Control of Bladder Function by Electrical Stimulation of Pudendal Afferents

by

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Date: December 11, 2009

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

Spinal cord injury (SCI) and other neurological disorders can cause urinary dysfunction that can cause serious health problems and reduce quality of life. Current methods for treating urinary dysfunction have major limitations or provide inadequate improvement in symptoms. Pudendal nerve stimulation is a potential means of restoring control of bladder function. Bladder contraction and relaxation can be evoked by pudendal afferent stimulation, and peripheral pudendal afferent branches may be ideal targets for a bladder control neural prosthesis. This dissertation investigates control of bladder function by selective electrical stimulation of pudendal afferents.

This study investigated the ability to improve both urinary continence and micturition by both direct and minimally-invasive electrical stimulation of selected pudendal afferents in α-chloralose anesthetized male cats. Direct stimulation of the pudendal afferents in the dorsal genital nerve (DGP), percutaneous DGP stimulation, and intraurethral stimulation were used to quantify the bladder response to selective activation of pudendal afferents. Finite element modeling of the cat lower urinary tract was used to investigate the effects of intraurethral stimulation location and intraurethral electrode configuration on the complement of pudendal afferents that was activated. The contribution of sympathetic bladder innervation to bladder activation by pudendal afferent stimulation was assessed through pharmacological and surgical block of sympathetic activity to clarify the mechanisms of bladder contraction evoked by pudendal afferent stimulation.

The DGN is an ideal target for restoring urinary function because stimulation at low frequencies (5-10 Hz) improves urinary continence, while stimulation at high

frequencies (33-40 Hz) improves urinary voiding. Intraurethral stimulation is a valid method for clinical investigation of bladder responses to selective activation of the DNP or cranial sensory branch (CSN) of the pudendal nerve. In the cat, intraurethral stimulation can activate the bladder via two distinct neural pathways, a supraspinal pathway reflex activated by the CSN and a spinal reflex activated by the DNP. Finite element modeling revealed that intraurethral electrode location (rather than electrode geometry) is the primary factor in determining whether the DNP and CSN can be selectively activated by intraurethral stimulation. Finally, the sympathetic bladder pathway does not play a significant role in mediating bladder activation by DNP stimulation. These findings imply that selective pudendal afferent stimulation is a promising approach for restoring control of bladder function to individuals with SCI or other neurological disorders.

Dedication

This dissertation is dedicated to my friends, my family, and my parents Tim and Jan

Woock for their unwavering support and encouragement.

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Over my life I have encountered countless professors, teachers, and coaches. So many left a lasting imprint that I cannot mention them all by name, but I thank them for

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1. Introduction

The spinal cord relays information about the state of the bladder to the brain as well as descending information to the somatic and autonomic innervation of the lower urinary tract. Spinal cord injury (SCI) can disrupt the neural pathways critical to urinary function, and urinary dysfunction reduces the quality of life of persons with SCI. There are a number of current approaches to treat or manage urinary dysfunction, and pudendal nerve stimulation (PNS) is a promising new approach to restore control of both continence (urine storage) and micturition (urine voiding) to individuals with SCI. One or more of the peripheral branches of the pudendal nerve may serve as an ideal stimulation target for a neural prosthesis to control urinary function, but the responses evoked by stimulation of these branches have not been characterized. As well, the mechanisms underlying bladder reflexes mediated by afferent (conveying information to the central nervous system [CNS]) fibers in the pudendal nerve remain unclear. The objective of this research is to characterize, in the male cat, the bladder reflexes evoked by selective activation of pudendal afferent branches, particularly the dorsal genital nerve.

1.1 Neural anatomy and normal urinary function

Normal bladder function consists of two phases: urine storage (continence) and voiding (micturition). These phases are controlled by neural signals relayed to and from the CNS by both autonomic (associated with involuntary control of the body) peripheral nerves (the pelvic and hypogastric nerves) and somatic (associated with voluntary control of the body) peripheral nerves (the pudendal nerve) (Figure 1.1) (de Groat 2006). The pelvic,

hypogastric, and pudendal nerves are involved in supraspinal and spinal neural pathways that maintain normal urinary function through coordinated activation of the bladder muscle (or detrusor) and urethral musculature, including the striated external urethral sphincter (EUS) (Barrington 1941).

The autonomic nervous system provides parasympathetic ("rest and digest") and sympathetic ("fight or flight") innervation to the bladder. Parasympathetic fibers originate from the sacral spinal cord and travel in the pelvic nerve, providing excitatory innervation to the bladder smooth muscle and inhibitory innervation to the urethral smooth muscle (de Groat et al. 1992). Preganglionic parasympathetic fibers activate post-ganglionic bladder efferent (conveying information from the CNS) fibers by releasing acetylcholine which activates post-ganglionic nicotinic receptors. Activation of bladder muscle by post-ganglionic efferent fibers occurs via release of acetylcholine to activate muscarinic receptors. A non-cholinergic, non-adrenergic pathway also exists by which bladder muscle is activated by release of ATP to activate purinergic receptors. Urethral inhibition is mediated by muscarinic receptors activated by the release of nitric oxide (de Groat 2006).

Bladder afferent fibers which relay information about bladder distension also originate in the sacral spinal cord and travel in the pelvic nerve. Normal micturition is mediated by a supraspinal reflex in which bladder distension activates pelvic afferent Aδ-fibers, and these fibers activate an ascending neural pathway that terminates at the pontine micturition center (PMC), located in the dorsolateral pontine tegmentum of the rostral brain stem (de Groat et al. 1992; Benevento et al. 2002). The PMC projects back down to the sacral parasympathetic nucleus then to the bladder via pelvic efferent fibers that drive contraction of the bladder (Thor et al. 2004). Conscious control of urinary function is

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mediated by projections from brain regions rostral to the pons, including the medial frontal

lobes and corpus callosum, to the PMC (de Groat et al. 1992; Benevento et al. 2002)

Sympathetic fibers travel in the hypogastric nerve, providing inhibitory innervation to

the bladder smooth muscle and vesical ganglia and providing excitatory innervation to the

bladder neck and urethral smooth muscle. Continence, or bladder filling, is maintained by

efferent hypogastric activity, which inhibits the bladder muscle directly (a ß-adrenergic

receptor pathway) and inhibits pelvic efferent fiber transmission at the vesicle

parasympathetic ganglia (an α-adrenergic receptor pathway) (de Groat et al. 1972). Bladder

neck and urethral smooth muscle activation are driven by α-adrenergic receptor activation (de

Groat 2006). While the hypogastric nerve is typically identified as the primary source of

sympathetic innervations to the bladder, a large number of sympathetic fibers have also been

identified in the pelvic nerve (Kuo et al. 1984), but the role of sympathetic fibers in the pelvic

nerve may be limited to modulating bladder vasomotor function (contraction and dilation of

blood vessels), suggested by histochemical findings that the primary sympathetic

contribution of the pelvic nerve to the bladder is adrenergic vasomotor nerves (Hamberger et

al. 1965). Sympathetic fibers originate in the rostral lumbar spinal cord and reach the

hypogastric nerve via the rostral lumbar and inferior mesenteric ganglia. The pelvic nerve

sympathetic fibers travel from the lumbar cord via the sacral sympathetic chain ganglia to the

pelvic nerve (de Groat 2006).

Somatic fibers originate in the sacral spinal cord and travel in the pudendal nerve,

providing sensory innervation (afferent fibers) to the urethra, genitalia, and perineum.

Additionally, the somatic pudendal nerve carries the efferent innervation to the perineal

musculature, including the external urethral and anal sphincters (Martin et al. 1974; Yoo et al. 2008b).

During normal urine voiding, activation of the bladder and relaxation of the EUS occur synergistically. Activation of pelvic afferents from the bladder activate a supraspinal pathway which results in inhibition of pudendal motor neurons to the EUS (de Groat 2006). The synergistic control of bladder contraction and EUS relaxation occurs because activation of the PMC results in bladder activation and EUS inhibition, despite separate areas of the dorsolateral pontine tegmentum projecting to parasympathetic and pudendal motor neurons (Holstege et al. 1986; Ding et al. 1995).

1.2 Spinal cord injury and urinary dysfunction

There are approximately 250,000 individuals living with SCIs in the United States, and an estimated 11,000 new SCIs occur annually (Jackson et al. 2004). SCI can disrupt the neural pathways involved in normal bladder function. Additionally, persons with multiple sclerosis, Parkinson's disease, stroke, and other neurological disorders may suffer urinary dysfunction similar to that in persons with SCI (Woodward 1996; Andrews et al. 1997), including lack of voluntary control of urination, neurogenic detrusor overactivity (involuntary bladder contractions during bladder filling), detrusor-sphincter dyssynergia (DSD, concomitant contraction of the bladder and urethral sphincter), and detrusor areflexia (inactive bladder) (Hinson et al. 1996; Watanabe et al. 1996; Fowler 1999). These abnormal or impaired reflexes can result in urinary retention, incontinence, frequency, and urgency, which reduce quality of life and can result in urinary tract infections and damage to the urinary tract and kidneys. Urologic complications, most often occurring in the form of

urinary tract infections, are a leading cause of rehospitalization of persons with SCI (Stover et al. 1995; Berkowitz et al. 1998; Cardenas et al. 2004). Also, lack of bladder or bowel autonomy was associated with higher incidence of complications and mortality (Pagliacci et al. 2007). A survey of persons with SCI found that recovering bladder and bowel function was a priority for improving their quality of life (Anderson 2004).

1.3 Bladder treatment and management

Several methods of treating or managing urinary dysfunction exist, including pharmacological therapy, surgical intervention, and both mechanical and electrical devices.

1.3.1 Treatment of urinary dysfunction

Treatments for urinary disorders resulting from neurological disease or SCI are limited. While treating urinary dysfunction can ameliorate the symptoms of urinary dysfunction, treatments do not restore any control of urinary function to the individual. Anti-cholinergic drugs block activation of muscarinic receptors in the bladder by acetylcholine, and these drugs are used to relax the bladder in persons with detrusor overactivity. However, anti-cholinergics cause unwanted side effects such as blurred vision, constipation, and dry mouth (Wein 1998).

Surgical interventions include bladder augmentation and sphincterotomy. Bladder augmentation (or augmentation cystoplasty) involves transplanting additional tissue (typically from the intestines) to increase the capacity of the bladder and lower intravesical pressure (Linder et al. 1983). Sphincterotomies involve cutting the EUS to reduce urethral resistance to urine flow (Reynard et al. 2003). These surgical procedures are irreversible and

the patient risks the ability to benefit from future therapies for treating or managing urinary dysfunction.

1.3.2 Management of urinary dysfunction

Management devices aim to restore some degree of control of urinary function. There are mechanical devices and electrical stimulation devices that vary in their success and limitations.

1.3.2.1 Mechanical devices

Urinary catheters are frequently used to empty the bladder in persons with voiding dysfunction. Suprapubic catheters are inserted through the abdomen into the bladder dome (Cook et al. 1976; MacDiarmid et al. 1995). Urethral catheterization can be indwelling or intermittent. Indwelling catheters are left in the urethra chronically and connected to an external reservoir to collect urine. Intermittent catheterization involves periodically inserting a catheter into the bladder via the urethra (Bennett et al. 1995; Gaunt et al. 2006). Catheterization is a reliable way of emptying the bladder but is associated with a high incidence of lower urinary tract infections. Also, persons with poor or limited hand function may be unable to perform intermittent catheterization without assistance (Gaunt et al. 2006).

In addition to catheters, mechanical devices to manage urinary dysfunction include urethral stents. Urethral stents are inserted into the urethra in the area of the EUS and prevent the sphincter from contracting the urethra and impeding urine flow (Benevento et al. 2002; Gaunt et al. 2006).

1.3.2.2 Electrical Devices

Restoration of bladder function with a neural prosthesis requires the ability to control continence and/or micturition. While many targets have been investigated for electrical stimulation devices for control of urinary function (e.g., intravesical stimulation, bladder wall stimulation, pelvic nerve stimulation) (Gaunt et al. 2006), the Finetech-Brindley method and InterStim method are the only clinically available devices.

1.3.2 .2.1 Finetech-Brindley method

The Finetech-Brindley (or Vocare) bladder system is a neural prosthesis that uses electrical stimulation of the sacral ventral nerve roots to provide effective bladder voiding in persons with SCI. The efferent fibers of the pelvic nerve that innervate the bladder are contained in the sacral ventral roots, so stimulation of the sacral roots drives contraction of the bladder. However, the sacral nerve roots also contain the efferent pudendal nerve fibers innervating the EUS and efferent fibers innervating leg musculature. Co-activation of the bladder and EUS occurs because the large myelinated fibers innervating the EUS are activated at lower stimulation intensity thresholds than the thresholds necessary to activate the small myelinated preganglionic pelvic efferent fibers that drive contraction of the bladder. The co-activation of the bladder and EUS by sacral root stimulation is overcome by utilizing intermittent stimulation, which involves interrupted trains (3-9 seconds on then off) of stimulation. Because the skeletal muscle of the EUS relaxes more quickly than the smooth muscle of the bladder, turning off the stimulation results in rapid relaxation of the EUS while bladder pressure is still high, resulting in post-stimulus voiding. The intermittent stimulation drives up bladder and urethral pressures, but during the "off" intervals bladder voiding is

achieved (Rijkhoff 2004; van Balken et al. 2004; Gaunt et al. 2006; Van Rey et al. 2008) with voiding efficiencies greater than 90% (Brindley et al. 1982; Brindley et al. 1986).

A major drawback of sacral root stimulation is requirement for a concomitant dorsal rhizotomy (transection of sacral dorsal nerve roots containing bladder afferent fibers) to prevent DSD and reflexive bladder contractions (Creasey et al. 2001). The dorsal rhizotomy also eliminates autonomic dysreflexia associated with urinary dysfunction. Despite the clinical efficacy of sacral root stimulation, many individuals still prefer self-catheterization because sacral root stimulation requires an invasive implantation procedure and an irreversible dorsal rhizotomy, which abolishes reflex erection, defecation, and any remaining perineal sensation (Gaunt et al. 2006). Additionally, complications associated with sacral root stimulation include infection from device implantation, increased lower limb spasticity, development of stress incontinence, and incontinence due to reflex bladder contractions which persist due to incomplete dorsal rhizotomy (Creasey et al. 2001)

1.3.2.2.2 InterStim

The InterStim (Medtronic, Inc) method utilizes stimulation of the sacral nerve to inhibit the urinary bladder (Spinelli et al. 2008). Activation of genital pudendal afferent fibers and anorectal pelvic afferents is believed to evoke an inhibitory bladder reflex (Fall et al. 1991). Originally developed for treatment of non-neurogenic urinary incontinence, sacral nerve stimulation appears to provide improvements in continence and bladder capacity in persons with SCI (Rijkhoff 2004; Gaunt et al. 2006). While this approach manages incontinence, it does not address voiding dysfunctions. InterStim therapy includes the risks of lead migration, pain at the neurostimulation site, infection, adverse change in bowel function, and a high surgical revision rate (33%) (Siegel et al. 2000).

