). The bladder contained a volume of approximately 125% of $V_{\text{thr}}$ for distention evoked bladder contractions when DP$_{thr}$ was determined. The electrical threshold to evoke a compound nerve action potential in the DP was measured in 2 cats by stimulating the DP and recording proximally to the stimulation site at the trunk of the ipsilateral pudendal nerve and fell in a similar range (100 $\mu$A to 300 $\mu$A). In these two experiments, DP$_{thr}$ ranged from 1.5 to 2.0 times the electrical threshold to evoke a compound nerve action potential. The data presented here were obtained at 2$\times$DP$_{thr}$ to 4$\times$DP$_{thr}$. In 3 cats, electroneurogram (ENG) activity of fibers innervating the EUS was recorded with a tripolar cuff electrode placed on the contralateral deep perineal nerve branch. The ENG was preamplified (100x, Stanford SR560, Sunnyvale, CA) and filtered (300 Hz – 10 kHz), further amplified (1,000x) and filtered (300 Hz – 3 kHz, Grass P511, Quincy, MA), sampled (24 kHz sampling frequency), and stored on digital tape. The ipsilateral DP was transected distal to the stimulating electrodes to
prevent direct activation of the EUS and the subsequent afferent response to the EUS ENG discharge that would be recorded by the contralateral cuff electrode.

The length tension properties of the detrusor muscle were determined in 3 cats by stimulating the whole sacral roots, both S1 and S2 simultaneously, over a range of bladder volumes and measuring the resulting bladder pressure. Stimuli were cathodic monophasic pulses with amplitude of 2 mA and duration of 100 ms applied as 5 s trains with a frequency of 20 Hz, based on parameters previously shown to be effective in eliciting maximal bladder pressures (Grill et al. 1999).

The feasibility of voiding was determined in 3 animals (2 cats prior to SCT and 1 cat post SCT) after unilateral transection of the DP and stimulation of the ipsilateral proximal stump to avoid direct activation of the EUS muscle. The bladder was filled at 1 ml/min and the urethral catheter was removed prior to voiding. Stimuli were cathodic monophasic pulses with amplitude of $2 \times \text{DP}_{\text{thr}}$ to $4 \times \text{DP}_{\text{thr}}$ and duration of 100 µs applied as 30 s to 120 s trains with a frequency of 33 Hz, based on parameters found to be effective in eliciting maximal sustained bladder pressures without increases in EUS ENG activity. At the conclusion of each experiment the animal was euthanized by an overdose of sodium pentobarbital, and the anatomy was verified by tracing the nerves proximally and distally.

2.3 Offline analysis of bladder pressure and electroneurogram

Data were analyzed using custom routines in MATLAB (The MathWorks, Natick, MA). Bladder responses to deep perineal nerve stimulation were assessed by measuring changes in bladder pressures (Figure 1). The analysis considered a bladder contraction to be evoked if the pressure increased over a threshold pressure ($P_{\text{th}}$):
\[ P_{th} = 2 \times \text{Std}(P_{\text{baseline}}) + \text{Avg}(P_{\text{baseline}}) \]

Baseline pressure \((P_{\text{baseline}})\) was defined as the pressure during the 2 s interval preceding the contraction, \(\text{Std}(P_{\text{baseline}})\) was the standard deviation of \(P_{\text{baseline}}\), and \(\text{Avg}(P_{\text{baseline}})\) was the mean of \(P_{\text{baseline}}\). When stimulation elicited sustained contractions, the bladder pressure increased initially and then oscillated slowly (0.2-0.3 Hz) before decaying to baseline upon termination of the stimulus. Bladder pressures were quantified by measuring the average pressure during this period of slow oscillation with respect to the \(P_{\text{baseline}}\). For stimulation evoked contractions, the contraction was defined to begin 2 s before the first peak of the contraction and terminate at the end of stimulus if the contraction was sustained (i.e. bladder pressure remained above \(P_{th}\) for the duration of stimulation) (Figure 1A). If the contraction was not sustained (i.e., the contraction ended before stimulation ceased), then the contraction was defined to terminate at the last peak (Figure 1C). If the stimulation failed to evoke a contraction, then the pressure was averaged over the interval of stimulation (Figure 1D). Distension evoked reflex contractions were defined to begin 2 s before the first peak of the contraction and terminate at the last peak (Figure 1B).

Electroneurogram responses of the contralateral deep perineal nerve innervating the EUS to stimulation of the ipsilateral deep perineal nerve were assessed by measuring changes in the rectified and integrated (RI) ENG. The rectified ENG was integrated with a time constant of 250 ms and the stimulus artifact (approximately 3 ms in duration) was replaced with an average of the rectified ENG from the 0.5 ms preceding each stimulus pulse. The analysis considered an EUS ENG discharge to be significant if the RI ENG increased over a threshold (RI ENG\(_{th}\)) defined as:
RI ENG_{th} = \text{Std}(\text{RI ENG}_{\text{baseline}}) + \text{Avg}(\text{RI ENG}_{\text{baseline}})

Baseline RI ENG (RI ENG_{baseline}) was defined as the RI ENG during the 2 s interval preceding stimulation or during the 2 s interval preceding the start of the bladder contraction (as defined above) in the case of distention evoked reflex bladder contraction. Std(RI ENG_{baseline}) was the standard deviation of RI ENG_{baseline}, and Avg(RI ENG_{baseline}) was the mean of RI ENG_{baseline}. If a bladder contraction was evoked in response to either bladder distention or DP stimulation, RI ENGs were quantified by measuring the average RI ENG during the bladder contraction with respect to the RI ENG_{baseline} (Figure 1A-C). If stimulation failed to evoke a bladder contraction, then RI ENG was averaged over the interval of stimulation (Figure 1D).

