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Bladder Activation by Selective Stimulation of Pudendal Nerve Afferents in the Cat

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Abstract

Bladder contractions evoked by pudendal nerve stimulation in both spinal intact and spinal transected cats support the possibility of restoring urinary function in persons with chronic spinal cord injury (SCI). However, electrically evoked bladder responses in persons with SCI were limited to transient contractions at relatively low pressures. This prompted the present study, which presents a detailed quantification of the responses evoked by selective stimulation of individual branches of the pudendal nerve at different stimulation frequencies. In spinal intact cats anesthetized with α -chloralose, selective frequency dependent electrical activation of the sensory ($2 \text{ Hz} \leq f \leq 50 \text{ Hz}$), cranial sensory ($f \leq 5 \text{ Hz}$), dorsal genital ($f \geq 20 \text{ Hz}$) and rectal perineal ($f \leq 10 \text{ Hz}$) branches of the pudendal nerve evoked sustained bladder contractions dependent on the stimulation frequency. Contractions evoked by selective electrical stimulation resulted in significant increases in voiding efficiency compared to bladder emptying by distension evoked contractions ($p_{\text{ANOVA}} < 0.05$). Acute spinal transection abolished reflex bladder contractions evoked by low frequency stimulation of the cranial sensory or rectal perineal branches, whereas contractions evoked by high frequency stimulation of the dorsal genital branch remained intact. This study presents evidence for two distinct micturition pathways (spino-bulbo-spinal vs. spinal reflexes) activated by selective afferent pudendal nerve stimulation, the latter of which may be applied to restore bladder function in persons with SCI.

Keywords

electrical stimulation; cranial sensory nerve; deep perineal branch; lower urinary tract physiology; external urethral sphincter; spinal cord injury; pudendo-pudendal reflex; spinal-bulbo-spinal; prostatic urethra; dorsal nerve of penis

Introduction

Neurogenic bladder dysfunction results from the loss of supraspinal inputs and neural reorganization following spinal cord injury (SCI), and is associated with urinary incontinence (neurogenic detrusor overactivity) and inefficient voiding of the urinary bladder (detrusor sphincter dyssynergia). Clean intermittent catheterization with concomitant anti-cholinergic medication is currently the most effective means of bladder management for most individuals with SCI. However, there are several limitations to this treatment including drug-related side

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effects, urethral damage, and recurrent urinary tract infections that can significantly diminish quality of life (Shingleton and Bodner, 1993).

Electrical stimulation of the pudendal nerve has been investigated in persons with SCI as an alternative approach to restoring urinary function. Low frequency activation (5 ~ 20 Hz) of the dorsal genital nerve results in robust inhibition of detrusor activity, significant increases in cystometric capacity in persons with SCI (Dalmose, et al., 2003, Fjorback, et al., 2006, Hansen, et al., 2005, Kirkham, et al., 2001, Vodusek, et al., 1986, Wheeler, et al., 1994), and is a potential treatment for urinary incontinence (i.e., urine storage). The same continence promoting inhibition of the bladder is evoked by low frequency stimulation (3 ~ 15 Hz) of the pudendal nerve trunk (Boggs, et al., 2006, Lindstrom, et al., 1983, Sundin, et al., 1974, Tai, et al., 2007, Walter, et al., 1993), or selective dorsal genital nerve (Woock, et al., submitted) in both spinal intact and chronically transected cats, and this enabled identification of potential underlying mechanisms (Craggs and McFarlane, 1999).

Conversely, activation of an excitatory pudendo-vesicle response has been studied as a means of restoring functional micturition (i.e., bladder emptying) in spinal intact and acute SCI (Boggs, et al., 2006, Gustafson, et al., 2003, Shefchyk and Buss, 1998, Yoo and Grill, 2007), and chronic SCI cats (Tai, et al., 2006). Electrical stimulation of the pudendal nerve at 20 – 50 Hz evoked robust sustained bladder contractions and voiding. However, the responses to high frequency pudendal nerve stimulation in humans with chronic SCI were limited to transient or small increases in bladder pressure (Gustafson, et al., 2004, Yoo, et al., 2007). Therefore, to develop an approach to restore functional bladder emptying in humans, we first sought to identify the specific afferent pathways responsible for the robust detrusor activation observed in animals.