1.4 Pudendal nerve stimulation

There remains a need for a neuroprosthesis that restores control of urinary function to persons with SCI, and this device must be as effective as existing treatments, achieve greater acceptance among potential candidates, and not require sacrifice of residual reflexes or sensation (i.e., no nerve transection(s)). Recent studies have investigated PNS as a potential means of restoring bladder function. The pudendal nerve is an ideal target because of its convergence with autonomic urinary pathways in the central nervous system, and mechanical ("natural") and electrical activation of the pudendal nerve have been observed to affect bladder function.

1.4.1 Convergence of pudendal and autonomic pathways

Bladder afferent activity elicits reflex activation of the sympathetic and parasympathetic efferent bladder pathways, resulting in inhibition and excitation of the bladder respectively (de Groat et al. 1992). The convergence of vesical (bladder) and pudendal afferent activity may provide the substrate for affecting these bladder efferent pathways with PNS. Spinal convergence is of particular interest for clinical management of the bladder in persons with complete SCIs. Vesicle and pudendal afferent projections overlap in the lateral dorsal horn and the dorsal gray commissure of the lumbosacral spinal cord in the rat, cat, and macaque monkey (Roppolo et al. 1985; Ueyama et al. 1985; Nunez et al. 1986; Kawatani et al. 1994; Rampin et al. 1997; de Groat 2006). Additionally, recordings of sacral interneurons revealed that 70% of sacral interneurons receiving input from the bladder branch of the pelvic nerve (vesical afferents) also received input from the pudendal afferents (Coonan et al. 1999).

However, in humans SCIs are often incomplete (Jackson et al. 2004), potentially sparing some degree of the ascending and descending CNS urinary pathways. Thus, supraspinal convergence of pudendal and bladder afferents is also relevant to clinical investigation. There is evidence of supraspinal convergence of bladder and pudendal afferent fibers in regions associated with control of lower urinary tract function. Penile afferent fibers project to the paragigantocellularis region of the medial reticular formation (Giuliano et al. 2000), and afferents activated by DNP stimulation, urethral infusion, and bladder distension converge on neurons in the medullary reticular formation (Kaddumi et al. 2006). The ventral medullary gigantocellular reticular nuclei (which consists partially of the lateral paragigantocellular nucleus) projects to the intermediate gray and sacral parasympathetic nucleus of the sacral spinal cord (Hermann et al. 2003).

1.4.2 Pudendovesical reflexes

Activation of pudendal afferent nerve fibers can engage spinal (Boggs et al. 2005) and spinobulbospinal (Barrington 1931; Barrington 1941) reflexes that are integral to the regulation of bladder function. These reflexes can be accessed by mechanical or electrical stimulation to evoke bladder excitation or inhibition and alter continence and micturition. As well, electrical stimulation has been used to access these pudendovesical reflexes to control bladder function.

1.4.2.1 Mechanically evoked pudendovesical reflexes

Fluid flow through the urethra activates pudendal afferents (Todd 1964), and this flow-driven activation can elicit bladder contractions and facilitate micturition (Barrington 1931; Robain et al. 2001; Shafik et al. 2003b). Urethral anesthesia with

lidocaine impairs urinary voiding (Shafik et al. 2003b) and the bladder response to urethral afferent activation (Robain et al. 2001; Shafik et al. 2003a), illustrating the importance of feedback between pudendal afferents innervating the urethra and the bladder (urethrovesical) in normal urinary function.

In addition to this urethrovesical reflex, a genitovesical reflex can also modulate bladder activity. Activation of pudendal afferent fibers innervating the perigenital region evokes an excitatory spinal bladder reflex in neonatal and chronic SCI cats (de Groat et al. 1981; Tai et al. 2006). However, a supraspinal serotonergic inhibitory pathway emerges in the adult cat, resulting in perigenital stimulation-evoked bladder inhibition (Thor et al. 1990).

1.4.2.2 Electrical stimulation-evoked pudendovesical reflexes

PNS can improve urinary continence and voiding in intact and chronic SCI cats (Boggs et al. 2006a; Wenzel et al. 2006; Tai et al. 2007b). In humans, stimulation of the dorsal genital branch of the pudendal nerve can improve urinary continence (Wheeler et al. 1992; Kirkham et al. 2001; Horvath et al. 2009). Activation of excitatory and inhibitory bladder reflexes by PNS is dependent on stimulation frequency and bladder volume.

1.4.2.2.1 Frequency dependence

Electrical stimulation of the compound pudendal nerve can activate or inhibit the bladder dependent on stimulation frequency, and these electrically evoked reflexes can be used to improve micturition and continence respectively (Boggs et al. 2005; Boggs et al. 2006a). The frequency of PNS determines the effect on the micturition reflex, with frequencies < 20 Hz producing inhibition and frequencies ≥ 20 Hz producing activation

of the micturition reflex (Boggs et al. 2006b; Tai et al. 2006). Electrical stimulation of pudendal urethral afferents in the cat (Shefchyk et al. 1998; Yoo et al. 2008a) and human (Gustafson et al. 2004) elicits bladder contraction. However, electrical stimulation of genital pudendal afferent fibers at 5-20 Hz inhibits the micturition reflex in humans with SCI, multiple sclerosis, Parkinson's disease, and other conditions resulting in neurogenic detrusor overactivity (Nakamura et al. 1984; Vodusek et al. 1986; Wheeler et al. 1992; Previnaire et al. 1996; Kirkham et al. 2001; Lee et al. 2002; Hansen et al. 2005; Fjorback et al. 2006). All of the preclinical and clinical studies activating specific pudendal afferent populations were limited in whether they examined bladder excitation or inhibition and the frequency range investigated (Table 1.1).

The discovery of the frequency dependent bladder response to PNS (Boggs et al. 2006b) suggests that subsequent investigations of electrically evoked pudendovesical reflexes should explore a broad range of stimulation frequencies. Therefore we selectively stimulated genital and urethral afferents at 1- 40 Hz to determine if bladder excitation and inhibition can be evoked dependent on stimulation frequency.

1.4.2.2.2 Volume dependence

Activation of the bladder by stimulation of pudendal afferent fibers occurs only when there is a sufficient volume of fluid in the bladder, both before and after SCI, as observed with PNS and stimulation of urethral and perineal pudendal afferent fibers in cats (Shefchyk et al. 1998; Boggs et al. 2005; Boggs et al. 2006b) and humans (Gustafson et al. 2004; Yoo et al. 2007b), and with contractions evoked by urethral fluid flow (Robain et al. 2001; Combrisson et al. 2007). The bladder volume dependence of the detrusor response is due to a neural mechanism rather than the length-tension properties of the

bladder (Boggs et al. 2005), and recordings from pelvic efferent fibers revealed that the ability of urethral afferent activity to elicit reflex discharges in bladder efferent pelvic nerves is similarly dependent on the level of background facilitation of the micturition reflex (Mazières et al. 1997). Additionally, bladder efferent fiber recordings revealed that PNS evoked responses in pelvic efferent fibers increased with increasing bladder pressure (McMahon et al. 1982a). The necessity of a sufficient bladder volume for stimulation evoked detrusor activation (Boggs et al. 2005; Tai et al. 2007c) is consistent with spinal convergence between vesical afferent fibers and pudendal afferent fibers (Coonan et al. 1999), as the volume dependence is observed following spinal cord transection. It has been suggested that a spinal gating mechanism exists in the sacral cord by which pelvic afferent activity modulates pudendal-pelvic transmission, facilitating activation of pelvic efferent fibers at sufficient bladder pressures (McMahon et al. 1982a).

1.4.2.3 Limitations

While PNS can improve continence and micturition in the preclinical model, several limitations must be overcome before this approach can be used to manage urinary dysfunction in persons with SCI. These limitations include the complexity of accessing and stimulating the pudendal nerve, the direct activation of the EUS by PNS, and the potential for co-activation of multiple reflex pathways by PNS.

1.4.2.3.1 Access

Pudendal nerve fibers emerge from the sacral spinal cord at the S1-S4 level, and the compound nerve runs adjacent to the sacrotuberous ligament as it enters the

ischiorectal fossa (Juenemann et al. 1988; O'Bichere et al. 2000). The pudendal nerve trunk is challenging to access surgically, making it less than ideal for clinical investigation of the pudendal-bladder reflexes. However, the nerve divides into several branches to innervate the lower urinary tract, genitalia, and perineum (Juenemann et al. 1988). In the cat, the pudendal nerve divides into two branches, the sensory branch and the rectal perineal branch. The sensory branch separates into the cranial sensory branch (CSN), innervating the membranous urethra, and the dorsal genital nerve (DGN), innervating the genitalia, perineum, and, in the male, the penile urethra. The rectal perineal branch also separates into two branches. These branches provide efferent innervation to the perineal musculature: the deep perineal branch innervates, among other muscles, the EUS, and the caudal rectal branch innervates, among other muscles, the external anal sphincter (Figure 1.2) (Martin et al. 1974; Yoo et al. 2008b).

Targeting these peripheral pudendal nerve branches may allow for more selective activation of pudendal afferents. Intraurethral stimulation has been investigated in the cat and clinically to activate urethral pudendal afferent fibers (Gustafson et al. 2003; Gustafson et al. 2004). The DGN can be stimulated percutaneously or transcutaneously in humans (Nakamura et al. 1984; Vodusek et al. 1986; Yang et al. 1998c; Goldman et al. 2008). While the compound pudendal nerve can be accessed minimally invasively (Yoo et al. 2007b), stimulating pudendal urethral and genital afferents provides easier access for selective stimulation of the two populations of afferent fibers. Because it innervates the external genitalia and perineum, the DGN is a very accessible stimulation target, making it ideal for preclinical and clinical investigation. Therefore, we investigated direct stimulation of the dorsal nerve of the penis (DNP, the male DGN) by stimulating at

a broad range of frequencies and determining if stimulation can evoke bladder excitation and inhibition. Additionally, we investigated activation of the DNP and CSN via intraurethral stimulation to validate intraurethral stimulation as a clinical means of minimally invasive selective activation of the pudendal afferent branches.

1.4.2.3.2 Efferent innervation of the urethral sphincter

Because the pudendal nerve innervates the EUS, PNS results in direct activation of the EUS, which constricts the urethra. During preclinical studies, voiding by PNS was evoked using intermittent stimulation (Boggs et al. 2006a), in which post-stimulus voiding occurs when the EUS relaxes while the bladder pressure is still elevated. However, additional sources of DSD may impede post-stimulus voiding, so an ideal target for a bladder neuroprosthesis should not directly activate the EUS. The DNP contains primarily afferent fibers and does not directly innervate the EUS (Martin et al. 1974; Yoo et al. 2008b), so may be a more suitable stimulation target than the compound nerve. The EUS EMG response to direct DNP stimulation was measured to determine the effect of DNP stimulation on EUS activity. Additionally, we determined if continuous DNP stimulation could evoke efficient bladder voiding in the preclinical model.

1.4.2.3.3 Co-activation of multiple reflex pathways

The pudendal nerve plays the primary afferent role in the urethrovesical and genitovesical reflexes. The pudendal anatomy suggests that the DGN, innervating the genitalia and perineum, is associated with the genitovesical reflex, and the CSN, innervating the membranous urethra, is associated with the urethrovesical reflex. PNS

may result in co-activation of the reflexes, potentially activating competing bladder reflexes, but stimulation of the individual pudendal afferent branches would allow selective activation of these distinct pudendovesical reflexes. Additionally, by stimulating the distal branches, potential side effects evoked by co-activation of unrelated (non-urinary) pathways may be minimized. We investigated selective activation of the DNP and CSN via intraurethral stimulation to determine the frequency characteristics of the DNP-bladder and CSN-bladder reflex and determine if these nerves are involved in separate pudendovesical pathways.

1.5 Effect of pudendal afferent activation on efferent activity in the pelvic and hypogastric nerves

The efferent activity of the autonomic innervation of the lower urinary tract regulates urinary continence and micturition, and PNS elicits bladder responses by altering the sympathetic and parasympathetic activity in the hypogastric and pelvic nerves, respectively. Low frequency PNS (5-10 Hz) inhibits bladder activity by activation of hypogastric bladder efferent fibers and inhibition of pelvic bladder efferent fibers (Lindström et al. 1983). However, the effect of high frequency (30-40 Hz) PNS, which can evoke bladder contractions and voiding, on hypogastric and pelvic bladder efferent fiber activity is unknown. Bladder contractions elicited by high frequency PNS may occur due to activation of pelvic bladder efferent fibers (i.e., exciting the exciter), inhibition of hypogastric bladder efferent fibers (i.e., inhibiting the inhibitor), or a combination of pelvic activation and hypogastric inhibition.

No studies have provided compelling evidence to suggest a particular mechanism for the frequency dependence of the bladder response to pudendal afferent activation.

Recordings from hypogastric nerve filaments revealed that PNS evoked reflex activation of hypogastric efferent fibers (and, consequently, bladder inhibition) at latencies of 35-150 ms and that optimal activation occurred with 4-6 Hz stimulation (Lindström et al. 1983). However, it was noted that there was a large reduction in spontaneous hypogastric activity between reflex responses. Pudendal nerve activation completely inhibited pelvic efferent activity in pelvic nerve filaments at similar frequencies (Lindström et al. 1983). The depression of the single hypogastric efferent reflex responses with increasing stimulation frequency (investigated up to 10 Hz) suggests that high frequency stimulation (>10 Hz) may reduce spontaneous hypogastric activity without eliciting robust reflex responses. Determining the effect of high frequency DGN stimulation on hypogastric and pelvic efferent bladder pathways will enhance our understanding of the roles of the hypogastric and pelvic nerves in the frequency dependent bladder responses evoked by pudendal afferent stimulation. Therefore we examined the effect of removal of the sympathetic bladder pathway on the excitatory pudendal-bladder reflex utilizing a combination of pharmacological sympathetic block and bilateral hypogastric nerve transection during pudendal afferent stimulation. This may provide important insight for screening clinical candidates and selection of future strategies that improve the efficacy of restoration of bladder function by selective stimulation of pudendal afferent fibers.