2.4 Distribution of experiments

Responses to DP stimulation were investigated in a total of 14 cats prior to SCT and 8 cats post SCT. The bladder volume dependence of the bladder pressure response to DP stimulation was investigated in 13 spinal intact cats. The relationship between the volume threshold (V_{thr}) for bladder contractions evoked by DP stimulation and the V_{thr} for distention-evoked contractions was investigated in 6 cats prior to SCT, and in 4 of the 6 cats the volume thresholds were examined in small 1 ml increments before and after SCT. In 3 cats, the bladder pressure changes generated by sacral nerve root stimulation, DP stimulation, and distension evoked reflex contractions were evaluated in 3 ml intervals. The bladder pressure response to the frequency at which the DP was stimulated was examined in 8 cats prior to SCT and in 6 of those 8 cats after SCT. Both bladder pressure and EUS ENG response to DP stimulation was measured in 3 cats before and after SCT.
The DP was transected and stimulated proximal and distal to the cut in 3 cats prior to SCT and in 1 cat post SCT. Voiding was validated in 3 cats: 2 cats prior to SCT and 1 cat after SCT.

2.5 Statistical analysis

Statistical analysis of bladder pressures and deep perineal ENG was performed using paired t-tests or ANOVA followed by Tukey-Kramer post hoc test. Analysis of bladder and EUS ENG activity characteristics was performed using Kruskal-Wallis test followed by nonparametric pairwise comparison based on Bonferroni inequalities (Gibbons 1993) because the data were not normally distributed. Values are expressed as means ± standard deviations.

3. Results

Stimulation of the deep perineal nerve (DP) evoked bladder contractions in 13 of 14 cats. The activity of the urethral sphincter decreased when DP stimulation generated bladder contractions before and after spinal cord transection at T12 (SCT) in all 3 of the cats in which the sphincter ENG was recorded. The bladder response to stimulation of the DP was robust, but dependent on bladder volume and stimulation frequency. Increasing stimulation intensity beyond twice the threshold to evoke a bladder contraction (2*DP_{thr}) did not increase magnitude of the bladder contractions nor did it change the ability of the stimulation to sustain a bladder contraction. DP stimulation evoked sustained bladder contractions that ended within 1 to 2 s of stimulus termination and a transient burst of stimulation was not sufficient to initiate a sustained bladder contraction. Occasionally, a bladder contraction would outlast stimulation but only when bladder volume was 150% to 200% of the volume threshold for distention-evoked reflex contractions. DP stimulation failed to elicit
bladder contractions immediately following SCT, but the micturition reflex evoked by DP stimulation returned as time post-SCT increased.

3.1 Micturition reflex evoked by deep perineal nerve stimulation in spinally intact cats

3.1.1 Volume dependence of the reflex

DP stimulation evoked bladder contractions only when the bladder contained more than a minimum threshold volume ($V_{\text{thr}}$). Figure 2 shows the bladder pressure responses to DP stimulation and bladder distention at different bladder volumes. The bladder was filled in increments and between each 1 ml bolus of saline, the DP was stimulated. In this example, DP stimulation first elicited a sustained contraction when bladder volume was increased to 16 ml (DP $V_{\text{thr}}$). The saline bolus first caused a distention-evoked reflex contraction, characterized by slow oscillation of the bladder pressure during the contraction, when the bladder volume was increased to 24 ml (distention-evoked $V_{\text{thr}}$). Volume thresholds varied among cats, but $V_{\text{thr}}$ for contractions evoked by DP stimulation ($7 \pm 6$ ml; 1 to 18 ml, n = 6 cats in which the volume thresholds were investigated in intervals of 3 ml or less) was always less than the $V_{\text{thr}}$ for distention-evoked reflex contraction ($10 \pm 8$; 2 to 24 ml, n = 6 cats). DP stimulation elicited contractions at $78 \pm 17\%$ of the volume required for the saline bolus to evoke a contraction (n = 4 cats in which the volume thresholds were investigated in 1 ml intervals).

The volume dependence may be due solely to the length tension properties of the detrusor muscle, which determine the maximum contraction pressures; or the volume dependence also may be mediated by a neural mechanism, which limits the contraction pressure generated by DP stimulation at low bladder volumes. These two possibilities were explored by measuring the bladder pressures evoked by bladder efferent (sacral nerve roots S1 and S2) stimulation (reflecting
length tension properties) and bladder pressures evoked by DP stimulation over a range of bladder volumes in 3 cats. The volume dependence of the bladder’s response to DP stimulation differed from the volume dependence of the response to sacral root stimulation and the volume dependence of distension-evoked reflex bladder contractions (p < 0.001) (Figure 3). Nerve root stimulation produced bladder pressures significantly larger than DP stimulation at low volumes (3 and 6 ml), and stimulation of either roots or DP generated similar bladder pressures at high volumes (9 and 12 ml) (Figure 3). Thus, the bladder response to DP stimulation did not depend only on the length tension properties of the detrusor muscle because the pressures elicited by stimulation of the deep perineal nerve were significantly less than the pressures produced by stimulation of the efferent neurons innervating the detrusor at low bladder volumes.

3.1.2 Bladder response was dependent on stimulation frequency

The character of the pressure responses (sustained or unsustained bladder contraction) varied according to the frequency at which the DP was stimulated (Figure 4, p < 0.001). Stimulation frequencies 20, 33 and 40 Hz produced a greater percentage of sustained contractions than 2 or 10 Hz (nonparametric pairwise comparison based on Bonferroni inequalities (Gibbons 1993)). The magnitudes of the pressures during sustained contractions were dependent on the frequency of stimulation (p < 0.005) (Figure 5); however, there were no significant differences between any two stimulation frequencies (post-hoc paired comparisons Tukey-Kramer).

3.1.3 Urethral sphincter response depended on stimulation frequency and bladder response

The character of the urethral sphincter ENG responses (increase or no increase in EUS ENG discharge during stimulation) varied according to the frequency at which the DP was stimulated (p
< 0.001 adjusted for ties, Kruskal-Wallis test, n = 190 trials across all 3 cats with a minimum of 56 trials per cat). A stimulation frequency of 33 Hz produced a lower percentage of increases in sphincter activity (23%) than 2 Hz (42%) (p < 0.05, nonparametric pairwise comparison based on Bonferroni inequalities (Gibbons 1993)). Stimulation at 33 Hz produced no significant change in EUS ENG in 2 cats (p > 0.05 for each cat, paired t-tests of EUS ENG before stimulation and during the stimulation-evoked bladder contraction, n = 18 and 21 comparisons in respective cats) (Figure 6A) and produced a decrease in EUS ENG in 1 cat (p < 0.01, paired t-test, n = 20 comparisons). Stimulation at 2 Hz produced an increase in EUS ENG in 2 cats (p < 0.001 for each cat, paired t-test, n = 10 and 11 comparisons in respective cats) (Figure 6B) and produced no significant change in EUS ENG in 1 cat (p > 0.05, paired t-test, n = 8 comparisons). Thus, high frequency stimulation was less likely to evoke EUS ENG discharges than low frequency stimulation irrespective of the bladder response.