The objective of this work was to quantify bladder responses evoked by selective activation of individual branches of the pudendal nerve. We measured the evoked bladder pressure in both spinal intact and acutely transected cats in response to selective electrical activation of distinct subsets of pudendal nerve afferents at various stimulation amplitudes and frequencies. The results demonstrate two distinct sensory pathways of the pudendal nerve, innervating the penis and the proximal urethra, each responding to different ranges of stimulation frequencies and exhibiting different degrees of preservation following acute spinal cord transection.

Materials and Methods

A series of non-survival experiments were conducted in 22 adult male cats (weight = 3.3–5.5 kg) and are summarized in Table 1. Anesthesia was induced with ketamine HCl (35 mg/kg, I.M.) and maintained with I.V. α -chloralose (initial 65 mg/kg supplemented at 15 mg/kg). Artificial respiration maintained end tidal CO₂ at 3–4 %, core temperature (39°C) was maintained with heating pads, arterial blood pressure was monitored via a catheter in the carotid artery, and fluids were administered continuously (~15 ml/kg/hr). At the conclusion of the experiment, animals were euthanized with an overdose of sodium pentobarbital (I.V.). Animal care and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Duke University.

Surgical Procedure

Following a midline abdominal incision, a 3.5 Fr catheter was introduced through the apex of the bladder and secured using a purse-string suture. The catheter was connected in series with a solid state pressure transducer (Deltran, Utah Medical, Midvale, UT) and a syringe pump, which was used to infuse room temperature 0.9 % saline solution. Both ureters were ligated, transected, and externally drained, and the abdomen was sutured closed in layers.

In a prone position, a longitudinal incision was made between the base of the tail and the ischial tuberosity. Transection of the gluteofemoralis muscle and blunt dissection of the ischiorectal fossa exposed the pudendal nerve, which divided into the sensory (SN) and rectal perineal (RP) branches (Figure 1). Further dissection of these two distal branches provided access to the cranial urethral sensory (CSN) and dorsal nerve of the penis (DNP), and the deep perineal (dPN) and caudal rectal (CR) branches (Martin, et al., 1974). A current pulse generator (Pulsar 6bp, FHC Inc., Bowdoin, ME) was used to deliver electrical stimuli to each nerve branch via nerve cuff electrodes that consisted of platinum contacts embedded within a silicone elastomeric cuff.

Electromyographic (EMG) signals were recorded from the external anal sphincter (EAS) with a pair of insulated stainless steel wires ipsilateral to the site of stimulation, and also from the bulbourethral sphincter (BUS) with a custom-made bipolar ring catheter electrode (1 mm Pt contacts separated by 1 cm). EMG activity of the BUS was recorded at the most constricted portion of the urethra, located 3–5 cm from the urethral meatus (Wang, et al., 1999), and EMG signals were amplified and filtered (gain = 1000, filter = 10 Hz to 3 kHz).

Isovolumetric Bladder Contraction Trials

In 5 spinally intact cats, the bladder volume was adjusted (fill rate = 1 ml/min) to approximately 90% of the volume at which distension-evoked reflex contractions began to occur and the responses to selective electrical stimulation of each nerve branch (SN, CSN, DNP, RP, dPN and CR) were measured. Trains of monophasic cathodic stimulus pulses (pulse width = 0.1 ms; duration = 20 s) were delivered through each nerve cuff electrode, while the bladder pressure and EMG responses were recorded (sampling rate = 12.5–20 kHz; Astromed 8Xe, Astro-Med Inc). At specific stimulation frequencies ($f = 2, 5, 10, 20, 33$ and 50 Hz), the stimulus amplitude was varied between values below the threshold for EAS/BUS activation and a preset limit (up to 1 mA) in pseudo-random order. Each stimulation paradigm was repeated at least three times. In one experiment, α -bungarotoxin (0.1 mg/kg, Sigma-Aldrich Inc.) was administered intravenously to differentiate the afferent vs. efferent effects of pudendal nerve branch stimulation.

The effects of acute spinal cord transection on electrically evoked reflex bladder activity were investigated in 4 additional cats. A laminectomy was made to access to the spinal cord at T10. The dura was opened, lidocaine was applied to the surface of the spinal cord, and the exposed cord was elevated and completely transected. Surgicel (Johnson & Johnson, New Brunswick, NJ) was used to promote coagulation and maintain separation between the two ends of the cord. Bladder contractions evoked by electrical stimulation of the DNP, CSN and RP branches were compared before and after spinal transection. At least 4 hours elapsed between spinal cord transection and stimulation to allow for at least partial resolution of spinal shock (Boggs, et al., 2005).