1.6 Specific Aims

SCI can disrupt normal bladder function resulting in impaired continence (urine storage) and micturition (urine voiding). PNS can selectively activate or inhibit the urinary bladder in intact and chronic spinalized cats and is currently being investigated as a means of

restoring control of urinary function to humans with neurological disease or SCI (Boggs et al. 2006b; Tai et al. 2006; Yoo et al. 2007b). However, non-selective stimulation of the compound pudendal nerve (PNS) evoked relatively low amplitude bladder contractions in persons with SCI (Yoo et al. 2007b). Further, PNS produces direct EUS activation presenting an additional challenge to achieving satisfactory bladder voiding. Finally, non-selective activation of the pudendal nerve trunk may activate multiple urinary and non-urinary pathways, potentially producing competing effects on the bladder and unwanted side effects. Selective activation of pudendal afferents in more peripheral branches of the pudendal nerve may provide a more ideal target for a neural prosthesis. The overall aim of this research was to characterize the bladder reflexes evoked by selective activation of pudendal afferent branches, specifically the DGN, in an animal model.

The DNP (the DGN in males) is an ideal target for a neural prosthesis because its readily accessible location renders it a potential target for transcutaneous, percutaneous, or intraurethral stimulation. In humans, transcutaneous stimulation of the DNP inhibits the bladder (Wheeler et al. 1992; Lee et al. 2002). However, whether or not DNP stimulation can activate the bladder is unknown. If the bladder response to DNP stimulation exhibits similar frequency dependent characteristics as the bladder response to PNS, then the DNP is an ideal target for a neural prosthesis for restoring control of bladder function. Therefore, experiments were conducted to characterize the bladder response to DNP stimulation, validate minimally invasive methods of pudendal afferent stimulation that enable translation to clinical studies, and improve our understanding of the neural pathways and mechanisms responsible for the frequency dependent bladder response to pudendal afferent branch

stimulation.

1.6.1 Characterized the bladder response to electrical stimulation of the dorsal nerve of the penis

The DNP was directly stimulated and the bladder pressure response measured in intact and acute spinal transected cats. These data allowed testing of the hypothesis that electrical stimulation of the DNP can elicit both bladder contraction and relaxation depending on the stimulation frequency and that these responses can be evoked following spinal cord transection. The EMG response to DNP stimulation and voiding efficiency with continuous DNP stimulation were measured to test the hypothesis that DNP stimulation does not evoke direct or reflex EUS activity during stimulation evoked contractions. The characterization of the bladder and EUS response to stimulation of the DNP provides new insight into the potential of the DNP as a target for a neural prosthesis to control bladder function.

1.6.2 Evaluated minimally invasive methods to stimulate pudendal afferents

Percutaneous stimulation and intraurethral stimulation were tested in intact and acute spinal transected cats to determine whether electrical stimulation of pudendal afferents by minimally-invasive methods can evoke bladder responses similar to those evoked by direct stimulation of the DNP or CSN. The *in vivo* experimental results were complemented by finite element analysis of intraurethral stimulation of the male cat urethra to determine the optimal electrode configuration and location for selective pudendal afferent fiber activation. These minimally-invasive methods provide a validated means of selectively activating pudendal afferents in humans.

1.6.3 Determined the role of the sympathetic bladder pathway in the frequency dependent bladder response to pudendal afferent stimulation

The effect of selective block of the sympathetic bladder pathway on DNP stimulation-evoked bladder excitation was examined in the adult male cat. The impact on the volume threshold for evoking distension evoked and stimulation evoked bladder contractions of selective pharmacological block with adrenergic antagonists or transection of the hypogastric nerve was determined. Additionally, the impact of sympathetic block on the magnitude of stimulation evoked and distension evoked bladder contractions was investigated. These studies provide an improved understanding of the neural pathways responsible for the frequency dependent bladder response to DNP stimulation. This allows for design of stimulus paradigms that exploit the characteristics of the response pathway. Also, the improved understanding enables the identification of appropriate clinical candidates.
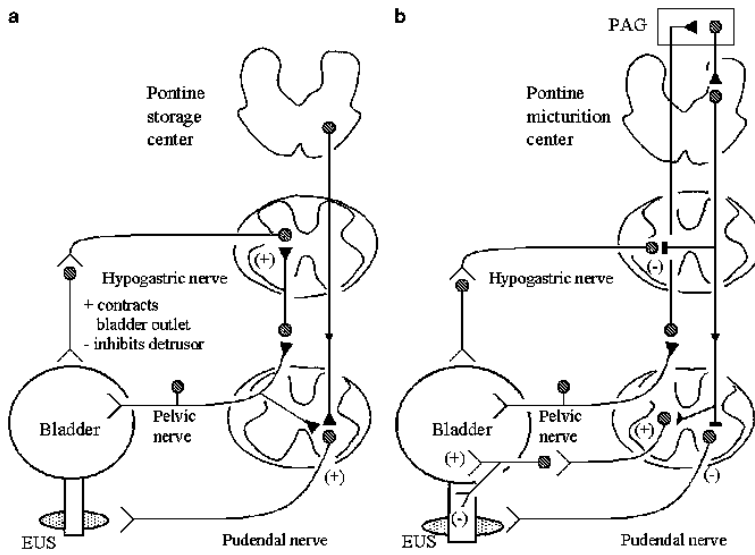
Figure 1.1. The neural pathways associated with bladder function. The pelvic, hypogastric, and pudendal nerves are critical to urinary function. These peripheral pathways transmit neural information between the CSN and the bladder and urethra, critical to controlling (a) continence and (b) micturition. Figure from(de Groat 2006).

Table 1.1. Limited frequency range of previous studies of pudendal afferent evoked bladder reflexes

Source	Model	Pudendal Stimulation Target	Inhibition/ Excitation	Frequencies
(Gustafson et al. 2003)	Cat	Urethral afferents	Excitation	2 Hz
(Shefchyk et al. 1998)	Cat	Urethral afferents	Excitation	10, 20, 50 Hz
(Gustafson et al. 2004)	Human	Urethral afferents	Excitation	2 and 20 Hz
(Vodusek et al. 1986)	Human	Genital afferents	Inhibition	5 and 10 Hz
(Wheeler et al. 1992)	Human	Genital afferents	Inhibition	5 Hz
(Previnaire et al. 1996)	Human	Genital afferents	Inhibition	5 Hz
(Kirkham et al. 2001)	Human	Genital afferents	Inhibition	15 Hz
(Hansen et al. 2005)	Human	Genital afferents	Inhibition	20 Hz
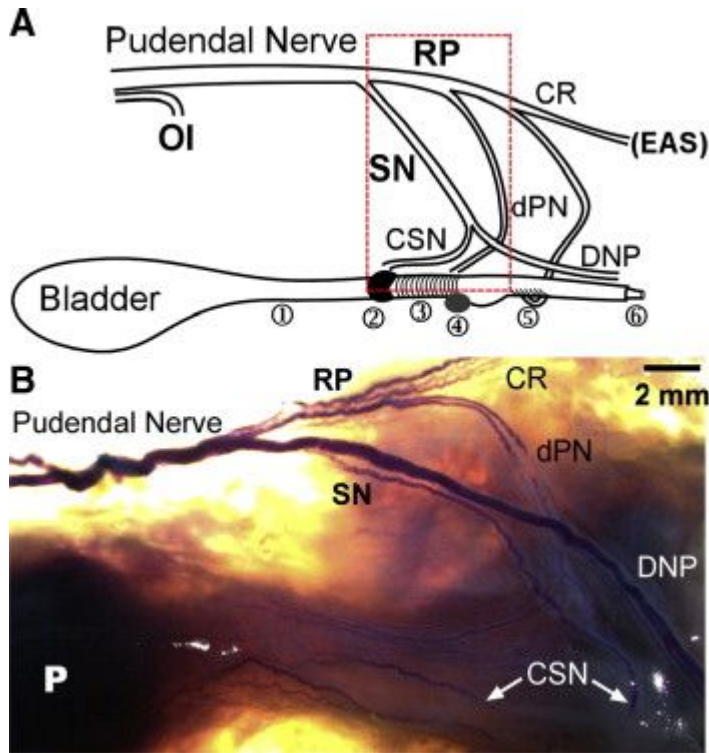
Figure 1.2. The innervation of the lower urinary tract by the pudendal nerve in the male cat. The nerve divides into the rectal perineal (RP) and sensory (SN) nerves. The SN further divides into the cranial sensory nerve (CSN), providing afferent innervation to the membranous urethra, and the dorsal nerve of the penis (DNP, the male dorsal genital nerve), and providing afferent innervation to the glans, distal urethra, and perineum. The RP branch provides efferent innervation to the external urethral sphincter via the deep perineal nerve (dPN) and efferent innervation to the external anal sphincter via the caudal rectal nerve (CR). Figure from (Yoo et al. 2008b).

2. Activation and inhibition of the micturition reflex by penile afferents in the cat

2.1 Introduction

The reciprocal coordination of the urinary bladder and the external urethral sphincter (EUS) to maintain continence and produce micturition is controlled by the pontine micturition center (Barrington 1931; Barrington 1941; Holstege et al. 1986). The spinobulbospinal micturition reflex is also subject to modulation by peripheral afferent activity (Kruse et al. 1991; Kruse et al. 1992) that can influence voiding efficiency (Peng et al. 2008b). Spinal cord injury and other neurologic disorders can disrupt the pathways associated with the spinobulbospinal micturition reflex and cause urinary dysfunction. Pudendal nerve stimulation is a potential means of restoring control of urinary function to persons with spinal cord injury. The objective of the present study was to quantify the contributions of genital afferents, traveling in the penile component of the somatic pudendal nerve, on the micturition reflex in the cat.

Electrical stimulation of pudendal afferents can evoke bladder contractions and bladder relaxation in cats and humans (Kirkham et al. 2001; Boggs et al. 2006b; Yoo et al. 2007b). These responses are present following spinal cord transection and decerebration in cats and are present in SCI humans (Shefchyk et al. 1998; Kirkham et al. 2001; Boggs et al. 2005; Tai et al. 2006; Yoo et al. 2007b) (Boggs, Tai, Schefchyk, Yoo). Specifically, stimulation of urethral pudendal afferents evokes bladder contractions in the cat (Shefchyk et al. 1998; Boggs et al. 2005) and human (Gustafson et al. 2004). However, stimulation of genital pudendal afferents has been observed to inhibit bladder contractions and improve urinary continence in persons with SCI, multiple sclerosis,

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Parkinson's disease, and other neurological disorders (Nakamura et al. 1984; Vodusek et al. 1986; Wheeler et al. 1992; Kirkham et al. 2001; Lee et al. 2002; Hansen et al. 2005; Fjorback et al. 2006). These observations are consistent with the role of urethrovesical feedback in normal micturition. Urethral fluid flow can evoke bladder contraction and is necessary for normal micturition (Barrington 1931; Robain et al. 2001; Shafik et al. 2003b).

Recent experiments in cats revealed that the frequency of electrical stimulation of afferents in the compound pudendal nerve determines their effect on the micturition reflex, with frequencies less than 20 Hz producing inhibition and frequencies above 20 Hz producing activation of the micturition reflex (Boggs et al. 2006b; Tai et al. 2006). However, the effects of genital afferent inputs on the micturition reflex and whether they exhibit similar frequency dependence is unknown. The present results demonstrate that, similar to effects produced by other pudendal afferents, activation of genital afferents produced either activation or inhibition of the micturition reflex in the α-chloralose anesthetized male cat, depending on the frequency of electrical stimulation of the afferents. The results reveal that genital afferent stimulation can activate both continence and micturition-like neural pathways, challenging the perception that the effect of stimulation of the genital afferents is solely inhibition of the micturition reflex.

2.2 Methods

2.2.1 Animal Preparation

All animal care and experimental procedures were followed according to the National Institutes of Health guidelines and were approved by the Institutional Animal

Care and Use Committee at Duke University. Twenty sexually intact, adult male cats

weighing 2.5-5.5 kg were anesthetized with ketamine HCl (Ketaset, 35 mg/kg i.m.) and

anesthesia was maintained with α-chloralose (Sigma-Aldrich, 65 mg/kg i.v.,

supplemented at 15mg/kg). The animals were intubated and respired artificially to

maintain end tidal CO_2 between 3.3 and 4.5%. Blood pressure was monitored via a

catheter in the carotid artery. Core body temperature was maintained at 38° with a

thermostatic heating pad, and IV fluids were administered (saline or lactated Ringer's

solution at 15cc/kg/hr).

The bladder was exposed through a midline abdominal incision and the ureters

were isolated, ligated, and cut proximal to the ligation. A suprapubic catheter (3.5 Fr)

was inserted into the bladder dome and secured with a purse-string suture, and the

abdominal incision was closed in layers. Intravesical pressures were measured with a

solid-state pressure transducer (Deltran, Utah Medical) connected to the catheter and

recorded (sampling rate=12.5-20 kHz, Astromed8Xe, Astro-Med, Inc).

2.2.2 EMG Recording

For isovolumetric experiments, the urethra was occluded with a 3.5 Fr or 5 Fr

catheter. In 5 cats, the catheter had three platinum ring electrodes positioned 6, 7, and 8

cm from the tip. The catheter was inserted so that two of the electrodes were within the

post-prostatic urethra and bulbourethra (3-5 cm from the urethral meatus (Wang et al.

1999)) and used to measure the activity of the periurethral musculature. This EMG

recording is termed the periurethral EMG (PU EMG) to reflect that it may include

contributions from multiple active muscles, including the external urethral sphincter

(EUS). Wire electrodes were also inserted into the external anal sphincter (EAS) to

record the anal sphincter EMG (EAS EMG). The EMG signals were amplified

(gain=1000), filtered (10 Hz-2 kHz), and sampled.

2.2.3 Spinal cord transection

In 2 cats the spinal cord was transected and responses to genital afferent

stimulation were measured 8-15 hours after spinal cord transection. The spinal cord was

exposed at the T10 vertebral level via laminectomy, the dura was incised, and lidocaine

was administered to the exposed cord. The spinal cord was elevated and transected, and

Surgicel was packed between the transected ends of the spinal cord.