The EUS response was also related closely to the bladder response to DP stimulation. At no time did stimulation evoke both a sustained bladder contraction and a significant increase in EUS ENG activity. Stimulation frequencies 20, 33, and 40 Hz produced a decrease in EUS ENG when they evoked sustained bladder contractions (p = 0.005, paired t test of ENG amplitude before stimulation and during bladder contraction, n = 84 comparisons across all 3 cats with a minimum of 18 comparisons per cat).

3.1.4 Afferent fiber stimulation produced voiding

Following transection of the DP nerve, stimulation proximal to the cut continued to elicit bladder contractions while stimulation distal to the cut produced no contractions (Figure 7) (n = 3 of 3 cats), demonstrating that the reflex was mediated via an afferent pathway. To confirm that the
bladder contraction and reduction in EUS activity evoked by DP afferent stimulation were indeed a micturition reflex, voiding was measured in 2 cats after transecting the deep perineal nerve and stimulating proximal to the cut. After removing the urethral catheter, stimulation of the proximal DP stump voided 63 ± 20% of the bladder’s volume (n = 3 trials across 2 cats) and distention-evoked reflex bladder contractions voided 32 ± 3% of the bladder’s volume (n = 4 trials across 2 cats). DP stimulation voided at least as much volume as distention-evoked reflex contractions (p = 0.05, Mann-Whitney test of volume voided by DP stimulation to volume voided by distension evoked reflex contraction).

3.2 Micturition reflex mediated by lumbosacral spinal cord

After acute spinal cord transection (SCT) at T12, bladder distention in the absence of DP stimulation failed to elicit reflex bladder contractions, but stimulation of DP afferents generated bladder contractions after SCT in all 8 SCT cats. Immediately after SCT (0-30 min), stimulation failed to elicit an increase in bladder pressure. However, the amplitude of post SCT contractions increased with time after spinalization, and in 3 cats, the contractions returned nearly to their pre-spatialization magnitude by eight hours post SCT (Figure 8). In another 4 cats, the bladder response did not return fully to pre-SCT magnitude over the times investigated, and 1 cat died less than five hours after SCT (Figure 9).

3.2.1 Volume dependence of response preserved after SCT

After SCT, there remained a threshold bladder volume (V_{thr}) above which deep perineal nerve stimulation evoked a bladder contraction and below which stimulation failed to elicit a contraction. DP stimulation after spinalization elicited contractions at 67 ± 18% of the volume
required for a distension evoked contraction before SCT (n = 4 cats). \( V_{\text{thr}} \) for DP stimulation post SCT (67 ± 18\%) was less but not significantly different than \( V_{\text{thr}} \) for DP stimulation pre-SCT (78 ± 17\%) (\( p > 0.100 \), Mann-Whitney test, n = 4 comparisons across 4 cats).

3.2.2 Probability of deep perineal stimulation evoking a bladder contraction increased after SCT

Following SCT, the character of the pressure responses still varied according to the frequency at which the DP was stimulated (\( p < 0.005 \)). Stimulation at 33 Hz produced the greatest percentage of sustained contractions, and 2 Hz produced the lowest percentage (Figure 10). Post SCT, all frequencies were more likely to evoke a bladder contraction than they were before SCT, and the ability of each frequency to evoke a sustained contraction also increased (Figure 10). As before SCT, the magnitudes of the sustained contraction pressure varied according to the frequency (\( p < 0.05 \)) (Figure 11), but there were no significant differences in the magnitude of bladder pressures produced by any two frequencies post SCT (Post-hoc paired comparisons Tukey-Kramer).

3.2.3 EUS response still depended on stimulation frequency and bladder response after SCT

Following spinal cord transection, the character of the urethral sphincter response (increase or no increase in EUS ENG during stimulation) still varied according to the frequency at which the DP was stimulated (\( p = 0.01 \) adjusted for ties, Kruskal-Wallis test, n = 77 trials across 3 cats with a minimum of 17 trials per cat) (Figure 12), but there were no significant differences between any two stimulation frequencies (post-hoc nonparametric paired comparison based on Bonferroni inequalities (Gibbons 1993)).

Within 8 hours following spinal transection, stimulation frequencies 20, 33, and 40 Hz produced sustained bladder contractions and a decrease in EUS ENG in 2 cats (\( p < 0.05 \), paired t
test, n = 36 comparisons between 2 cats with a minimum of 17 comparisons per cat) and an increase in EUS ENG in 1 cat (p < 0.001, paired t test, n = 16 comparisons within 1 cat). Within 16 hours following SCT, stimulation frequencies 20, 33, and 40 Hz produced sustained bladder contractions and a decrease in EUS ENG in all 3 cats (p < 0.05, paired t test, n = 44 comparisons across all 3 cats with a minimum of 8 comparisons per cat).

3.2.4 Afferent fiber stimulation produced voiding after spinalization

Following transection of the DP nerve in one SCT cat, stimulation proximal to the cut continued to elicit bladder contractions while stimulation distal to the cut did not produce contractions. After removing the urethral catheter, stimulation of the proximal DP voided 65 ± 8% of the bladder’s volume (n = 4 trials within 1 cat). This was comparable to DP stimulation evoked voiding before SCT (63 ± 20%).

4. Discussion

The present study confirms the presence of a spinal reflex mediating a coordinated contraction of the bladder and relaxation of the sphincter, and demonstrates that afferents in the deep perineal nerve (DP) can activate this reflex under appropriate conditions. The excitatory bladder response was only apparent at larger bladder volumes and most often evoked with stimulation frequencies consistent with firing rates recorded from urethral flow receptors (Todd 1964). The reflex pathways between deep perineal afferents and bladder and sphincter efferents must lie caudal to T12 as the reflex persisted following acute spinalization. The existence of a spinal micturition reflex activated by coincident activity in bladder stretch receptors and DP afferents supports the existence of a micturition pathway not involving the brainstem. Somatic DP and visceral pathways converge
within the marginal zone, intermediate gray, and dorsal gray commissure of sacral segments S1 and S2 (Roppolo et al. 1985; Thor et al. 1989), regions containing putative interneurons known to be active during micturition-like activity and pudendal nerve stimulation in animals with an intact neuraxis (Grill et al. 1998). Neurons in these regions may mediate the spinal micturition-like reflex elicited through activation of DP afferents.