Bladder Voiding Trials

In 12 cats, the voiding achieved by selective stimulation of individual nerve branches was investigated. With the bladder filled (rate = 1 ml/min) just below the threshold for distension evoked reflex bladder activity, the lack of which was verified by a minimum 5 second pause, continuous or intermittent trains of stimulus pulses were subsequently delivered to one of the nerve branches to initiate voiding. Stimulation was terminated following the end of the void, and both the voided volume (collection vial) and residual volume (withdrawn via the suprapubic bladder catheter) were measured to calculate the voiding efficiency (VE):

$$VE(\%) = \frac{\text{Voided Volume}}{\text{Infused Bladder Volume}} \times 100$$

Based on results from the isovolumetric stimulation trials, stimulation was either with low ($2 \text{ Hz} \leq f \leq 10 \text{ Hz}$) or high ($10 \text{ Hz} < f \leq 50 \text{ Hz}$) frequency trains. For control trials (i.e., distension evoked voiding), the bladder was filled until a sustained bladder contraction or a continuous stream of fluid was observed. Following an initial control voiding trial, subsequent stimulation evoked or control voiding was repeated at least twice and performed in pseudo-random order. In 7 cats, voiding was measured for SN or RP branch stimulation, and in 5 cats voiding was measured with DNP or CSN branch stimulation.

Data Analysis

A sustained bladder contraction was defined as an increase in bladder pressure of $10 \text{ cmH}_2\text{O}$ that remained until the end of stimulation. The mean pressure during a bladder contraction was computed between the point where the bladder pressure exceeded $10 \text{ cmH}_2\text{O}$ above the baseline pressure and the end of stimulation, and was averaged over three repeated trials. The effectiveness of electrical stimulation was also quantified by the percentage of trials that resulted in a bladder contraction for each nerve branch and stimulation frequency. Grouped data were compared using an analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test corrected for multiple comparisons. Experimental values are expressed as mean $\pm$ standard deviation, unless specified otherwise.

Results

Isovolumetric Bladder Responses to Nerve Stimulation

At or above 90% of the volume threshold for distension evoked bladder contractions, sustained bladder contractions were evoked in every experiment by electrical stimulation of at least one of the primary pudendal nerve branches (SN and RP) or one of the secondary components (CSN and DNP) of the SN branch. Both the bladder pressures measured during a contraction (mean = $26.8 \pm 9.2 \text{ cmH}_2\text{O}$, range = $12.6 \text{ cmH}_2\text{O}$ to $48.7 \text{ cmH}_2\text{O}$) and the stimulus threshold ($I = 0.03 \text{ mA} - 0.4 \text{ mA}$) required to evoke these bladder contractions varied across all experiments.

Electrical stimulation of the SN branch elicited isovolumetric bladder contractions using trains of both low ($f \leq 10 \text{ Hz}$) and high ($f \geq 20 \text{ Hz}$) frequency pulses (Figure 2A). This resulted in a flat frequency tuning curve that exhibited a uniform pattern of stimulation evoked contractions across all tested frequencies (Figure 3A). Electrical activation of the proximal stump of the transected SN branch still evoked bladder contractions (2 experiments), confirming that these responses were a result of afferent nerve activity.

Selective electrical stimulation of nerves distal to the SN branch revealed differential frequency-dependent bladder activation patterns (Figure 2B): high frequency DNP stimulation or low frequency CSN stimulation evoked bladder contractions. These frequency-dependent characteristics were confirmed by the frequency tuning curves (Figure 3C,D), where the range of excitatory stimulation frequencies were $\geq 20 \text{ Hz}$ for the DNP and $\leq 5 \text{ Hz}$ for the CSN. Thus the broad frequency tuning of bladder responses evoked by SN branch stimulation resulted from the superposition of responses evoked by the individual afferent components.