2.2.4 Nerve Stimulation

The dorsal nerve of the penis (DNP) was stimulated unilaterally either by placing

a cuff electrode directly around the nerve (n=13) or by percutaneous insertion of a wire

electrode adjacent to the nerve (n=7). With the animal in a supine position, a midline

incision was made from the caudal border of the gracilis muscle to a few millimeters

cranial to the caudal end of the prepuce. For direct stimulation, the DNP was dissected

free from the body of the penis caudal to the bulb of the penis, and a monopolar cuff

electrode (platinum contacts embedded in a silicone elastomeric cuff) was placed around

the nerve. Indirect stimulation was delivered through an insulated stainless steel wire

inserted via a 22G needle between the prepuce and the glans penis and directed along the

dorsolateral body of the penis 2.5-3.0 cm from the tip of the glans penis. The

percutaneous electrode was inserted so that the DNP was likely activated unilaterally, and

no characteristics of the observed responses suggested otherwise. A 20G stainless steel

needle was inserted into the ipsilateral leg of the animal and served as the anode during

percutaneous and cuff electrode stimulation. Stimuli were 20-30 second trains of monophasic constant current cathodic pulses (100μs pulse width) delivered at varying amplitudes (direct stimulation: 10μA-1mA, percutaneous stimulation: 100μA-8mA) and frequencies (1-40 Hz). The train lengths and frequency range were chosen based on previous data on the effect of stimulus parameters on bladder response (Boggs et al. 2006b).

2.2.5 Experimental Design

Experiments were performed either with the urethra occluded (isovolumetric experiments) or unobstructed. All artificial bladder filling was performed with room temperature saline. Isovolumetric experiments were performed on 18 cats, of which 18 cats included stimulation delivered when the bladder was relaxed (direct stimulation in 11 cats, percutaneous stimulation in 7 cats) and 16 cats included stimulation delivered during distension evoked bladder contractions (direct stimulation in 9 cats, percutaneous stimulation in 7 cats).

Volume thresholds were investigated systematically in 8 cats. The bladder was filled in discrete 1 ml increments, and stimulation was applied during a 2-3 minute interval in between filling. The minimum volume at which electrical stimulation of the DNP evoked bladder contractions was defined as the stimulation threshold volume (STV). The minimum volume at which reflex bladder contractions ($>10cmH_2O$) occurred was defined as the distension threshold volume (DTV). Stimulation for determining thresholds was delivered at 33 Hz and 2 times the amplitude threshold for eliciting an EAS response.

2.2.6 Quantification of Bladder Responses

For isovolumetric experiments, stimulation evoked bladder responses were defined as increases in intravesical pressure that exceeded the baseline intravesical pressure by at least 10% and were sustained until the end of stimulation. The baseline intravesical pressure was defined as the mean intravesical pressure over the 2 seconds prior to stimulation onset. The mean pressure of a stimulus-evoked contraction was calculated as the mean pressure from the point in which the 10% threshold was crossed until the end of the stimulation. Inhibitory (bladder relaxation) responses were defined as occurring when stimulation initiated during a distension evoked bladder contraction resulted in at least a 50% decline from the peak intravesical pressure (the peak pressure after stimulus onset) within the first 5 seconds of stimulation. Relaxation was determined to have failed if the intravesical pressure increased back above 50% of the peak contraction pressure before stimulation ended.

The area under the intravesical pressure curve (the pressure-time product, PTP) was measured to quantify the effects of stimulation delivered during distension evoked contractions (inhibition or augmentation). The onset of a distension-evoked contraction was defined by averaging the intravesical pressure in one second increments and subsequently searching for a 10% increase in the intravesical pressure in the absence of stimulation. For a detected distension evoked contraction, the pressure baseline was defined as the two seconds preceding the onset of the distension evoked contractions, and all PTPs were computed from the intravesical pressure (minus baseline) for 20 seconds following onset of the distension evoked contractions. Distension evoked bladder contractions were only included in the analysis if they lasted at least 10 seconds in the

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absence of stimulation, and stimulation trials were only included if stimulation began within 10 seconds of the onset of the distension evoked contraction. Trials that occurred in the same animal, at the same volume, and within three minutes of one another were grouped together. Each group of trials included one or more distension evoked contraction during which stimulation did not occur. The PTPs in each group were normalized by dividing by the average PTP of the distension evoked contractions.

Direct DNP stimulation was performed in 4 cats with an unobstructed urethra to investigate the effects of genital afferent activity on bladder storage and voiding. The bladder was filled continuously at 1 ml/minute and failure of urine storage was defined as the point when urine leakage was observed or a sustained distension evoked contraction occurred (defined as a contraction lasting at least 20 seconds and having a pressure increase $\geq$15 cmH$_2$O). In the urine storage trials, continuous stimulation was applied starting at 50-80% of the previously determined volume at which failure of storage occurred (in absence of stimulation) and the stimulation was stopped when failure of storage occurred. Distension evoked voiding was measured by stopping bladder filling when failure of storage occurred and allowing distension evoked voiding to complete (3-5 minutes after the last volume was voided). Stimulation evoked voiding was measured by stopping filling when failure of urine storage occurred and immediately applying stimulation. Stimulation was applied at a single frequency and at varying amplitudes until stimulus evoked contractions (which resulted in voiding) could no longer be elicited. For each cat, a distension evoked voiding/storage trial was performed first, and subsequent trials for the storage and voiding studies were randomized.

Voiding efficiencies were defined by dividing the difference between the initial

bladder volume and the post-void residual volume by the initial bladder volume:

$$Voiding(\%) = \frac{V_{initial} - V_{residual}}{V_{initial}} \times 100 \,.$$

2.2.7 Statistical Analysis

Statistical analysis of the dependence of bladder responses on frequency was

performed using a One-way Kruskal-Wallis test with the null hypothesis that the percent

of trials showing a specific response did not vary across stimulus frequencies. Post hoc

paired comparisons between individual frequencies were made using Bonferroni

inequalities. Comparison of mean contraction amplitudes (across frequencies, across cats,

and across frequencies for individual cats) was made using a One-way Kruskal-Wallis

test or a Wilcoxon rank sum test (for individual cats if contraction occurred at only 2

frequencies). Statistical analysis of the normalized PTPs computed for distension evoked

contractions with and without stimulation was done by a One-way Kruskal-Wallis test

and post hoc paired comparisons were made using Bonferroni inequalities. Continent

and voided volumes in the presence and absence of stimulation were compared separately

for each cat using a Welch two-sample t-test. All reported values are mean ± standard

deviation.

2.3 Results

The dorsal nerve of the penis (DNP) originated from the sensory branch of the

pudendal nerve, passed superficially along the ventral insertion of the ischiocavernosus

muscle into the bulb of the penis, and continued towards the glans penis. As it coursed

along the body of the penis, the DNP branched into three discernible fiber populations (Figure 2.1). Superficial fibers traveled along the dorsal aspect of the body of the penis and innervated the glans and the prepuce. Lateral fibers branched off deeper into the penile body and gave off branches directed towards the ventral aspect of the penis, innervating the urethra as well as the glans. Also, a branch of the DNP was directed off of the body of the penis and had cutaneous terminations. Similar DNP anatomy was observed in all 13 cats in which the DNP was isolated for stimulation with a cuff electrode. Some variability was observed in the distance caudal to the bulb of the penis before the branches of the DNP could be distinguished from one another. The cuff electrode was placed on the DNP proximal to any branching unless otherwise specified.

Activation of penile afferents in the DNP elicited stimulation-frequency dependent activation or inhibition of the micturition reflex. Bladder contraction was elicited by direct or percutaneous stimulation of the DNP in 17 of 20 cats, and inhibition of distension evoked bladder contractions was elicited in 16 of 18 cats. No response to DNP stimulation was observed in 2 cats, and only activation (bladder contraction) or only inhibition (bladder relaxation) was observed in 1 cat each (these cats were omitted from further analysis). Three cats were excluded from quantitative analysis because urethral leakage occurred during bladder contractions despite the presence of the urethral catheter. Transecting the DNP cranial to the stimulating electrode (3 cats) or transecting the compound pudendal nerve (3 cats) abolished both the bladder and EMG responses to DNP stimulation.

The ability to elicit bladder activation by electrical stimulation of the DNP was dependent on the bladder volume. Stimulation threshold volumes (3-10ml, n=8 cats in

which thresholds were investigated in 1ml increments) were less than distention threshold volumes (4-13ml), and STVs averaged 70%±7% of DTVs (range = 61-80%).

2.3.1 Stimulation at bladder volumes above the STV elicited frequency dependent activation of the micturition reflex

Direct electrical stimulation of the DNP activated the micturition reflex and elicited bladder contractions in 9 of 11 cats (Figure 2.2A). The effect of stimulation frequency was examined systematically in 7 cats. The ability to elicit sustained bladder contractions depended on stimulation frequency (Figure 2.2B, $p<0.001$, Kruskal-Wallis test, n=453 trials across 7 cats, stimulation amplitude at 2-4 times threshold). Stimulation at 20, 33, and 40Hz elicited contractions more consistently than stimulation at any of the lower frequencies ($\leq$10 Hz) ($p<0.01$, Bonferroni inequalities). However, the response to stimulation at 20 Hz was not consistent across all cats. In 5 of 9 cats, 20 Hz stimulation elicited contractile responses (contraction occurred in >80% of the trials), while in 4 of 9 cats it did not elicit contractile responses (contraction occurred in <15% of the trials). The lower percentage of trials in which 20 Hz stimulation evoked contractions compared to 33 and 40 Hz is a reflection of this interanimal variability and not the effectiveness of 20 Hz stimulation within individual cats. The threshold stimulation amplitudes for eliciting bladder responses ranged from 50μA-300μA. The ability to elicit contractions and contraction magnitudes increased when stimulation amplitude was increased from threshold to 2 times threshold, but stimulation at 2-4 times threshold evoked similar bladder contractions.

The mean increase in intravesical pressure evoked by stimulation at 20, 33, and 40 Hz was 33.5 cmH$_2$O ($\pm$15.4 cmH$_2$O, n=283 contractions across 7 cats, stimulation

amplitude at 2-4 times threshold), and did not vary significantly across these frequencies (p=0.207, Kruskal-Wallis). While the magnitude of stimulus evoked contractions varied from cat to cat (p<0.01, Kruskal-Wallis), there was no significant difference between contraction amplitudes elicited by stimulation at 20, 33, or 40 Hz in 6 of the 7 cats (p>0.05, Kruskal-Wallis, n=24-67 contractions per cat). In one cat, stimulation at 40 Hz elicited larger contractions than stimulation at 33 Hz (32.5±8.0 vs. 27.1±8.5 cmH$_2$O, p<0.05, Wilcoxon rank sum test, n=60 contractions) while 20 Hz stimulation did not evoke bladder contraction.

2.3.2 Stimulation at volumes above the DTV elicited frequency dependent bladder relaxation and contraction

At bladder volumes above the DTV, direct stimulation of the DNP elicited bladder contraction and relaxation in 8 of 9 cats (Figure 2.3A,B). The effect of stimulation frequency was examined systematically in 7 cats, and the bladder response depended on stimulation frequency (Figure 2.3C, p<0.001, Kruskal-Wallis, n=212 trials across 7 cats). Stimulation at 5-10 Hz consistently inhibited distension evoked bladder contractions, and stimulation at these frequencies inhibited distension evoked bladder contractions more consistently than stimulation at a lower frequency (2 Hz) and higher frequencies (33-40 Hz) (p<0.01, Bonferroni inequalities). Stimulation during distension evoked contractions at 20 Hz inhibited bladder contractions in 3 of 8 cats, augmented bladder contractions in 4 cats, and did not elicit either response in 1 cat. Bladder relaxation was evoked at threshold amplitudes of 80μA-400μA, but was elicited more consistently at 2-4 times the amplitude threshold for relaxation.

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At volumes above the DTV, stimulation at higher frequencies elicited bladder contractions when delivered between distension evoked contractions and augmented bladder contractions when applied during distension evoked bladder contractions (Figure 2.3A,B). The ability to augment distension evoked bladder contractions was dependent on stimulation frequency (Figure 2.3C, $p < 0.001$, Kruskal-Wallis test, n=212 trials across 6 cats). Stimulation at 33 and 40 Hz augmented contractions in a greater percentage of trials than stimulation at 2-20 Hz ($p < 0.05$, Bonferroni inequalities). Augmentation of distension evoked contractions resulted in maximum intravesical pressures similar to maximum stimulation evoked pressures (in absence of distension evoked contractions), but in some cases stimulation elicited a brief period of relaxation prior to contraction (Figure 2.3B).

The effect of DNP stimulation on distension evoked contractions was investigated systematically in 6 cats. The average pressure-time product (PTP) of bladder contraction differed significantly across stimulation groups (Figure 2.4, Kruskal-Wallis, $p < 0.001$). Inhibition of a contraction resulted in a mean PTP of 0.47 ($\pm.017$), which was significantly less than that of the unstimulated contractions (1.0 ± 0.07) and the augmented contractions (1.47 ± 0.29) ($p < 0.01$, Bonferroni inequalities). Also, the augmented contractions had significantly larger relative PTPs than the unstimulated contractions ($p < 0.01$, Bonferroni inequalities).

2.3.3 The reflex response of periurethral musculature to direct stimulation of the DNP

Direct stimulation of the DNP did not directly activate the periurethral musculature but elicited reflex PU EMG response in 4 of 5 intact cats. Whether electrical

stimulation of the DNP elicited PU EMG responses depended on stimulation frequency

(Figure 2.5). Low frequency stimulation (1-5 Hz) elicited reflex responses in the

bulbourethra with reflex latencies of ~ 10-12 ms. The persistence of the reflex response

throughout the trial diminished at higher frequencies (7.5-10 Hz), and reflex responses

were elicited only by the first 1-4 stimulation pulses at high frequencies (20-40 Hz). The

magnitude of reflex responses appeared to decrease with increasing bladder volume and

over the course of repeated stimulation during the experiment. Stimulation of the DNP

also evoked a reflex response in the EAS in 4 of 5 cats at a latency of 8-9 ms. Similar

frequency dependence was observed in the EAS reflex response to DNP stimulation

(Figure 2.5).