4.1 Limitations of the present study

Experiments were conducted in cats anesthetized with $\alpha$-chloralose because it interferes less with autonomic function that other anesthetics and maintains spinal reflexes (Balis and Monroe, 1964; Bonvento et al., 1994). The bladder pressures elicited by DP stimulation in the present study were similar to pressures elicited by distention-evoked bladder contractions in cats anesthetized with $\alpha$-chloralose (Rudy et al. 1991), but slightly less than pressures elicited by bladder distention and urethral sensory nerve stimulation in decerebrate cats (Rudy et al. 1991; Shefchyk and Buss 1998). Anesthesia may also effect the coordination between the bladder and the external urethral sphincter, and $\alpha$-chloralose caused dyssynergic contraction of the bladder and EUS during distention-evoked micturition (Rudy et al. 1991), while Angel et al. showed synergic EUS relaxation and bladder contraction under with $\alpha$-chloralose (Angel et al. 1994). In the present study the EUS ENG activity during distention-evoked bladder contractions was observed to be synergic in some instances and dyssynergic in others. However, synergy between the bladder and EUS was observed every time DP stimulation evoked a sustained contraction prior to SCT, and was also observed in all animals within 16 hours post SCT in this study. Thus, DP stimulation evoked a synergic micturition-like response despite the tendency of $\alpha$-chloralose to induce dyssynergia in previously synergic preparations.
The present study was limited to acute measurements before and up to 16 hours after spinal transection, and it did not investigate the properties of the reflex following chronic spinal cord injury (SCI). Spinal shock was not assessed directly, but the time course of the recovery of the bladder’s response to DP stimulation suggests that spinal shock initially depressed the bladder’s response and as the animals recovered, the bladder response to DP stimulation increased (Figure 9) (Hassouna et al. 1992; Zvara et al. 1998). In chronic SCI, plasticity in neural and muscle properties lead to hyper reflexia, bladder-sphincter dyssynergia, and bladder hypertrophy (de Groat et al. 1981; Krenz and Weaver 1998), and these changes could alter the properties of the reflex in chronic SCI. However, a similar reflex, mediated by pudendal afferents in the proximal urethra, is present in persons with chronic SCI (Gustafson et al. 2004).

4.2 Bladder response was dependent on bladder volume

Deep perineal nerve afferent stimulation produced bladder contractions at high bladder volumes but not at lower volumes, analogous to the effects produced by urethral fluid flow (Barrington 1931, 1941). When bladder volume was high, fluid flow through the urethra elicited a bladder contraction, and conversely when bladder volume was low, fluid flowing through the urethra inhibited bladder activity (Garry et al., 1959). The volume threshold to evoke bladder contractions by DP stimulation was 78 ± 17% of the volume threshold for distention evoked contractions, and urethral fluid flow evoked bladder contractions at bladder volumes 65 ± 17% of the volume required to evoke contractions with rapid infusion of saline into the bladder (Robain et al. 2001). Similarly, electrical stimulation of the urethral sensory nerve elicited excitation of bladder preganglionic parasympathetic neurons only if the bladder contained a sufficient volume (Mazieres et al. 1997), and an excitatory bladder response to urethral sensory nerve stimulation was observed.
at large bladder volumes (Shefchyk and Buss 1998). In humans intra-urethral stimulation produced bladder contractions only when the bladder contained a sufficient volume (Gustafson et al. 2004).

The differences between pressures evoked by DP stimulation and sacral root stimulation at low bladder volumes demonstrate that the length tension properties did not account for the absence of sustained bladder contractions evoked by DP stimulation at low bladder volumes (Figure 3). This difference in bladder response to efferent and afferent stimulation demonstrates that the reflex is modulated by the bladder volume and that the bladder volume threshold results from a neural mechanism. The volume dependence may be mediated by convergence of deep perineal and bladder stretch afferents within the spinal micturition circuitry (Morgan et al. 1981; Roppolo et al. 1985), and must exist in the lumbosacral spinal cord because the volume dependence was preserved following acute spinal cord transection at T12.

4.3 Bladder response to deep perineal nerve stimulation was dependent on frequency

Higher frequency stimulation (20 Hz – 40 Hz) elicited sustained bladder contractions more consistently than lower frequency stimulation (2 Hz – 10 Hz). Deep perineal nerve stimulation presumably activated afferents from fluid flow receptors in the region of the EUS (Todd 1964; Talaat 1937), and the frequency dependence of the electrically-evoked reflex is consistent with recordings of afferent fibers in the pudendal nerve showing peak firing rates between 20 Hz and 40 Hz during fluid flow through the urethra (Todd 1964). Spinalization increased the percent of stimuli that elicit sustained bladder contractions at all stimulation frequencies (Figure 10). The increase in sustained contractions following SCT may be due to the decrease in the minimum volume necessary for DP stimulation to elicit bladder contractions or to removal of descending inhibitory inputs (Holstege et al. 1986).
4.4 Urethral sphincter response to deep perineal stimulation is related to bladder response

Activation of deep perineal afferents initiated and sustained a synergic micturition-like reflex including a sustained bladder contraction and an absence of increased urethral sphincter ENG. These results confirm and expand on previous studies showing a decrease in the electromyographic activity of the urethral sphincter evoked by pudendal nerve stimulation during a bladder contraction before and after spinalization (Rampal and Mignard 1975a, b). At the onset of stimulation, the EUS ENG increased and then either returned to baseline or below as bladder pressure rose (Figure 6A) or remained elevated above baseline if bladder pressure did not increase (Figure 6B). The initial increase in EUS ENG activity prior to bladder contraction and the decrease in EUS ENG activity concomitant with bladder contraction are consistent with the response evoked by stimulation of the rostral urethral sensory branch of the pudendal nerve (Shefchyk and Buss, 1998), and similar to the biphasic EUS ENG response evoked by high frequency stimulation of pelvic detrusor nerve afferents after spinalization (Bradley and Teague 1972). Typically, no bladder contraction was elicited by low frequency DP stimulation and the EUS activity remained elevated above baseline for the duration of stimulation. The frequency dependence of the EUS ENG response is supported by earlier findings that activation of DP afferents with stimulation frequencies less than 20 Hz evoked an increase in contralateral EUS ENG activity while frequencies greater than 20 Hz did not (Bradley and Teague 1972). In the present study, the sphincter activity appeared more closely linked to bladder activity than to stimulation frequency because high frequency stimulation would occasionally elicit an increase in EUS ENG but only if a bladder contraction was not evoked.
4.5 The spinal reflex was mediated by activation of deep perineal afferents