Electrical activation of the RP branch also generated reflex bladder contractions, but mainly at frequencies below 20 Hz (Figure 2A,C). This yielded a frequency tuning curve indicating a bias to low frequency pulse trains ($f \leq 10 \text{ Hz}$, Figure 3B). The absence of any response evoked by selective stimulation of the caudal rectal (CR) branch showed that this bladder reflex was mediated specifically by the deep perineal (dPN) branch (Figure 2C). Selective bilateral transection of the dPN branches further revealed that these reflex bladder contractions were in fact generated by afferent pudendal nerve activity evoked by the motor response arising from

afferent dPN stimulation. This was demonstrated by the following procedures (Figure 2C): stimulation of the proximal transected stump of the dPN branch failed to evoke a bladder response; while subsequent activation of the distal cut end of the ipsi- or contralateral dPN branch evoked bladder contractions. Finally, intravenous injection of α -bungarotoxin (0.1 mg/kg) in one cat completely abolished bladder activity evoked by distal dPN/RP branch stimulation. Consistent with this assertion, selective low frequency stimulation of the CSN branch continued to generate bladder contractions following bilateral transection of the RP branches (Figure 2C).

EMG Responses to Selective Nerve Stimulation

Electrical stimulation ($f = 1$ Hz) of pudendal nerve branches evoked EMGs that were identified as either direct or reflex responses, based on the measured latency being less or greater than 3 ms, respectively. Activation of the SN branch elicited only reflex responses in the external anal sphincter (EAS) and bulbourethral sphincter (BUS) (Figure 4A), confirming the afferent composition of this nerve branch. Selective stimulation of the RP branch evoked both direct and reflex EMGs (Figure 4B). Subsequent stimulation of the distal transected stump of the RP branch did not alter the evoked direct and reflex EMGs, while stimulation of the proximal cut end abolished all EMG responses. This indicated that, like the bladder response, the reflex EMG generated by RP branch stimulation was caused by afferent activity resulting from the motor response to efferent stimulation. Further selective activation of the dPN branch (derived from the RP) indicated direct efferent innervation of the BUS muscles with minimal evoked reflex activity (Figure 4C).

Bladder Voiding Generated by Nerve Branch Stimulation

The bladder voiding efficiency (VE) generated by selective stimulation of the SN and RP branches (7 cats), or the CSN and DNP branches (5 cats) was measured. Electrical stimulation commenced following termination of bladder filling, and generally resulted in a continuous stream of urethral flow that often switched to discrete spurts or drops of fluid as the reflex EAS response re-emerged at the end of the contraction (Figure 5).

The VE achieved by distension evoked bladder contractions was consistently lower than the VE evoked by selective nerve branch stimulation (range = 9.3 % to 48 %, $n = 12$ cats, Figure 6). Stimulation evoked voiding was observed in all cats, but the percent of experiments in which stimulation produced voiding varied across nerve branches. Stimulation at low frequencies ($1 \text{ Hz} \leq f \leq 15 \text{ Hz}$) resulted in more efficient voids compared to distension evoked contractions (Figure 6): SN (VE = 19.6 to 77 %, 5 of 7 cats), CSN (VE = 50 to 91.4 %, 4 of 5 cats) and RP (VE = 23 to 99 %, 4 of 7 cats) branches. Efficient voiding was also achieved by high frequency ($f > 15 \text{ Hz}$) stimulation of the SN (VE = 16 to 80 %, 6 of 7 cats), DNP (VE = 51 to 79.4 %, 5 of 5 cats) and CSN (VE = 56.3 %, 1 of 5 cats) branches. The VE generated by high frequency CSN stimulation was not significantly greater than distension evoked voiding (Figure 6). Electrical nerve stimulation also failed to achieve bladder voiding in two other cases: low frequency DNP stimulation that evoked reflex bladder inhibition, and high frequency RP stimulation that resulted in dyssynergic activation of the EUS.

Effects of Acute Spinal Transection

Following acute spinal transection (Figure 7A), low frequency stimulation of the CSN or RP branches continued to evoke excitatory bladder responses (range = 0.9 to 5.3 cmH₂O, 4 of 4 cats) that exhibited an early peak followed typically by sustained low pressure activity. High frequency stimulation of the DNP branch elicited more robust bladder contractions that were sustained for the duration of stimulation (range = 12.9 to 21.5 cmH₂O, 2 of 4 cats). The average residual evoked pressures (Figure 7B) in response to selective DNP, CSN and RP stimulation

in the acute SCT cats were 34%, 14% and 4% of the pre-spinalization bladder responses, respectively.