2.3.4 Stimulation controlled urine storage and voiding

Urine storage and voiding were elicited by direct stimulation of the DNP in 4 cats

(Figure 2.6). Bladder volumes at incontinence in the presence of low frequency (5-10

Hz) stimulation were 21±10 ml compared to bladder volumes at incontinence in the

absence of stimulation of 15±7 ml ($p < 0.02$ for each cat, n=25 trials across 4 cats with at

least 3 trials of each type per cat, Welch Two-Sample t-test). Direct stimulation of the

DNP at higher frequencies (33 and 40 Hz), begun at the volume at which continence was

lost, resulted in an increase in percent bladder voiding compared to distension evoked

percent bladder voiding in 4 of 4 cats ($p < 0.05$, n=27 trials across 4 cats with at least 3

trials of each type per cat, Welch Two-Sample t-test). Distension evoked voiding

resulted in 37% (±13.5%) bladder voiding (19-60%), while stimulation evoked voiding

resulted in 64% (±12%) voiding (49% -84%).

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2.3.5 Percutaneous stimulation of the DNP elicited frequency dependent bladder responses

Electrical stimulation of the DNP with a percutaneous wire electrode evoked activation and inhibition of the micturition reflex (Figure 2.7) in 6 of 7 cats. These responses exhibited the same characteristics as those evoked by direct stimulation of the DNP with a cuff electrode. The threshold amplitude for eliciting responses ranged from 1.5 to 4 mA. Whether stimulation evoked bladder contraction or relaxation depended on the stimulation frequency ($p<0.001$ for augmentation and inhibition, Kruskal-Wallis test, n=520 trials across 6 cats). Stimulation at 20-40 Hz elicited bladder contraction more effectively than stimulation at 2-10 Hz ($p<0.01$, Bonferroni inequalities). Stimulation at 7.5 and 10 Hz inhibited distension evoked contractions in a greater percentage of trials than stimulation at 33 and 40 Hz ($p<0.01$, Bonferroni inequalities).

2.3.6 Selective stimulation of the penile body branches of the DNP elicited bladder responses

The cutaneous branch of the DNP, observed to branch off of the penile body within a centimeter of the bulb (Figure 2.1), was stimulated selectively, separate from the 2 larger branches of the DNP that continued to course along the penile body. No EAS or bladder responses were elicited by stimulation of the cutaneous branch in 6 of 6 cats. Conversely, co-stimulation of the 2 penile branches (separately from the cutaneous branch) elicited bladder contractions and EAS responses comparable to those evoked by stimulation of the DNP in 5 of 6 cats. In 3 of 4 cats in which distension evoked contractions occurred during stimulation of the divisions of the DNP, inhibition of distension evoked contractions was elicited by low frequency co-stimulation of the 2 penile branches.

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2.3.7 Responses to DNP stimulation were preserved after acute spinal cord transection

At 8-15 hrs following spinal cord transection (SCT), direct stimulation of the DNP elicited bladder contractions in 2 of 2 cats (Figure 2.8A). Bladder contractions were evoked at stimulation frequencies of 20, 33, and 40 Hz, while stimulation at lower frequencies failed to elicited bladder contractions (Figure 2.8B). The ability to elicit bladder contractions depended on stimulation frequency in a manner similar to prior to SCT ($p<0.001$, Kruskal-Wallis test, n=75 trials across 2 cats), and stimulation at 20, 33, and 40 Hz were significantly more likely to elicit contractions than stimulation at 2, 5, and 10 Hz ($p<0.05$, Bonferroni inequalities). In one cat the minimum bladder volume at which stimulation evoked bladder contractions occurred was between the STV and DTV observed before the spinal transection, and in the second cat the volume was 20% higher (12 ml vs. 10 ml) than the DTV before the spinal transection.

2.4 Discussion

Stimulation of penile afferents in the dorsal nerve of the penis (DNP) activated reflexes that elicited either contraction or relaxation of the bladder. Bladder activation and inhibition were evoked differentially by electrical stimulation of the DNP dependent on the stimulation frequency. Low frequency (5-10 Hz) stimulation inhibited distension-evoked bladder contractions and promoted urine storage, while high frequency stimulation (33-40 Hz) elicited bladder contractions, augmented distension evoked contractions, and produced bladder voiding. These results demonstrate that a somatic sensory input, heretofore asserted to only cause bladder inhibition, can also cause bladder contraction, and that the evoked response is strongly dependent on the frequency of

electrical stimulation of the afferent inputs. The present study provides the first known confirmation of that both bladder inhibition and excitation can be elicited by electrical stimulation of the DNP in the male cat.

2.4.1 Bladder volume dependence of DNP-bladder reflex

DNP stimulation evoked bladder contractions only when the bladder volume was above 70% ± 7% of the distension threshold volume, similar to the volume threshold observed for perineal nerve stimulation in cats (78% ± 17%) (Boggs et al. 2005), urethral fluid flow in ewes (65% ± 17%) (Robain et al. 2001). Similar bladder volume dependence was observed for the bladder response to urethral fluid flow in humans (Karlson 1953) and to intraurethral stimulation in humans (Gustafson et al. 2004). Additionally, stimulation of pudendal afferents only elicited a reflex response in the parasympathetic preganglionic neurons when the bladder contained sufficient volume (Mazières et al. 1997). The bladder volume dependence results from a neural mechanism (Boggs et al. 2005), and this mechanism must exist in the lumbosacral spinal cord because the volume dependence is present following SCI.

2.4.2 The DNP-bladder pathway

Contraction of the bladder evoked by penile afferent activation in the intact cat could reflect activation of a spinobulbospinal excitatory pathway, a spinal pudendovesical reflex, or the simultaneous inhibition of the supraspinal serotonergic inhibition of the spinal pudendovesical reflex (Thor et al. 1990) and activation of the spinal reflex. In the acute SCT cat, the activation is through a spinal reflex, which may only be accessible after removal of supraspinal inhibitory inputs following spinal

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transection, implying convergence between bladder (pelvic) and penile afferents in the spinal cord. In addition to the volume dependence of the bladder response to DNP stimulation, studies have shown that pudendal stimulation evoked inhibition of bladder activity in chronic spinalized cats and DGN stimulation evoked inhibition of the pelvic C-fiber bladder reflex in acute spinalized cats can be enhanced by increasing the stimulation intensity at inhibitory stimulation frequencies (Mazieres et al. 1998; Tai et al. 2006). The dependence of DNP stimulation evoked bladder inhibition on stimulus amplitude has also been illustrated in humans with SCI (Previnaire et al. 1996). Consistent with the spinal convergence of pudendal and pelvic afferents (Chapter 1), afferent fibers from the DNP of the rat project to the dorsal horn, the dorsal gray commissure, and the sacral parasympathetic nucleus (Nunez et al. 1986; Rampin et al. 1997), and clitoral afferents in the cat terminate in the dorsal gray commissure (Kawatani et al. 1994). Activation of penile afferents results in Fos labeling of neurons in the sacral parasympathetic nucleus after spinal transection, consistent with supraspinal inhibition of DNP-activated spinal neurons (Rampin et al. 1997). The convergence of penile somatic and bladder parasympathetic pathways is not surprising considering that DNP afferents and parasympathetic efferents are involved in erectile function (Rampin et al. 1997). The present results extend the role of DNP afferents to urinary function, as well.

2.4.3 The DNP-bladder reflex is frequency dependent

Stimulation of the DNP caused bladder contraction at high frequencies (33 and 40 Hz) and bladder relaxation at 5-10 Hz, similar to the frequency dependence for bladder contraction and relaxation evoked by stimulation of afferents in the compound pudendal nerve (Boggs et al. 2006b; Tai et al. 2006; Yoo et al. 2007a). However, the bladder

responses to direct DNP stimulation appear more frequency specific than responses to compound pudendal nerve stimulation (Boggs et al. 2006b). No studies have provided evidence to suggest a particular mechanism for this frequency dependence. The frequency dependence of the bladder response to penile afferent stimulation may be due to frequency-dependent interactions between DNP afferents and parasympathetic bladder afferents and efferents. Low frequency (5-10 Hz) activity in DNP afferents may evoke primary afferent depolarization (PAD) of bladder afferents (Angel et al. 1994) or post-synaptic inhibition of interneurons activated by bladder afferents. At higher stimulation frequencies a second pathway may be excited by activation of an interneuron via temporal summation of the DNP afferent activity. This pathway would result in the inhibition of the inhibitory PAD in addition to driving further activation of the micturition reflex pathway associated with bladder afferents. This mechanism of frequency dependent pathway activation is similar to that proposed for differential motor responses evoked by different frequencies of epidural spinal cord stimulation (Jilge et al. 2004). Another potential mechanisms for the observed frequency dependence is a synaptic mechanism described previously in the hippocampus (Mori et al. 2004). A biphasic response to afferent activation depended on stimulation frequency; at 10 Hz disynaptic inhibition predominated, while at frequencies greater than 20 Hz the inhibition was depressed and monosynaptic excitation was dominant. A similar mechanism could exist at the convergence of pudendal afferents and sacral interneurons. Recordings from parasympathetic preganglionic neurons and sacral spinal interneurons reveal the existence of inhibitory and excitatory inputs from pudendal and pelvic afferents (de Groat et al. 1969; Lu et al. 2000).

The behavior of the bladder elicited by excitatory stimulation during distension evoked bladder contractions supports the proposed model of the micturition reflex in which the activity from the bladder afferent pathway and the urethral sensory afferent pathway converge to drive bladder efferent activity. The addition of excitatory pudendal activation during distension evoked contractions could result in augmentation of the magnitude of the bladder contraction (Figure 2.5). However, these augmented contractions were similar in amplitude to the stimulus evoked contractions (in the absence of distension evoked contractions), and the augmentation disappeared at higher volumes as distension evoked contraction amplitudes matched the amplitudes of stimulus evoked contractions. This behavior suggests the additive effect of the DNP afferent and bladder afferent input to the bladder efferents can drive bladder contractions up to a limit, and it is possible that this limit is determined by the bladder volume.

2.4.4 DNP stimulation improves continence and micturition

Direct stimulation of the DNP resulted in increases in the continent bladder volume of 35% -77% over no stimulation. A previous study in intact cats with pudendal afferent stimulation found volume increases of 18% over no stimulation (Wenzel et al. 2006), and in chronic spinalized cats the increase in continence volume during pudendal nerve stimulation was 147% (Tai et al. 2007b). Similar increases (22-366%) in continent bladder volumes result from genital afferent stimulation in the human (Vodusek et al. 1986; Wheeler et al. 1992; Kirkham et al. 2001; Hansen et al. 2005; Fjorback et al. 2006). Bladder relaxation was likely caused by inhibition of the Aδ-fiber mediated micturition reflex via reflex activation of sympathetic hypogastric efferents (at low intravesical pressure) or inhibition of pelvic efferents (at high intravesical pressure) (Fall et al. 1978;

Lindström et al. 1983). The micturition reflex after chronic SCI is mainly mediated by c-fiber bladder afferents (de Groat et al. 1998; Cheng et al. 1999; de Groat et al. 2006), but stimulation of the DGN can inhibit both the Aδ and the c-fiber mediated reflexes (Mazieres et al. 1998). Based on results in previous studies, it is likely that an even greater increase in continence volume can be achieved using conditional stimulation of the DNP (Kirkham et al. 2001; Wenzel et al. 2006)

Electrical stimulation of the DNP at high frequencies (33 and 40 Hz) improved the voiding efficiency from 37% (±14%, no stimulation) to 64% (±12%, DNP stimulation). Voiding via stimulation of the DNP was achieved with continuous stimulation and without transection of any nerves via reflex activation of parasympathetic pelvic efferents (Barrington 1931; Barrington 1941). The stimulation evoked voiding efficiencies are comparable with voiding percentages reported from stimulation of perineal afferents of the pudendal nerve (63% ± 20%) and the compound pudendal nerve (64% ± 14%) in the α-chloralose anesthetized male cat (Boggs et al. 2005; Boggs et al. 2006a). Incomplete voiding and the consistent post-void residual bladder volume may be due to the effects of the α-chloralose (Rudy et al. 1991) or the reduced drive to the micturition reflex from the bladder afferents in the pelvic nerve when the bladder volume decreases as voiding occurs.

2.4.5 PU response to DNP stimulation is frequency dependent

The ability to elicit voiding with continuous DNP stimulation demonstrates an apparent absence of stimulation induced detrusor-sphincter dyssynergia (DSD), which is consistent with the present PU EMG recordings and previous EUS recordings during pudendal afferent stimulation (Shefchyk et al. 1998; Boggs et al. 2005; Boggs et al.

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2006b). The PU EMG response was strongly dependent on stimulation frequency and did not persist at stimulus frequencies $\geq$ 20 Hz, consistent with previous findings (Cook et al. 1991b; Cook et al. 1991a; Boggs et al. 2006b). The lack of direct activation of the urethral sphincter as measured in the PU EMG combined with the ability to void during continuous stimulation suggest that stimulation of the DNP in the intact cat does not result in DSD. However, the bulbocavernosus reflex and EUS response to DNP stimulation during bladder contraction are enhanced in patients with DSD (Dyro et al. 1986; Walter et al. 1994), so the effect of high frequency DNP stimulation evoked bladder contractions on already present DSD is unclear. Intermittent stimulation would provide a means of achieving voiding despite the reflex urethral activation caused by stimulation of the DNP in persons with SCI if the reflex activation persisted at excitatory stimulation frequencies (Boggs et al. 2006a).

2.4.6 Similarity of human and feline DNPs

Observations of the anatomy of the DNP lead to the conclusion that there are similarities and differences between the penile neuroanatomy in the cat and the human. The branching of the DNP into two main populations of fibers (dorsal fibers and lateral fibers) is consistent with observations in the human (Yang et al. 1998b). Based on neuroanatomic and electrodiagnostic observations in the human, the lateral branches from the DNP provide some innervation to the urethra (Bradley et al. 1973; Yang et al. 1998b). However, the presence of a cutaneous branch originating from the DNP in the cat is not consistent with the penile neuroanatomy of the DNP in humans. This cutaneous branch could play a role in the ability to elicit bladder excitation if the direct activation of this branch at high frequencies activates a response related to the perineal excitatory reflex

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observed in neonatal cats and chronic spinal cord injured cats (de Groat et al. 1981; Tai et al. 2006). However, selective stimulation of the cutaneous branch did not evoke bladder responses, while stimulation of the remaining penile branches did evoke frequency dependent bladder contraction and relaxation. Transcutaneous stimulation of the DNP in humans (Vodusek et al. 1986; Wheeler et al. 1992; Kirkham et al. 2001; Hansen et al. 2005; Fjorback et al. 2006) may excite the DNP distal enough that it elicits activity in the different branches of the DNP in a manner which may not be comparable to the excitation of the DNP achieved in this study.