The results of nerve transection demonstrated that the reflex was activated through afferent fibers (Figure 7). Bladder and sphincter activity may have also been influenced by retrograde activation of pudendal motoneurons. About half of EUS motoneurons have recurrent axon collaterals that terminate in or around Onuf’s nucleus and lamina VIII in sacral levels S1 and S2 (Sasaki 1994). The axon collaterals terminating within Onuf’s nucleus could modify the activity of ipsilateral EUS motoneurons but would not influence the analysis of EUS ENG data because activity was recorded from the contralateral DP. The collaterals terminating in lamina VIII could synapse onto putative interneurons and ultimately modify the activity of both EUS motoneurons and the preganglionic neurons innervating the bladder detrusor muscle.

4.6 Stimulation of deep perineal afferents elicits micturition

Bladder emptying achieved by stimulation of the deep perineal nerve afferents was approximately twice as effective as bladder emptying achieved by distention-evoked bladder contractions in α-chloralose anesthetized cats. The difference in voiding efficacy is consistent with the conclusion that DP stimulation elicits synergic bladder contraction and sphincter relaxation facilitating bladder evacuation while distention-evoked bladder contractions evoke dyssynergic sphincter contractions obstructing voiding under the influence of α-chloralose (Rudy et al. 1991; Thor and Katofiasc, 1995). The increased voiding percentages produced by DP stimulation also may be related to the capacity of DP stimulation to evoke bladder contractions at lower volumes than bladder distention (Figure 2 and 3). The volume remaining after DP stimulation may be the result of bladder volume decreasing below \( V_{\text{thr}} \) for DP stimulation evoked bladder contractions or α-chloralose induced decreased bladder pressures (Rudy et al. 1991) or both. This lumbosacral
reflex loop, shown to decrease urethral sphincter ENG activity and initiate sustained bladder contractions producing micturition, has clinical relevance for the development of a neural prosthesis to restore bladder emptying in persons with spinal cord injury.
Acknowledgements

This work was supported by NIH R21 NS-43450 (WMG), NIH K25 HD-40298 (KJG), and the Whitaker Graduate Student Fellowship (BJW). The authors thank Kerri Leder for outstanding technical assistance.
References


Barrington FJF. The component reflexes of micturition in the cat III. Brain 64: 239-243, 1941.

Barrington FJF. The component reflexes of micturition in the cat, Parts I and II. Brain 54: 177-188, 1931


Figure 1 Analysis of bladder pressure (black top trace) and external urethral sphincter electroneurogram (EUS ENG) (gray bottom trace) responses to electrical stimulation of the deep perineal nerve (black bar below the pressure and ENG traces). The analysis characterized a sustained bladder contraction in (A), a distension-evoked reflex contraction in (B), an unsustained contraction in (C), and no contraction in (D). In (A), (B) and (C) the bladder contractions were defined to begin 2 seconds prior to the first peak in the pressure trace and end at the last pressure peak in (B) and (C) and with the termination of the stimulus train in (A). The quantified bladder pressure is indicated by the gray horizontal line within each pressure trace. The analysis detected an increase in EUS ENG discharge in (B) and (D), but no discharge increase in (A) and (C). An increase in EUS ENG discharge was defined as the baseline ENG plus one standard deviation (“T” in ENG trace: the base of the “T” is the mean, and the horizontal bar forming the top of the “T” represents the +1 standard deviation level). Baseline was defined using the 2 seconds preceding stimulation in (A), (C), and (D), and as the 2 seconds preceding the bladder contraction in (B). The stimulation parameters were 33 Hz and 500 μA in (A), 20 Hz and 500 μA in (C) and 10 Hz and 500 μA in (D). The bladder volume was 20 ml in (B) and 8 ml in (A), (C), and (D).

Figure 2 Bladder responses to stimulation of the DP denoted by bars and bladder distention (infusion of 1 ml of saline in 1 second denoted by stair-steps below pressure trace). (A) The bladder contained 9 ml at the beginning of the trace. Initially, stimulation of the DP evoked small unsustained contractions denoted by gray bars (A) and (B), and distention produced similar single peak increases in bladder pressure denoted by gray steps in (A) and gray arrows in (B) and (C). When bladder volume was increased to 16 ml and greater, DP stimulation produced sustained
contractions of increasingly larger magnitudes denoted by black bars in (A), (B), and (C). At 16 ml and greater, the contractions elicited by DP stimulation were characterized by an increase in pressure that remained elevated significantly above baseline plus twice the standard deviation for the duration of stimulation (as defined in Methods). When bladder volume was increased to 24 ml and greater, bladder infusions generated distention-evoked contractions with multiple peaks, characterized by the period of slow oscillation, of similar magnitude denoted by the black steps in (A) and black arrow in (C). The first single peak contraction evoked by distention triggered reflex occurred when bladder volume was increased to 23 ml (C). Note that the volume thresholds observed in response to saline infusion are likely dependent on the rate of volume infusion, such that rapid infusion may elicit distention-evoked contractions at lower threshold volumes than slower filling during urine production. Ripples in the bladder pressure trace can be observed from the artificial ventilation in (B) and (C). DP stimulation parameters were 33 Hz and 700 μA for 10 seconds.