Discussion

The results of this study provide a detailed quantification of the reflexes evoked by activation of pudendal afferents that extends our current understanding of the excitatory pudendo-vesicle reflex evoked by electrical nerve stimulation. In the spinal intact cat, there were two distinct afferent pudendal nerve pathways that evoked isovolumetric sustained bladder contractions in response to selective nerve stimulation: high frequency ($20 \text{ Hz} \leq f \leq 50 \text{ Hz}$) stimulation of the dorsal genital branch (DNP) and low frequency ($2 \text{ Hz} \leq f \leq 5 \text{ Hz}$) stimulation of the cranial sensory (CSN) branch. The distinct excitatory frequency range of these neural pathways was consistent with the flat frequency tuning curve obtained from SN branch stimulation (Figure 3A), and with the full frequency range of afferent neural discharge recorded during urethral fluid flow (Todd, 1964). The bladder responses evoked by selective electrical stimulation of pudendal afferents following acute spinal transection (SCT) further differentiated these two micturition pathways: high frequency activated DNP (direct spinal) pathway that still evoked robust sustained bladder contractions and the low frequency CSN (spino-bulbo-spinal) pathway that evoked transient bladder contractions analogous to those observed in humans with chronic SCI pressure (Gustafson, et al., 2004, Yoo, et al., 2007). These results (summarized in Figure 8) indicate that subsequent efforts to restore micturition function in persons with chronic SCI should focus on activation of the direct spinal pathway mediated by DNP afferents.

Sustained Bladder Contractions Evoked by Pudendal Afferents

This is the first study to identify the CSN as the afferent pathway responsible for evoking excitatory bladder contractions by low frequency ($f \leq 10 \text{ Hz}$) pudendal nerve trunk stimulation in spinal intact cats (Boggs, et al., 2006, Mazieres, et al., 1997, Yoo and Grill, 2007). The absence of any excitatory bladder activity in response to DNP activation at these stimulus frequencies further supports the notion of the CSN as an independent component of the SN branch. Based on earlier studies, the afferent fibers of the CSN branch are thought to extend cranially along the anterior urethra (Bradley, et al., 1973) and innervate urethral mechanoreceptors (e.g., pacinian corpuscles) located in the membranous and prostatic urethra (Todd, 1964).

Following acute SCT, the response to CSN branch stimulation was reduced to transient low pressure increases in bladder pressure (Figure 7A), which corresponded to the responses generated by urethral sensory (urSPud = CSN) stimulation in SCT cats (Shefchyk and Buss, 1998). That robust sustained bladder contractions could not be evoked in the acute SCT cat indicated this low frequency pudendo-vesicle reflex is associated with the A δ afferent fiber mediated spino-bulbo-spinal micturition reflex (Barrington, 1931, de Groat, et al., 1990). In cats, this supraspinal micturition reflex loop involves an initial convergence of pudendal and pelvic afferents in the sacral spinal column (de Groat, 2006, Thor, et al., 1989, Ueyama, et al., 1987), an ascending projection to the pontine micturition center (Blok, et al., 1995), and a descending projection terminating in the sacral parasympathetic nucleus (SPN) (Holstege and Mouton, 2003). As a consequence of the loss of these connections, limited bladder responses are evoked by low frequency pudendal nerve trunk stimulation in the SCI cat (Tai, et al., 2007) and in persons with chronic SCI (Gustafson, et al., 2004, Yoo, et al., 2007).

The micturition reflex pathway associated with electrical stimulation of the feline dorsal genital nerve (DNP) is distinct from the CSN pathway, as robust bladder contractions can be evoked both prior to and following SCT of the thoracic spinal cord (Figure 7). The high degree of variability associated with evoking this DNP mediated pudendo-vesicle reflex following SCT

is in agreement with previous work (Boggs, et al., 2005, Boggs, et al., 2006) and provides evidence that both supraspinal (A δ fiber) and spinal (c fiber) micturition pathways are involved (Mazieres, et al., 1998). In neonatal cats, this direct spinal pudendo-vesicle reflex is described as a mechanically induced perigenital reflex that facilitates bladder activity (DeGroat, et al., 1975). During maturation, a serotonergic descending inhibitory pathway masks this neonatal reflex (Thor, et al., 1990), but the pathway re-emerges in the chronic SCI cat (Tai, et al., 2007). The present data indicate that this pathway can be activated electrically following acute SCT.