2.4.7 Percutaneous DNP stimulation effectively activates DNP-bladder reflex

Percutaneous stimulation of the DNP was illustrated as a minimally-invasive means of exciting and inhibiting the bladder in the intact cat. Stimulation of the DNP at high frequencies (20-40 Hz) elicited bladder contractions and augmentation of distension evoked bladder contractions, while stimulation at 5-10Hz consistently inhibited distension evoked bladder contractions. These results were similar to those obtained for direct stimulation of the DNP. This method may be more suitable than transcutaneous stimulation in humans. Excitation of the urinary bladder was likely elicited by activation of the urethral afferents in the DNP, eliciting a reflex analogous to the excitatory bladder reflex elicited by fluid flow in the urethra (Barrington 1931). While there exists a similar urethrovesical reflex in humans (Shafik et al. 2003b), this reflex has only been accessed by activation of nerves in the human posterior urethra (Geirsson et al. 1999; Shafik et al. 2003a; Gustafson et al. 2004) and no evidence has been presented that suggests this reflex can be accessed by activation of urethral afferents in the anterior human urethra. If the stimulation evoked bladder excitation observed in this study was due to activation of

the urethral afferents present in the DNP, maximizing the activation of these fibers in humans may allow for stimulation evoked bladder excitation to be achieved. Percutaneous stimulation will allow the DNP to be stimulated more proximally than transcutaneous stimulation, which may minimize the number of afferents that branch off to innervate the anterior urethra before the site of stimulation (Yang et al. 1998c; Yang et al. 2000).

2.4.8 Spinal reorganization following SCI

While reorganization of the sacral spinal components of the micturition reflex pathway (Beattie et al. 1993; de Groat et al. 1998) may affect the ability to elicit bladder contractions by electrical stimulation of the DNP, recent results suggest that pudendal afferent mediated reflex activation of the bladder is preserved in humans with chronic SCI (Gustafson et al. 2004; Yoo et al. 2007b). Also, perigenital electrical stimulation in the chronic spinal transected cat can activate and inhibit the bladder at similar frequencies as DNP stimulation (Tai et al. 2007a). This method of stimulation in the female cat is likely activating the dorsal clitoral nerve, the female analog of the DNP, suggesting that the frequency dependent bladder responses to electrical stimulation of the DNP observed in the intact and acute cat are still present in the chronic spinal cord injured cat.

2.4.9 Conclusions

Stimulation of the DNP at high frequencies (20-40 Hz) elicited bladder contractions and augmentation of distension evoked bladder contractions, while stimulation at 5-10Hz consistently inhibited distension evoked bladder contractions. These findings may find application in electrical restoration of bladder function – both

storage and emptying, and electrical stimulation of the DNP is an especially compelling approach because of the ease of access to the dorsal genital nerves in human (Yang et al. 1998b).

Access to the DGN for electrical stimulation can be achieved more readily than access to the compound pudendal nerve or other urethral pudendal afferents (Martin et al. 1974; Yang et al. 1998c; Schraffordt et al. 2004), and the proximity of the DGN to the skin makes it an ideal target for percutaneous stimulation (Yang et al. 1998c). Percutaneous stimulation of the DNP produced bladder responses equivalent to those generated by direct nerve stimulation. In addition, transection or block of the nerve distal to the stimulating electrode is not necessary because the DGN does not innervate the EUS (Martin et al. 1974). However, DNP stimulation may only be applicable to a limited population as certain individuals may be unable to tolerate stimulation at an adequate intensity (Fjorback et al. 2006). Further, the excitatory bladder response to DNP stimulation has only been accessed in cats, and the existence of a similar excitatory bladder pathway from penile afferents in humans is unknown.
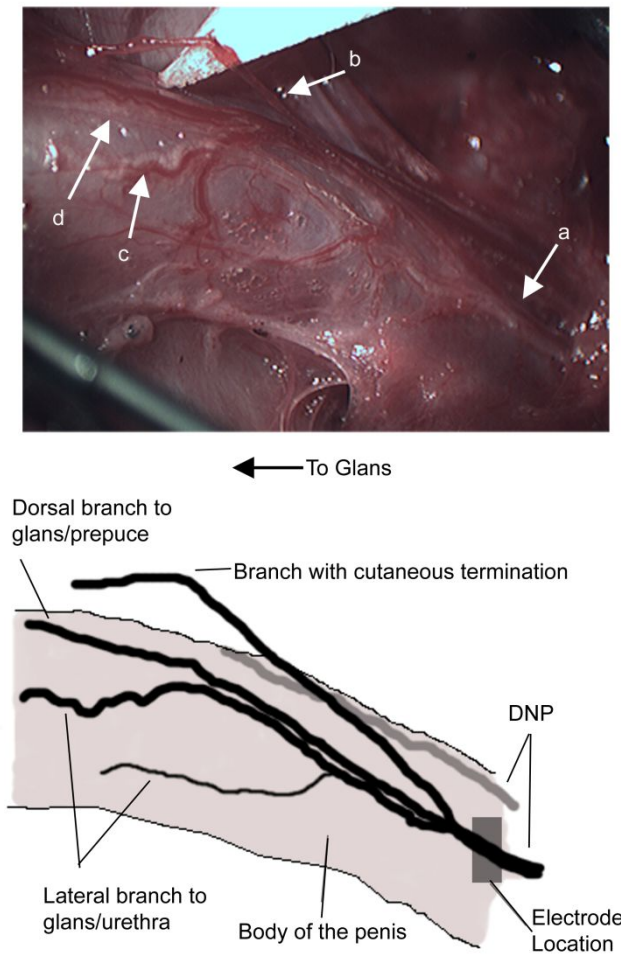
Figure 2.1. Anatomy of the dorsal nerve of the penis (DNP) of the cat.

Dorsolateral view of the DNP (a) and the penile body from the ventral side of the cat. A branch of the DNP was observed leaving the body of the penis and innervating the skin of the prepuce and perineum (b). Another branch of the DNP coursed along the lateral body of the penis to the glans and gave off nerves towards the urethra (c). A third, more superficial branch traveled along the dorsal aspect of the body of the penis to the glans penis and prepuce (d).

A.

B.
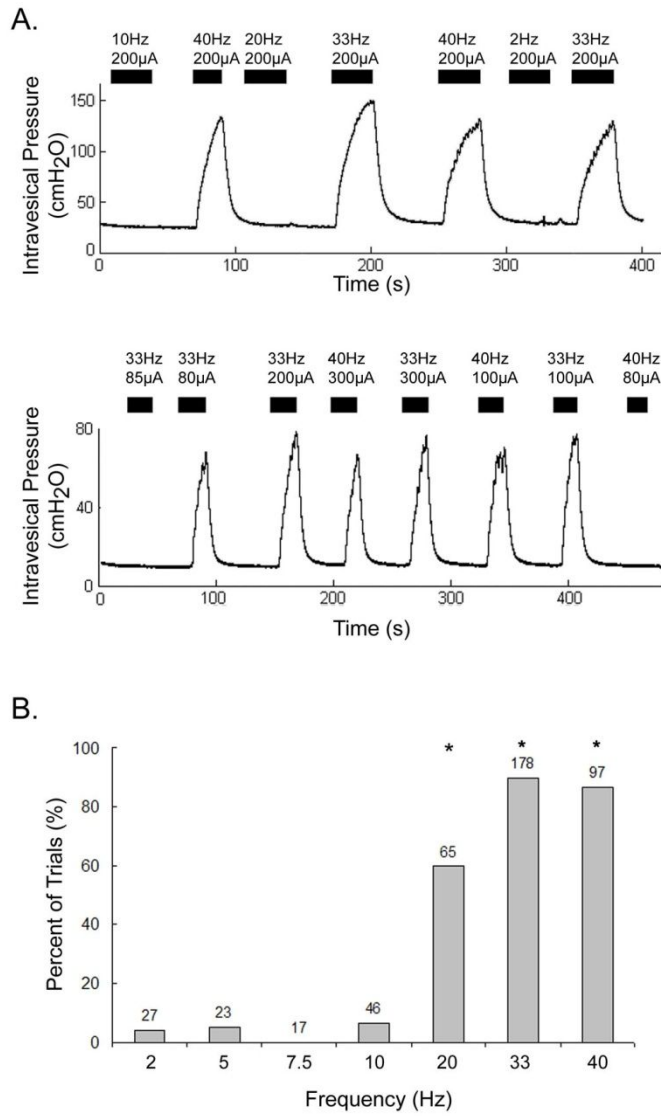
Figure 2.2

Figure 2.2. Frequency dependent bladder responses to direct electrical stimulation of the DNP. (A) Direct stimulation of the DNP is shown evoking detrusor contractions at bladder volumes between the STV and DTV in 2 different cats. Contractions were generated within 5 seconds of the onset of high frequency stimulation (33 and 40 Hz in CAT A, top, 20-40 Hz in CAT B, bottom) and ended with the termination of stimulation or shortly thereafter. Stimulation at low frequencies ($\leq$10 Hz) did not elicit contractions. The black bars indicate the duration of stimulation, which consisted of 20 or 30 second trains at the frequency and amplitude above each bar. **(B)** Percent of trials in which direct stimulation of the DNP elicited detrusor contractions at different stimulus frequencies. The ability to elicit detrusor contraction by stimulation of the DNP was dependent on stimulation frequency ($p<0.001$, Kruskal-Wallis test, n=453 trials across 7 cats). Stimulation at 33 and 40 Hz consistently evoked detrusor contractions when stimulation was applied at appropriate bladder volumes and stimulus amplitude. Stimulation at 20 Hz evoked detrusor contractions in 4 of the 7 cats represented in the figure. Stimulation at 20, 33, and 40 Hz elicited contractions in a significantly greater percentage of trials than stimulation at 2-10 Hz (*$p<0.01$, Bonferroni inequalities). Bladder volumes were above STVs and below DTVs. Stimulus amplitudes ranged from 150μA-600μA (all amplitudes were 2-4x the threshold to elicit a bladder response). The number above each bar is the number of trials.
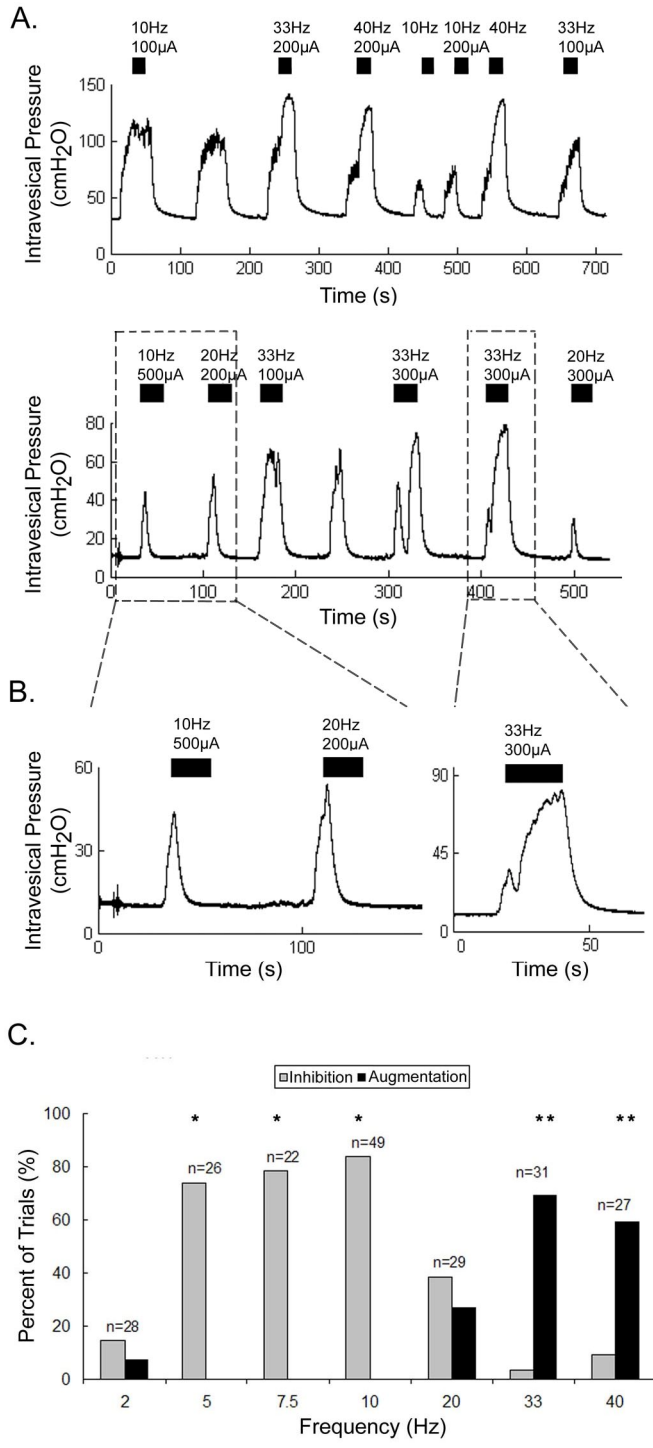
Figure 2.3