Figure 3 Bladder pressure changes (means ± standard deviations) generated by sacral nerve root stimulation (◇), DP stimulation (□), and distension evoked reflex contractions (▲) (n = 3 cats) as a function of bladder volume. Bladder pressures evoked by DP stimulation were significantly (* p < 0.001) lower than pressures evoked by sacral nerve root stimulation at low bladder volumes (3 and 6 ml) but not at higher volumes (9 and 12 ml). Bladder pressures evoked by DP stimulation were significantly (* p < 0.001) greater than pressures elicited by distension evoked reflex contractions at a mid range bladder volume (9 ml) but not at lower volumes (3 and 6 ml) or a higher volume (12 ml). Bladder pressures evoked by sacral nerve root stimulation were significantly greater than pressures elicited by distension evoked contractions at low to mid range bladder volumes (3, 6, and
9 ml) but not at a higher volume (12 ml). Therefore the volume dependence of the bladder response to DP stimulation did not depend only on the length tension properties of the detrusor muscle and was therefore mediated by a neural mechanism. All DP stimulation amplitudes were between \(2 \times \text{DP}_{\text{thr}}\) and \(4 \times \text{DP}_{\text{thr}}\), and the DP was stimulated at 33 Hz. The S1 and S2 nerve roots were stimulated at 20 Hz and 2 mA. The numbers in the symbols represent the number of trials. Statistical significance (* \(p < 0.001\)) was determined by two-way ANOVA of the null hypothesis that bladder pressure was independent of stimulus type for all bladder volumes, across 3 cats. Significant variance among bladder pressures was determined by post-hoc paired comparisons (Tukey-Kramer).

Figure 4 Histogram of the bladder responses evoked by DP stimulation at different stimulation frequencies (n = 330 across 8 cats). The percentage of sustained contractions and type of bladder contractions were dependent on the stimulation frequency. All stimulus frequencies elicited sustained (gray) and unsustained (black) contractions. The higher frequencies (20 Hz – 40 Hz) were more successful in generating bladder contractions that lasted for the duration of the stimulus. All stimulation amplitudes were between \(2 \times \text{DP}_{\text{thr}}\) and \(4 \times \text{DP}_{\text{thr}}\) and all bladder volumes were above threshold for distention-evoked bladder contractions. Statistical significance (p < 0.001 adjusted for ties) was determined with a Kruskal-Wallis test of the null hypothesis that the bladder response to DP stimulation was independent of stimulation frequency, n = 330 trials across 8 cats.

Figure 5 Bladder pressure of sustained contractions elicited by DP stimulation (means ± standard deviations) at different stimulation frequencies (n = 190 across 8 cats). Though the pressures of sustained contractions varied with stimulation frequency, no two stimulation frequencies produced
significantly different bladder pressures. All stimulation amplitudes were between 2*DP_{thr} and 4*DP_{thr} and all bladder volumes were above threshold for distention-evoked bladder contractions. The numbers in the boxes represent the number of trials. Statistical significance (p < 0.005) was determined by a one-way ANOVA of the null hypothesis that bladder pressure was independent of stimulation frequency, n = 190 sustained contractions across 8 cats.

Figure 6 Bladder pressure and rectified and integrated electroneurogram (ENG) from the external urethral sphincter (EUS) evoked by deep perineal nerve stimulation (indicated by bar below pressure and ENG trace). (A) EUS ENG activity initially increased during deep perineal nerve stimulation at 33 Hz (→), but returned to baseline as bladder pressure increased. EUS ENG activity remained within pre-stimulus baseline activity plus 1 standard of deviation (“T” in ENG trace: the base of the “T” is the mean, and the horizontal bar forming the top of the “T” represents the +1 standard deviation level) for the duration of the bladder contraction. (B) EUS ENG activity increased during deep perineal nerve stimulation at 2 Hz and remained above +1 standard deviation of the pre-stimulus baseline ENG for the duration of the bladder contraction. Stimuli were 20 second trains of 100μs, 900μA pulses, and bladder volume was above threshold for distension-evoked reflex contractions.

Figure 7 Bladder pressures evoked by deep perineal nerve stimulation (indicated with bars below pressure trace) were mediated by afferent nerve fibers. (A) Bladder contractions were elicited by deep perineal nerve stimulation prior to nerve transection. (B) After nerve transection, bladder contractions were elicited by stimulation proximal to the cut but not by stimulation distal to the cut.
Stimuli were 10 second 33 Hz trains of 100μs, 600μA pulses. Bladder volume was above threshold for distension-evoked reflex contractions.

Figure 8 Bladder responses to deep perineal nerve stimulation were mediated by circuits in the lumbosacral spinal cord. Stimulation of the deep perineal nerve elicited a bladder contraction before (A), 1h after (B), 5h after (C), and 15h after (D) transection of the spinal cord at T12. Stimuli were 20 second 33 Hz trains of 100μs, 900μA pulses. Bladder volume was above threshold for distension-evoked reflex bladder contractions prior to spinal cord transection.

Figure 9 Recovery of bladder contractions evoked by stimulation of the deep perineal nerve (DP) following spinal cord transection (SCT) at T12. All cats produced bladder contractions in response to DP stimulation following acute SCT (n = 8 cats). DP stimulation initially failed to evoke bladder contractions immediately following SCT, but the response of the bladder to DP stimulation returned as time progressed. Both the time course of recovery and the extent to which the reflex returned varied across cats. The contractions returned nearly to their pre-spinalization magnitude by eight hours post SCT in 3 cats; in 4 cats, the bladder response did not return fully to pre-SCT magnitude, and one cat died less than five hours after SCT. Each symbol represents a different cat. Bladder volumes were above threshold for distension-evoked bladder contractions.

Figure 10 Histogram of the bladder responses evoked by DP stimulation at different stimulation frequencies following acute spinal cord transection (SCT) (n = 176 across 6 cats). Post-SCT, all stimulus frequencies elicited sustained (gray) and unsustained (black) bladder contractions most of the time, but the percentage and type of bladder contractions elicited by stimulation depended on
stimulation frequency. High frequency (33 Hz) stimulation was more successful in generating bladder contractions that lasted for the duration of the stimulus than low frequency (2 Hz) stimulation (post hoc comparison). All stimulation amplitudes were between $2*DP_{\text{thr}}$ and $4*DP_{\text{thr}}$ and all bladder volumes were above threshold for distention-evoked bladder contractions. Statistical significance ($p < 0.005$ adjusted for ties) was determined by a Kruskal-Wallis test of the null hypothesis that the bladder response to DP stimulation was independent of stimulation frequency, n = 176 trials across 6 cats. The post hoc nonparametric pairwise comparison was based on Bonferroni inequalities (Gibbons 1993).