A summary of bladder responses evoked by either electrical or mechanical stimulation of pudendal afferents is presented in Table 2. Mechanical perigenital (PG-M) evoked differential bladder responses in neonatal, adult, and SCI animals, presumably a reflection of the plasticity and ensuing shifts in the frequency tuning properties of the spinal neural networks. The occurrence of this spinal reorganization, however, is not apparent in the consistent frequency dependent bladder responses evoked by direct electrical stimulation of the compound pudendal nerve, its distal branches or the perigenital area (Table 2). In all cases low frequency stimulation evoked bladder inhibition (Adult CP/DNP, and Chronic SCT PG-E/CP), and high frequency stimulation evoked bladder excitation (Adult CP/DNP, and Chronic SCT PG-E/CP). This is particularly evident in the excitatory bladder responses evoked by high frequency stimulation in acute SCT cats, in which there was certainly insufficient time for significant plastic changes to occur in the sacral cord. Thus, if we consider that mechanical PG-M stimulation can co-activate pudendal afferent fibers associated with both bladder inhibitory and excitatory reflexes, the differential bladder responses to electrical or mechanical stimulation can be explained as the sum of various excitatory or inhibitory inputs converging onto the efferent sacral parasympathetic nuclei. Accordingly, the excitatory perigenital (PG-M) reflex in the neonatal cat becomes inhibitory in the spinal intact cat as a result of the added inhibitory effect of the supraspinal serotonergic descending pathway (Thor, et al., 1990), whereas the excitatory high frequency CP reflex in the spinal intact cat is the outcome of the combined excitatory spinal neonatal and supraspinal micturition pathways over the inhibitory serotonergic pathway. This analysis is critical for clinical translation, as the feasibility of this approach will depend on identifying the presence of analogous spinal pudendo-vesicle reflexes in persons with chronic SCI.

The Effects of RP Branch Stimulation

Low frequency electrical stimulation of the RP branch, specifically the deep perineal (dPN) component, also evoked SBCs (Figure 3B) and augmented bladder voiding (Figure 6) in spinal intact cats. Selective activation of the bilaterally transected dPN nerve branches (Figure 2C) and application of a nicotinic acetylcholine receptor antagonist (α -bungarotoxin) both confirmed that the electrically evoked excitatory bladder responses were secondary to skeletal muscle contractions, analogous to the urethrovesical reflex in humans (Shafik, et al., 2003). It is important to point out that low frequency DNP stimulation did not evoke bladder contractions, even at frequencies ($f \leq 2$ Hz) below those associated with reflex bladder inhibition (Tai, et al., 2006, Woock, et al., submitted). This consistent non-responsiveness to low frequency DNP stimulation suggests a paucity of mechanoreceptors in the anterior urethra that could possibly limit the role of the anterior urethra, between the base and the glans of the penis, in the micturition reflex.

The low frequency dPN mediated pudendo-vesicle reflex is another pudendal nerve pathway that has not been described previously. Both the absence of bladder contractions in response to high frequency stimulation ($f > 20$ Hz) and the lack of any bladder contractions evoked by CR branch stimulation at any frequency (Bradley and Teague, 1972), provide evidence that

reflex bladder contractions are not mediated by dPN afferents, as previously suggested (Boggs, et al., 2005), but rather result from re-afference.

Pudendo-Pudendal Reflex Arc

The branching patterns of the SN and RP branches in the cat (Figure 1) were consistent with the recorded EMG responses (Figure 4), suggesting a functional division of the pudendal nerve trunk into afferent sensory and efferent motor branches, similar to that reported in the rat (McKenna and Nadelhaft, 1986). The range of latencies (7.5 to 16.1 ms) measured from the SN branch evoked pudendo-anal reflex was consistent with reflexes associated with compound pudendal nerve trunk and dorsal genital nerve stimulation in cats (Cook, et al., 1991, Rampal and Mignard, 1975). We also observed a stimulus amplitude dependent shift in latency of the pudendo-anal reflex that was also consistent with that previously reported in cats (Rampal and Mignard, 1975) and in human subjects with or without SCI (Dyro and Yalla, 1986, Rampal and Mignard, 1975). In this study, selective activation of the CSN branch also unmasked a urethro-anal reflex that exhibited significantly longer latencies, compared to DNP stimulation, that were analogous to reflexes evoked by intraurethral stimulation at the level of the prostatic urethra in humans (Bradley, et al., 1973, Dick, et al., 1974). The persistence of both reflexes following acute spinal transection and the differential latencies elicited by DNP and CSN stimulation support the notion that these are spinal oligosynaptic and polysynaptic reflexes, respectively (Dyro and Yalla, 1986).