Figure 2.3. Direct stimulation of the DNP inhibited or augmented distension evoked detrusor contractions dependent on the stimulation frequency. (A) Intravesical pressure in 2 cats shows inhibition and augmentation of distension evoked detrusor contractions by stimulation of the DNP. Stimulation at 10 Hz (and 20Hz in the lower trace) inhibited distension evoked contractions, while stimulation at 33 and 40 Hz augmented distension evoked contractions. Bladder volumes were above the DTVs. The black bars indicate the duration of the DNP stimulation, which consisted of 20 or 30 second trains at the frequency and amplitude above each bar. **(B)** A more detailed view of the detrusor inhibition (left panel) and augmentation (right panel) from the lower trace in (A). Stimulation at 10 and 20 Hz after the onset of distension evoked contractions caused the intravesical pressure to rapidly return to baseline. Stimulation at 33 Hz during a distension evoked contraction caused a rapid rise (after a brief decrease) in the intravesical pressure. **(C)** Percent of trials in which direct stimulation inhibited or augmented distension evoked detrusor contractions at different stimulus frequencies. Stimulation at 5-10 Hz inhibited detrusor contractions in a greater percentage of trials than stimulation at 2, 20, 33, and 40 Hz (***** $p<0.01$, Bonferroni inequalities, n=7 cats). Stimulation at 33 and 40 Hz augmented contractions in a greater percentage of trials than stimulation at 2-20 Hz (****** $p<0.05$, Bonferroni inequalities, n=7 cats). Bladder volumes were above the DTV and stimulus amplitudes ranged from $160\mu A$-$800\mu A$ (all amplitudes were 2-4x threshold). Numbers above each bar are the number of trails at each frequency.
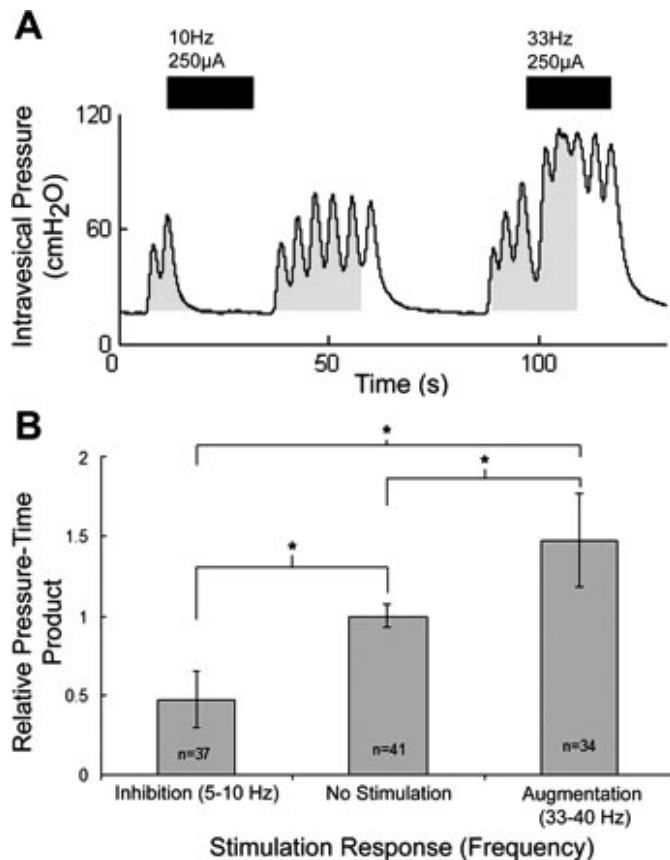
Figure 2.4. Effect of direct stimulation of the DNP on distension evoked detrusor contractions. (A) The pressure-time produce (PTP) was computed as the area under the intravesical pressure trace (minus baseline pressure) for 20 seconds after the onset of a distension evoked contraction. The intravesical pressure trace shows the PTP (shaded area) for 10 Hz stimulation, a distension evoked contraction with no stimulation, and 33 Hz stimulation. **(B)** The normalized pressure-time products (PTPs) of the first 20 seconds of distension evoked detrusor contractions with or without stimulation are shown (mean ± standard deviation). The relative PTP of the detrusor contraction was dependent on the stimulation frequency ($p<0.001$, Kruskal-Wallis test, $n=112$ trials across 6 cats). Inhibitory stimulation (5-10 Hz) within the first 10 seconds of a distension evoked contraction reduced the PTP of distension evoked detrusor contractions significantly, while excitatory stimulation (20-40 Hz) increased detrusor PTP (*$p<0.01$, Bonferroni inequalities).
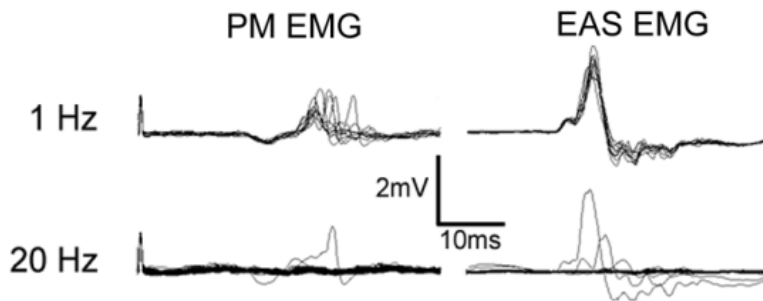
Figure 2.5. Periurethral (PU) and external anal sphincter (EAS) electromyograms evoked by direct electrical stimulation of the DNP. Stimulation was delivered at either 1 or 20 Hz (10 second trains, 100µs pulses, 200µA) with the bladder empty. Reflex responses were elicited following each pulse during 1 Hz stimulation, but the response disappeared after the first 2-4 pulses during 20 Hz stimulation.
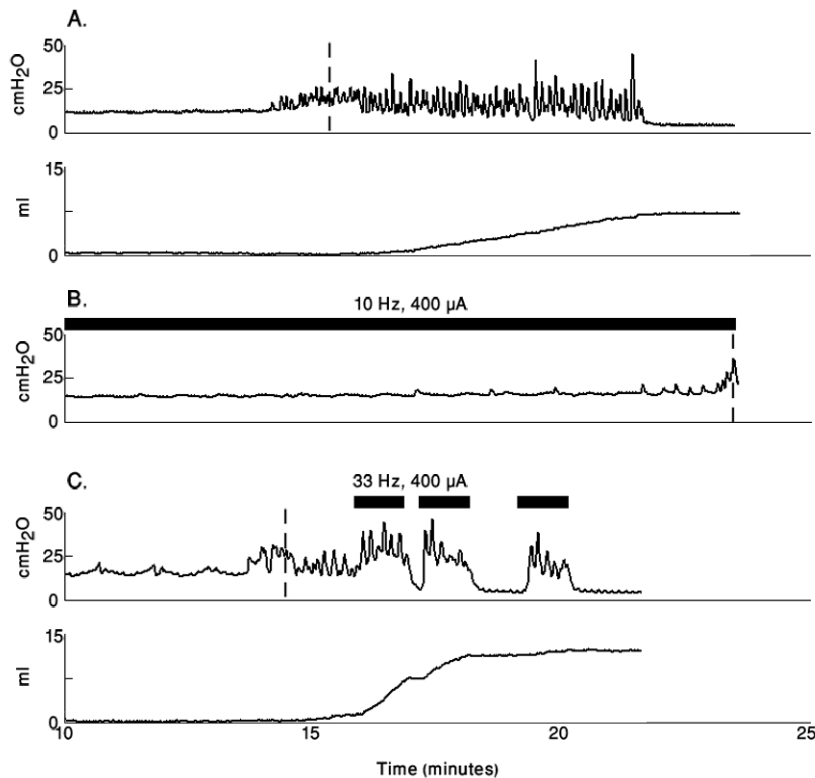
Figure 2.6. Direct stimulation of the DNP improved continence and voiding.
Cystometrograms are shown for a single cat with no stimulation, inhibitory stimulation, and excitatory stimulation. The bladder was filled at 1ml/min. (**A**) Intravesical pressure during bladder filling in the absence of stimulation (upper plot) and the volume voided by the distension evoked contractions (lower plot). Continence was lost at 15.3ml (indicated by the dashed vertical line in the upper plot) and the total volume voided was 8.2ml. (**B**) Intravesical pressure during bladder filling with continuous 10 Hz stimulation of the DNP starting at 8ml infused volume. A sustained detrusor contraction occurred at 23.4ml (indicated by the dashed vertical line).   (**D**) Intravesical pressure during bladder filling in the absence of stimulation but with stimulation evoked bladder voiding. Continence was lost at 14.2ml (indicated by the dashed vertical line in the pressure trace), and the total volume voided was 11.0ml. Stimulation trains are indicated by the black bars above the intravesical pressure traces.
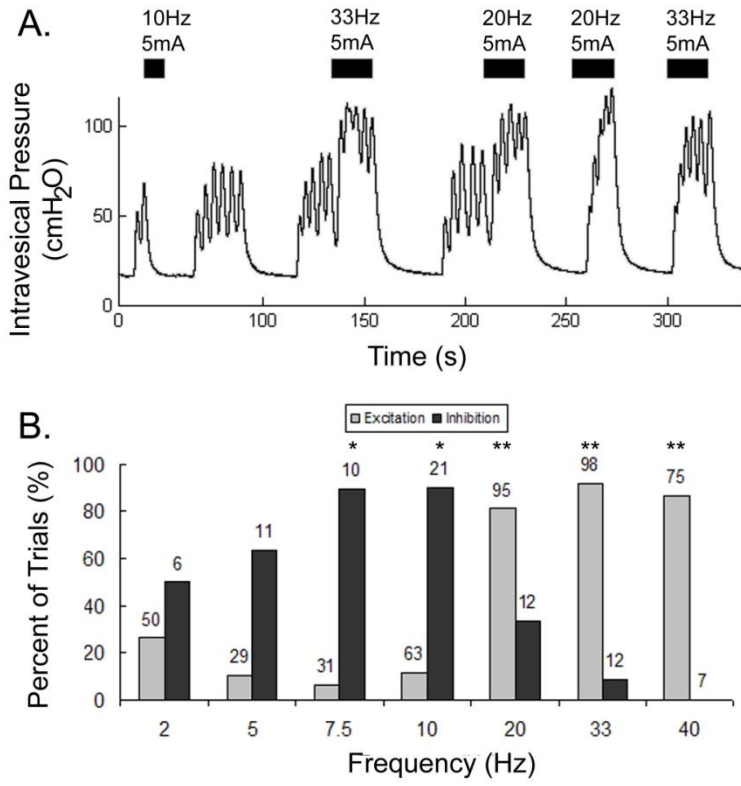
Figure 2.7

Figure 2.7. Bladder response evoked by percutaneous stimulation of the DNP. Percutaneous stimulation elicited similar responses to those observed with direct stimulation of the DNP. (**A**) The intravesical pressure trace showing an example of inhibition of a distension evoked contraction (10 Hz stimulation), augmentation of distension evoked contractions (20 and 33 Hz stimulation), and contraction of the detrusor (20 and 33 Hz stimulation). Bladder volume was above the DTV. The black bars indicate the duration of the percutaneous DNP stimulation, which consisted of 10 or 20 second trains at the frequency and amplitude indicated. (**B**) The proportion of trials in which percutaneous stimulation elicited bladder responses varied across stimulus frequencies ($p<0.001$ for both relaxation and contraction, Kruskal-Wallis one-way ANOVA, n=6 cats with 441 trials for the contractile data and 79 trials for the relaxative data). The gray bars show the percentage of trials resulting in detrusor contraction at each frequency, and the black bars show the percentage of detrusor relaxation. Stimulation at 7.5 and 10 Hz was more effective at inhibiting detrusor contractions than stimulation at 33 and 40 Hz (*$p<0.01$, Bonferroni inequalities), while stimulation at 20-40 Hz was more effective at exciting the detrusor than stimulation at 2-10 Hz (**$p<0.01$, Bonferroni inequalities). The relaxative and contractile percentages for each frequency do not add up to 100% because relaxation could only be determined during trials in which the bladder volume was above the DTV while contraction was possible in all trials in which the volume was above the STV.

A. Pre-SCT

20cmH₂O

10 seconds

B. 15 Hours Post-SCT

C. Post-SCI Frequency Response
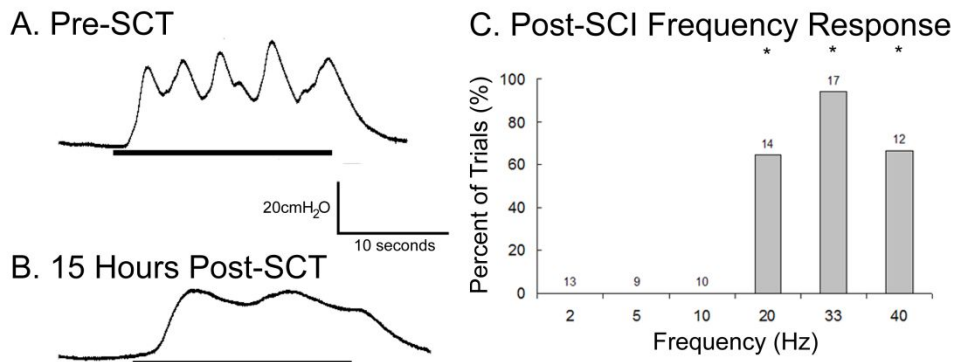
Figure 2.8. The contractile detrusor response to direct electrical stimulation of the DNP was present following acute SCI. The intravesical pressure responses to 20 second trains of stimulation at 33 Hz, 200µA before SCT (**A**) and 15 hours after SCT at T10 (**B**) in one cat. (**C**) The percent of trails that elicited detrusor contractions at different frequencies shows that detrusor contraction was dependent on stimulation frequency ($p<0.0001$, Kruskal-Wallis test, n=75 trials across 2 cats). Stimulation at 20, 33, and 40 Hz elicited contractions significantly more frequently than stimulation at 2, 5, and 10 Hz (*$p<0.05$, Bonferroni inequalities). Stimulation at 2, 5, or 10 Hz did not elicit any detrusor contractions following SCT.

3. Intraurethral stimulation evokes bladder responses via two distinct reflex pathways

3.1 Introduction

Spinal cord injury (SCI) or neurological disorders disrupt the neural control of continence and micturition and may cause urinary retention and/or urinary incontinence. Complications secondary to urinary dysfunction (e.g., frequent urinary tract infections) can lead to significant morbidity and substantially reduced quality of life. Electrical stimulation is a promising approach to restore control of urinary function to individuals with SCI or neurological disorders (Jezernik et al. 2002).