Figure 11 Bladder pressure of sustained contractions elicited by DP stimulation (means + standard deviations) at different stimulation frequencies following acute spinal cord transection (n = 147 across 6 cats). The pressures of sustained contractions varied with stimulation frequency, but no two stimulation frequencies produced significantly different bladder pressures. All stimulation amplitudes were between $2*DP_{\text{thr}}$ and $4*DP_{\text{thr}}$ and all bladder volumes were above threshold for distention-evoked bladder contractions. The numbers of trials are shown in the boxes. Statistical significance ($p < 0.05$) was determined by a one-way ANOVA of the null hypothesis that bladder pressure was independent of stimulation frequency, n = 147 sustained contractions across 6 cats.

Figure 12 Bladder pressure and rectified and integrated electroneurogram (ENG) from the external urethral sphincter (EUS) evoked by deep perineal nerve stimulation (indicated with bars below pressure and ENG trace) 16 hours after spinal cord transection (SCT). (A) EUS ENG decreased below baseline as bladder pressure increased during deep perineal nerve stimulation at 33 Hz. (B) EUS ENG activity increased during deep perineal nerve stimulation at 2 Hz and remained above
pre-stimulus baseline plus 1 standard deviation (“T” in ENG trace: the base of the “T” is the mean, and the horizontal bar forming the top of the “T” represents the +1 standard deviation level) for the duration of the bladder contraction. Stimuli were 15 second trains of 100μs, 900μA pulses, and bladder volume was above the pre-SCT threshold for distension-evoked reflex contractions.
Figure 1 Analysis of bladder pressure (black top trace) and external urethral sphincter electroneurogram (EUS ENG) (gray bottom trace) responses to electrical stimulation of the deep perineal nerve (black bar below the pressure and ENG traces). The analysis characterized a sustained bladder contraction in (A), a distension-evoked reflex contraction in (B), an unsustained contraction in (C), and no contraction in (D). In (A), (B) and (C) the bladder contractions were defined to begin 2 seconds prior to the first peak in the pressure trace and end at the last pressure peak in (B) and (C) and with the termination of the stimulus train in (A). The quantified bladder pressure is indicated by the gray horizontal line within each pressure trace. The analysis detected an increase in EUS ENG discharge in (B) and (D), but no discharge increase in (A) and (C). An increase in EUS ENG discharge was defined as
the baseline ENG plus one standard deviation ("T" in ENG trace: the base of the "T" is the mean, and the horizontal bar forming the top of the "T" represents the +1 standard deviation level). Baseline was defined using the 2 seconds preceding stimulation in (A), (C), and (D), and as the 2 seconds preceding the bladder contraction in (B). The stimulation parameters were 33 Hz and 500µA in (A), 20 Hz and 500µA in (C) and 10 Hz and 500µA in (D). The bladder volume was 20 ml in (B) and 8 ml in (A), (C), and (D).
Figure 2 Bladder responses to stimulation of the DP denoted by bars and bladder distention (infusion of 1 ml of saline in 1 second denoted by stair-steps below pressure trace). (A) The bladder contained 9 ml at the beginning of the trace. Initially, stimulation of the DP evoked small unsustained contractions denoted by gray bars (A) and (B), and distention produced similar single peak increases in bladder pressure denoted by gray steps in (A) and gray arrows in (B) and (C). When bladder volume was increased to 16 ml and greater, DP stimulation produced sustained contractions of increasingly larger magnitudes denoted by black bars in (A), (B), and (C). At 16 ml and greater, the contractions elicited by DP stimulation were characterized by an increase in pressure that remained elevated significantly above baseline plus twice the standard deviation for the duration of stimulation (as defined in Methods). When bladder volume was increased to 24 ml and greater, bladder infusions generated distention-evoked contractions with multiple peaks, characterized by the period of slow oscillation, of similar magnitude denoted by the black steps in (A) and black arrow in (C). The first single peak contraction evoked by distention triggered reflex occurred when bladder volume was increased to 23 ml (C). Note that the volume thresholds observed in response to saline infusion are likely dependent on the rate of volume infusion, such that rapid infusion may elicit distention-evoked contractions at lower threshold volumes than slower filling during urine production. Ripples in the bladder pressure trace can be observed from the artificial ventilation in (B) and (C). DP stimulation parameters were 33 Hz and 700 µA for 10 seconds.
Figure 3 Bladder pressure changes (means ± standard deviations) generated by sacral nerve root stimulation (△), DP stimulation (■), and distension evoked reflex contractions (▲) (n = 3 cats) as a function of bladder volume. Bladder pressures evoked by DP stimulation were significantly (* p < 0.001) lower than pressures evoked by sacral nerve root stimulation at low bladder volumes (3 and 6 ml) but not at higher volumes (9 and 12 ml). Bladder pressures evoked by DP stimulation were significantly (* p < 0.001) greater than pressures elicited by distension evoked reflex contractions at a mid range bladder volume (9 ml) but not at lower volumes (3 and 6 ml) or a higher volume (12 ml). Bladder pressures evoked by sacral nerve root stimulation were significantly greater than pressures elicited by distension evoked contractions at low to mid range bladder volumes (3, 6, and 9 ml) but not at a higher volume (12 ml). Therefore the volume dependence of the bladder response to DP stimulation did not depend only on the length tension properties of the detrusor muscle and was therefore mediated by a neural mechanism. All DP stimulation amplitudes were between 2*DP_{thr} and 4*DP_{thr}, and the DP was stimulated at 33 Hz. The S1 and S2 nerve roots were stimulated at 20 Hz and 2 mA. The numbers in the symbols represent the number of trials. Statistical significance (* p < 0.001) was determined by two-way ANOVA of the null hypothesis that
bladder pressure was independent of stimulus type for all bladder volumes, across 3 cats. Significant variance among bladder pressures was determined by post-hoc paired comparisons (Tukey-Kramer).