Effects of Anesthesia on Bladder Voiding

As previously demonstrated in decerebrate cats (Rudy, et al., 1991), the voiding efficiency achieved by distension evoked bladder contractions was profoundly impaired by α -chloralose (Figure 6). Electrical pudendal nerve stimulation improved the voiding efficiency by activating reflex micturition pathways to the pelvic efferent output that were also presumably blunted by the anesthetic agent. This view of an inhibited spinal or supraspinal drive to the urinary bladder as being the main reason for inefficient distension evoked voiding is supported by the continuous stream of fluid observed during stimulation (Figure 6), which offered no evidence of dyssynergic EUS activity. As previously discussed (Boggs, et al., 2006, Tai, et al., 2006), α -chloralose is the most appropriate anesthetic for demonstrating the physiological effects of electrical nerve stimulation in the lower urinary tract.

Clinical Outlook

The current study identified two distinct afferent branches of the pudendal nerve (CSN and DNP) that can mediate electrically evoked bladder contractions. The similarity of bladder inhibition by low frequency DNP stimulation in both cats and human subjects (see Introduction), suggests the possibility of analogous excitatory pathways also existing in persons with SCI. The identification of this DNP-mediated spinal micturition pathway will depend on the use of higher stimulation frequencies (> 20 Hz) and more selective activation of nerve fibers by utilizing minimally-invasive methods, such as intraurethral or percutaneous needle stimulation (Gustafson, et al., 2004, Yoo, et al., 2007).

The CSN-mediated spino-bulbo-spinal pathway presents an additional means of restoring urinary function, specifically for individuals with incomplete spinal injuries. According to the National Spinal Cord Injury Statistical Center, this extent of injury comprises a significant percentage of all individuals diagnosed with SCI (estimated 34.1 % of tetraplegics and 18.5% of paraplegics). The precise anatomical innervation of the afferent pudendal nerve branches, particularly the CSN pathway, has not yet been identified.

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Figure 1.

The feline pudendal nerve trunk consists of an afferent sensory (SN) and efferent rectal perineal (RP) branch. The SN component further branches into the cranial urethral sensory nerve (CSN) and the dorsal nerve of the penis (DNP), while the RP separates into the deep perineal (dPN) and the caudal rectal (CR) nerves. The labels were based on previous anatomical work in cats (Martin, et al., 1974).

Figure 2.

Bladder contractions evoked by selective pudendal nerve branch stimulation. (A) Selective activation of the SN generated bladder responses at all frequencies (≥ 0.05 mA), whereas primarily low frequency ($f \leq 10$ Hz) RP branch stimulation evoked contractions. (B) Selective activation of the DNP and CSN branches revealed differential frequency tuning between the two components of the SN branch. (C) Selective transection of the RP branch showed that bladder activity could only be elicited by activating the distal stump of the nerve. Note CSN stimulation could still evoke bladder responses following bilateral section of the RP branch. [Bar = 20 second stimulus pulse train]

Figure 3.

Analysis of the overall percentage of stimulation trials that evoked a sustained bladder contraction (SBC, $n = 5$ cats, mean $\pm$ SE). Statistically, there was no difference across stimulation frequencies in the percent of trials that generated a bladder contractions ($p = 0.78$) during (A) SN branch stimulation. In contrast, selective activation of the (B) RP, (C) DNP and (D) CSN branches exhibited stimulus frequency dependent bladder excitation: $f \leq 10$ Hz, $f \geq 20$ Hz, and $f \leq 5$ Hz, respectively. [* and + denote $p < 0.001$ and $p < 0.002$, respectively; ANOVA with post-hoc Bonferroni test]

Figure 4.

EMGs evoked by selective stimulation of pudendal nerve branches. (A) Activation of the SN branch evoked only reflex (latency > 3 ms) EMGs in both the EAS and BUS. (B) Electrical stimulation of the RP branch elicited both direct (latency < 3 ms) and reflex EMGs. (C) The BUS response persisted during selective dPN branch stimulation, but the EAS response was absent.

Figure 5.