Figure 4 Histogram of the bladder responses evoked by DP stimulation at different stimulation frequencies (n = 330 across 8 cats). The percentage of sustained contractions and type of bladder contractions were dependent on the stimulation frequency. All stimulus frequencies elicited sustained (gray) and unsustained (black) contractions. The higher frequencies (20 Hz – 40 Hz) were more successful in generating bladder contractions that lasted for the duration of the stimulus. All stimulation amplitudes were between 2*DP_{thr} and 4*DP_{thr} and all bladder volumes were above threshold for distention-evoked bladder contractions. Statistical significance (p < 0.001 adjusted for ties) was determined with a Kruskal-Wallis test of the null hypothesis that the bladder response to DP stimulation was independent of stimulation frequency, n = 330 trials across 8 cats.
Figure 5 Bladder pressure of sustained contractions elicited by DP stimulation (means ± standard deviations) at different stimulation frequencies (n = 190 across 8 cats). Though the pressures of sustained contractions varied with stimulation frequency, no two stimulation frequencies produced significantly different bladder pressures. All stimulation amplitudes were between $2 \times DP_{thr}$ and $4 \times DP_{thr}$ and all bladder volumes were above threshold for distention-evoked bladder contractions. The numbers in the boxes represent the number of trials. Statistical significance ($p < 0.005$) was determined by a one-way ANOVA of the null hypothesis that bladder pressure was independent of stimulation frequency, $n = 190$ sustained contractions across 8 cats.
Figure 6 Bladder pressure and rectified and integrated electroneurogram (ENG) from the external urethral sphincter (EUS) evoked by deep perineal nerve stimulation (indicated by bar below pressure and ENG trace). (A) EUS ENG activity initially increased during deep perineal nerve stimulation at 33 Hz (-), but returned to baseline as bladder pressure increased. EUS ENG activity remained within pre-stimulus baseline activity plus 1 standard of deviation ("T" in ENG trace: the base of the "T" is the mean, and the horizontal bar forming the top of the "T" represents the +1 standard deviation level) for the duration of the bladder contraction. (B) EUS ENG activity increased during deep perineal nerve stimulation at 2 Hz and remained above +1 standard deviation of the pre-stimulus baseline ENG for the duration of the bladder contraction. Stimuli were 20 second trains of 100μs, 900μA pulses, and
bladder volume was above threshold for distension-evoked reflex contractions.
Figure 7 Bladder pressures evoked by deep perineal nerve stimulation (indicated with bars below pressure trace) were mediated by afferent nerve fibers. (A) Bladder contractions were elicited by deep perineal nerve stimulation prior to nerve transection. (B) After nerve transection, bladder contractions were elicited by stimulation proximal to the cut but not by stimulation distal to the cut (C). Stimuli were 10 second 33 Hz trains of 100μs, 600μA pulses. Bladder volume was above threshold for distension-evoked reflex contractions.
Figure 8 Bladder responses to deep perineal nerve stimulation were mediated by circuits in the lumbosacral spinal cord. Stimulation of the deep perineal nerve elicited a bladder contraction before (A), 1h after (B), 5h after (C), and 15h after (D) transection of the spinal cord at T12. Stimuli were 20 second 33 Hz trains of 100μs, 900μA pulses. Bladder volume was above threshold for distension-evoked reflex bladder contractions prior to spinal cord transection.
Figure 9 Recovery of bladder contractions evoked by stimulation of the deep perineal nerve (DP) following spinal cord transection (SCT) at T12. All cats produced bladder contractions in response to DP stimulation following acute SCT (n = 8 cats). DP stimulation initially failed to evoke bladder contractions immediately following SCT, but the response of the bladder to DP stimulation returned as time progressed. Both the time course of recovery and the extent to which the reflex returned varied across cats. The contractions returned nearly to their pre-spinalization magnitude by eight hours post SCT in 3 cats; in 4 cats, the bladder response did not return fully to pre-SCT magnitude, and one cat died less than five hours after SCT. Each symbol represents a different cat. Bladder volumes were above threshold for distention-evoked bladder contractions.
Figure 10 Histogram of the bladder responses evoked by DP stimulation at different stimulation frequencies following acute spinal cord transection (SCT) (n = 176 across 6 cats). Post-SCT, all stimulus frequencies elicited sustained (gray) and unsustained (black) bladder contractions most of the time, but the percentage and type of bladder contractions elicited by stimulation depended on stimulation frequency. High frequency (33 Hz) stimulation was more successful in generating bladder contractions that lasted for the duration of the stimulus than low frequency (2 Hz) stimulation (post hoc comparison). All stimulation amplitudes were between $2 \times \text{DP}_{\text{thr}}$ and $4 \times \text{DP}_{\text{thr}}$ and all bladder volumes were above threshold for distention-evoked bladder contractions. Statistical significance (p < 0.005 adjusted for ties) was determined by a Kruskal-Wallis test of the null hypothesis that the bladder response to DP stimulation was independent of stimulation frequency, n = 176 trials across 6 cats. The post hoc nonparametric pairwise comparison was based on Bonferroni inequalities (Gibbons 1993).
Figure 11 Bladder pressure of sustained contractions elicited by DP stimulation (means + standard deviations) at different stimulation frequencies following acute spinal cord transection (n = 147 across 6 cats). The pressures of sustained contractions varied with stimulation frequency, but no two stimulation frequencies produced significantly different bladder pressures. All stimulation amplitudes were between 2*DP_{thr} and 4*DP_{thr} and all bladder volumes were above threshold for distention-evoked bladder contractions. The numbers of trials are shown in the boxes. Statistical significance (p < 0.05) was determined by a one-way ANOVA of the null hypothesis that bladder pressure was independent of stimulation frequency, n = 147 sustained contractions across 6 cats.
Figure 12 Bladder pressure and rectified and integrated electroneurogram (ENG) from the external urethral sphincter (EUS) evoked by deep perineal nerve stimulation (indicated with bars below pressure and ENG trace) 16 hours after spinal cord transection (SCT). (A) EUS ENG decreased below baseline as bladder pressure increased during deep perineal nerve stimulation at 33 Hz. (B) EUS ENG activity increased during deep perineal nerve stimulation at 2 Hz and remained above pre-stimulus baseline plus 1 standard deviation (“T” in ENG trace: the base of the “T” is the mean, and the horizontal bar forming the top of the “T” represents the +1 standard deviation level) for the duration of the bladder contraction.
Stimuli were 15 second trains of 100µs, 900µA pulses, and bladder volume was above the pre-SCT threshold for distension-evoked reflex contractions.