Bladder voiding achieved by electrical stimulation of the CSN branch. In this example, continuous electrical nerve stimulation (solid bar: 5 Hz and 0.2 mA) was initiated at approximately 10 seconds after the bladder was filled to 12 ml. This resulted in a voided volume of 9.2 ml and a voiding efficiency of 77 %. Activation of the pundendo-pudendal reflex prior to and following sustained activation of the bladder, as depicted by the rectified-average EMG (grey trace) of the external anal sphincter, confirmed the absence of any distension evoked bladder activity prior to stimulation. The absence of any direct EMG (black trace) indicated the afferent nature of the CSN branch.

Figure 6.

Analysis of voiding efficiencies (VE, mean $\pm$ SE) achieved by distension evoked (DE) and electrically evoked bladder contractions. Compared to the VE achieved by DE contractions ($28.1 \pm 4.3\%$, $n = 12$ cats), electrical stimulation of the SN ($1 \text{ Hz} \leq f \leq 50 \text{ Hz}$, $49.8 \pm 7.1\%$ in 7 cats), CSN ($f \leq 15 \text{ Hz}$, $61.4 \pm 10\%$ in 5 cats), DNP ($f > 15 \text{ Hz}$, $63.4 \pm 6.6\%$ in 5 cats), and RP ($f \leq 15 \text{ Hz}$, $49.7 \pm 12.1\%$ in 7 cats) branches generated significant improvements in VE (*, $p < 0.05$). Both low frequency DNP and high frequency RP branch stimulation failed to achieve bladder emptying (denoted by +).

Figure 7.

The effects of acute spinal transection on bladder activation evoked by selective stimulation of pudendal afferents. (A) Compared to the spinal intact cat, selective electrical stimulation of the DNP, CSN and RP branches resulted in weaker bladder contractions. (B) Quantitatively, a pair-wise comparison (pre- vs. post- SCT) of the mean bladder pressures (mean ± SD) evoked by selective pudendal nerve branch stimulation showed a significant reduction in the evoked pressures: DNP (32.5 ± 7.9 vs. 11 ± 8 cmH₂O), CSN (26.3 ± 9.1 vs. 3.6 ± 1.9 cmH₂O), and RP (24.8 ± 9.4 vs. 1 ± 0.2 cmH₂O). [* and + denote $p < 0.01$ and $p < 0.003$, respectively; ANOVA with post-hoc Bonferroni test]

Figure 8.

Summary of pudendal nerve mediated spinal (DNP) and spino-bulbo-spinal (CSN) micturition pathways in the cat. The excitatory stimulation frequency ranges are indicated for both the ascending sensory (DNP and CSN) and descending rectal perineal (dPN) pathways. Electrical stimulation of the caudal rectal (CR) branch did not evoke bladder contractions. [SPN = sacral parasympathetic nucleus, PMN = Pudendal Motor Nucleus]

Table 1

Summary of experimental procedures.

PROTOCOL	No. cats
Isovolumetric Bladder Contraction	5
Nicotinic Acetylcholine Antagonist (α -bungarotoxin)	1
Acute Spinal Cord Transection	4
Bladder Voiding	12
- SN/RP branches	(7)
- DNP/CSN branches	(5)

Table 2

Summary of pudendo-vesicle reflexes in the cat.

Mode of Stimulation	CAT			
	NEONATAL	ADULT	ACUTE SCT	CHRONIC SCT
MECHANICAL	Perigenital (PG-M)	Inhibition ²	N/A	Excitation ³
	Perigenital (PG-E)	N/A	N/A	Inhibition (5–7 Hz) ³ Excitation (20–40 Hz) ³
ELECTRICAL	Compound Pudendal (CP)	Inhibition (10 Hz) ⁴ Excitation (1–40 Hz) ⁵	Excitation (33 Hz) ⁴	Inhibition (3 Hz) ⁶ Excitation (20 Hz) ⁶
	Dorsal Genital (DNP)	N/A	Excitation* (20–40 Hz)	N/A
	Cranial Sensory (CSN)	N/A	No excitation*	N/A

* The presence of an inhibitory pathway cannot be investigated due to lack of distension evoked bladder activity, N/A ≡ not applicable.

¹ de Groat 1981,

² Thor 1990,

³ Tai 2007,

⁴ Boggs 2006,

⁵ Yoo 2007,

⁶ Tai 2006,

⁷ Woeck 2008