Electrical activation of somatovesical reflexes by stimulation of pudendal nerve afferents allows both inhibition and activation of the bladder. Two distinct sensory pathways, originating from the cranial sensory branch (CSN) and the dorsal genital branch (DNP) of the pudendal nerve, can produce bladder activation and inhibition in adult cats (Chapter 2) (Yoo et al. 2008a). The CSN and DNP innervate the membranous and penile urethra (Martin et al. 1974), and stimulation of the CSN and DNP evoke reflex bladder contractions through a supraspinal and spinal pathway, respectively (Yoo et al. 2008a). In persons with SCI, robust bladder inhibition is evoked by stimulation of the DNP (Wheeler et al. 1992; Kirkham et al. 2001). Conversely, urethral fluid flow (Bump 2000), urethral distension (Shafik et al. 2003a), and intraurethral electrical stimulation (IES) (Gustafson et al. 2004) can evoke bladder contractions, but the neural pathways mediating the responses to intraurethral activation are unclear. The objective of this study in the male cat was to determine the neural pathways mediating the effects of IES and to validate IES as a tool to investigate analogous pathways in human. The

parameters of stimulation (frequency, location) enabled selective activation of the CSN and DNP pathways, and in humans IES may provide a means to determine the parameters of pudendal nerve stimulation that enable control of urinary function

3.2 Methods

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at Duke University. Sixteen sexually intact male cats (2.8-4.6 kg) were anesthetized with ketamine HCl (35 mg/kg i.m.) and α-chloralose (65 mg/kg i.v. supplemented at 15 mg/kg as needed). The animals were intubated and respired artificially to maintain end tidal CO_2 between 3.5 and 4.5 %. A thermostatic heating pad was used to maintain body temperature at 38ºC, a catheter was inserted in the carotid artery to monitor the blood pressure, and a catheter was inserted in the cephalic vein for administration of IV fluids (lactated Ringer's solution or saline/5% dextrose/sodium bicarbonate solution) at 15 cc/kg/h. Following a midline abdominal incision, a 3.5 Fr or 5 Fr catheter was inserted into the bladder dome and secured with a purse-string suture. The abdomen was sutured closed in layers. The catheter was connected to a solid-state pressure transducer (Deltran, Utah Medical) and a pump for infusion of room temperature saline into the bladder.

3.2.1 Intraurethral and direct nerve stimulation

A 3.5 Fr catheter customized by adding a concentric platinum ring electrode 2-4 cm from the catheter tip was inserted into the urethra via the urethral meatus. Monophasic, cathodic stimulation pulses (10, 20, or 30 second trains of 100 µs pulses)

were delivered at varying locations along the urethra (1-7 cm from the urethral meatus), frequencies (2, 10, or 33 Hz), and intensities (0.5-10 mA).

The pudendal nerve was isolated in the ischiorectal fossa, and the sensory branch of the pudendal nerve was identified and traced until it branched into the CSN and DNP. Individual branches of the pudendal nerve were directly stimulated with monopolar cuff electrodes (platinum contact embedded in a silicon elastomeric cuff), and a needle in the hindlimb served as the return electrode for all stimulation.

3.2.2 Spinal cord transection

In 3 animals the spinal cord was transected at the T10 vertebral level following collection of pre-spinalization data. A laminectomy was performed, the dura was incised, the spinal cord was elevated and transected with a scalpel, and a barrier of Avitene and Surgicel was placed between the transected ends of the spinal cord.

3.2.3 Data analysis

Changes in bladder pressure in response to 20 second trains of electrical stimulation were analyzed to quantify the bladder response to IES. The ability to evoke bladder contractions was investigated at volumes between 85 and 100% of the minimum volume at which distension evoked contractions occurred, and bladder inhibition was investigated at volumes at which distension evoked bladder contractions occurred. The mean bladder pressure for 3 seconds prior to stimulation was defined as the baseline pressure. A bladder contraction was determined to be evoked if the bladder pressure increased 10 cmH_2O above the baseline pressure within 8 seconds of stimulation onset and remained above this threshold until stimulation ended. Bladder relaxation was

determined to have occurred if stimulation during a distension evoked contraction caused the bladder pressure to decrease by 50% of the peak distension evoked pressure within 10 seconds of the onset of stimulation and remained below this threshold until stimulation ended. The mean contraction pressures were calculated from when the pressure reached the threshold until stimulation ended. Statistical analysis of bladder pressures at different stimulation locations and frequencies was done by one-way ANOVA with post-hoc comparisons made using Tukey-Kramer. All data are mean ± SE.

3.3 Results

Intraurethral electrical stimulation (IES) evoked bladder responses in 13 of 16 cats, and the bladder responses to stimulation at 2, 10, and 33 Hz at locations 1-7 cm from the urethral meatus were investigated systematically in 11 cats. The ability to evoke a bladder response and the characteristics of the response depended on stimulation frequency, intensity, and location (Figure 3.1). Excitatory bladder responses were evoked only when the bladder volume was greater than ~70% of the threshold volume for distension evoked bladder contractions (Chapter 2) (Boggs et al. 2005; Yoo et al. 2008a), while inhibitory bladder responses were only apparent when the bladder was contracting (i.e., at bladder volumes greater than the threshold volume for distension evoked bladder contractions). Average stimulation evoked bladder contraction magnitudes (27±1 cmH$_2$O, range = 11 to 60 cmH$_2$O) and baseline pressures (13±1 cmH$_2$O, range = 8 to 20 cmH$_2$O) varied across experiments.

3.3.1 Frequency dependence of bladder responses

Bladder contractions were consistently evoked by 2 Hz stimulation in the membranous urethra, but 2 Hz stimulation did not evoke bladder relaxation at any location or intensity (Figure 3.1C). IES at 10 Hz evoked either bladder contraction or bladder relaxation depending on location (Figure 3.1D).   10 Hz stimulation in the membranous urethra evoked bladder contraction while 10 Hz stimulation in the penile urethra evoked relaxation. IES at 33 Hz consistently evoked bladder contractions at all locations but did not consistently evoke bladder relaxation at any location or intensity (Figure 3.1E). Stimulation with excitatory parameters during distension evoked contractions often augmented distension evoked contraction magnitude (Figure 3.1A).

The distance from the urethral meatus to several anatomical landmarks was measured post-mortem in 6 cats (Figure 3.2). When positioned 1-3 cm from the urethral meatus the electrode was within the penile urethra, and at 5-7 cm from the urethral meatus the electrode was within the membranous urethra, extending into or just proximal to the prostate.

3.3.2 Role of DNP and CSN in IES evoked bladder responses

Selective transection of individual branches of the pudendal nerve was used to determine the pathways mediating the IES evoked responses. Bilateral transection of the DNP abolished the responses evoked by stimulation in the penile urethra, while bilateral transection of the CSN abolished responses evoked by stimulation in the membranous urethra (n=2 cats, Figure 3.1B). Direct stimulation of the proximal stump of the transected DNP at 33 Hz or the proximal stump of the transected CSN at 2, 10, and 33 Hz still evoked bladder contractions (n=2 cats, Figure 3.1B), demonstrating the continued

functionality of the reflexes. Administration of α-bungarotoxin (2 cats, 100 μg/kg i.v.) abolished the reflex EMG evoked in the external anal sphincter by IES, but the bladder responses remained intact.

3.3.3 Effect of stimulation parameters on evoked responses

The stimulation intensity threshold to evoke a bladder contraction varied depending on stimulation location (Figure 3.3). Stimulation thresholds were highest 2-3 cm from the urethral meatus, and in some instances no bladder responses were evoked by stimulation at 2 cm (1 cat) or 3 cm (2 cats) from the urethral meatus, possibly because the thresholds were greater than 10 mA. In one cat stimulation thresholds were investigated above 10 mA, and the threshold to evoke contractions with 33 Hz stimulation at 3 cm from the urethral meatus was 12 mA. Thresholds were substantially lower in the membranous urethra (Figure 3.3), and stimulation at 2, 10, and 33 Hz evoked bladder responses at the same thresholds.

Contraction magnitudes were dependent on stimulation frequency and intensity. Stimulation in the penile urethra at 33 Hz and stimulation at 10 Hz and 33 Hz in the membranous urethra evoked larger bladder contractions than stimulation in the membranous urethra at 2 Hz (Figure 3.4). Stimulation at threshold intensities for evoking a bladder response evoked smaller bladder contractions (19 ± 2 cmH$_2$O) than stimulation at 1.5-2x threshold (28 ± 2 cmH$_2$O) and 2.25-3x threshold 29 ± 3 cmH$_2$O).

3.3.4 Bladder response to IES following SCT

At 8-13 hours following SCT, IES at 33 Hz in the penile urethra evoked bladder contractions (Figure 3.5), while stimulation at 2 or 10 Hz did not evoke bladder

contractions at any location. Stimulation in the membranous urethra occasionally evoked

small magnitude (<5 cmH$_2$O), transient rises in bladder pressure but never evoked a

robust bladder response. In 2 SCT animals, the magnitude of bladder responses evoked

by IES following unilateral DNP transection decreased by 23% (from 18±3 cmH$_2$O to

14±1 cmH$_2$O) and 62% (from 16±1 cmH$_2$O to 6±0 cmH$_2$O) , and following bilateral

transection of the DNP, IES no longer evoked bladder contractions. In SCT animals

direct 33 Hz stimulation of the proximal stump of the transected DNP continued to evoke

bladder contractions (2 cats), while direct stimulation of the proximal stump of the

transected CSN did not produce contractions (1 cat).

3.4 Discussion

3.4.1 Frequency and location dependent bladder response to IES

IES evoked bladder responses through two distinct reflex pathways. IES at 2, 10,

and 33 Hz in the membranous urethra evoked bladder contractions via afferents in the

CSN branch of the pudendal nerve. These responses were no longer evoked following

transection of the CSN or acute SCT. IES in the penile urethra at 10 Hz evoked bladder

relaxation and at 33 Hz evoked bladder contraction via afferents in the DNP branch of the

pudendal nerve. These responses were no longer evoked following DNP transection;

however, following acute SCT, stimulation in the penile urethra at 33 Hz continued to

evoke bladder contraction.   Direct stimulation of the CSN and DNP (Chapter 2) (Yoo et

al. 2008a) evoked bladder contraction and relaxation at similar frequencies as IES in the

membranous and penile urethra, respectively, and direct stimulation of the proximal

stumps of the transected nerves in this study confirmed that the IES evoked responses

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were via sensory activation of reflex pathways. Direct CSN stimulation at 33 Hz was

previously found not to evoke bladder contractions consistently across animals (Yoo et

al. 2008a), but 33 Hz IES in the membranous urethra did consistently evoke bladder

contractions across animals in this study. This difference is likely due to the different

stimulation methods. Intraurethral stimulation may activate a different complement of

sensory fibers in the CSN than direct CSN stimulation. Also, IES can activate the CSN

bilaterally, and IES would be more effective at evoking bladder contractions if there is

functional asymmetry in the CSN-bladder pathway (Hamdy et al. 1999) or if bilateral

activation is more effective at evoking bladder contractions than unilateral activation.

3.4.2 Effect of α-chloralose anesthesia

Administration of α-chloralose anesthesia can effect bladder function leading to

increased baseline pressures and reduced contraction magnitudes (Rudy et al. 1991). The

contractions observed here are consistent with previous results in α-chloralose

anesthetized cats, and a similar frequency dependence of somatovesical reflexes was

observed in awake chronic SCI cats (Tai et al. 2008).

3.4.3 IES evoked responses require pudendal afferent activation

IES activated the pudendal, pelvic, and hypogastric nerves in the cat, evoking

stronger hypogastric and pelvic nerve responses than pudendal nerve responses when

stimulation was proximal to the prostate (Bradley et al. 1973). In the present study, the

pudendal nerve was targeted by limiting IES to 1-7 cm from the urethral meatus, a length

consistent with the urethral areas most heavily innervated by pudendal afferents (Figure.

2) (Yoo et al. 2008b), and transection of the DNP and CSN verified that activation of

pudendal afferents was required to evoke bladder contractions, and any spillover of stimulation to pelvic or hypogastric afferents was not sufficient to generate the observed responses.

Stimulation of efferent fibers in the deep perineal branch of the pudendal nerve activates the external urethral sphincter, and this motor response can generate sufficient sensory activation of the CSN to generate a bladder response (Yoo et al. 2008a). Deep perineal nerve activation by IES in the area of the external urethral sphincter may have therefore contributed to the bladder responses observed in this study. However, evoked bladder responses were preserved following administration of α-bungarotoxin, demonstrating that this pathway did not contribute to the responses evoked by IES.

In a previous study of the bladder response to IES, stimulation at 2 Hz in the membranous urethra evoked bladder contractions in a SCT cat (Gustafson et al. 2003), but a similar response was not observed in this study. Direct CSN stimulation evoked small magnitude, transient bladder responses in acute SCT cats (Shefchyk et al. 1998; Yoo et al. 2008a), and in chronic SCI cats, 2 Hz stimulation did not evoke bladder contractions (Tai et al. 2006), consistent with the observed lack of robust bladder responses evoked by IES of the membranous urethra. In the previous cat study of IES, the nature of the contractions evoked by 2 Hz stimulation in the proximal urethra in the SCT cat was not described in detail, so it is likely that the contractions were small magnitude, transient responses (Shefchyk et al. 1998; Yoo et al. 2008a).

3.4.4 Dependence on bladder volume and stimulation intensity

The ability to evoke bladder responses depended on bladder volume and stimulation intensity. Similar bladder volume dependence was observed in the previous

studies of bladder responses evoked by pudendal afferent activation (Chapter 2) (Boggs et al. 2005; Yoo et al. 2008a), as well as previous human IES experiments (Gustafson et al. 2004), and the volume dependence is mediate by neural rather than biomechanical factors (i.e. length-tension properties) (Boggs et al. 2005). Stimulation intensity thresholds were smaller in the membranous urethra than in the penile urethra, possibly because the post-prostatic and prostatic urethra are more heavily innervated by pudendal afferents than the penile urethra (Todd 1964; Yoo et al. 2008b) or because the CSN trunk is in closer proximity to the membranous urethral lumen than the DNP trunk is to the penile urethral lumen (Yoo et al. 2008b).

3.4.5 Implications for clinical investigation of the pudendovesical reflex

These results suggest that the DNP pathway should be the target for restoring bladder function in SCI humans. Clinical studies investigating the effect of DNP stimulation on bladder inhibition and urinary continence are promising (Wheeler et al. 1992; Kirkham et al. 2001), but the ability to evoke bladder contractions and improve bladder voiding (i.e. treat urinary retention) by pudendal afferent stimulation has not been adequately investigated. High frequency direct stimulation of the DNP in the cat evoked bladder contraction and improved voiding (compared to distension evoked voiding) (Chapter 2), but it is unknown how pudendal afferent evoked bladder contractions will effect bladder voiding and detrusor-sphincter dyssynergia in humans with SCI.

The CSN-to-bladder reflex may return at longer times post-SCT. While CSN stimulation does not appear to evoke bladder contractions in SCT cats, the organization of feline and human pudendal-to-bladder reflexes may differ. Bladder contractions evoked by IES in persons with SCI may have been evoked by activation of the human

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analog of the CSN. It is unclear if a human analog of the CSN exists, but the urethrovesical reflex in humans suggests somatic innervation of the proximal urethra (Shafik et al. 2003a; Shafik et al. 2003b).

3.4.6 Conclusions

The bladder responses evoked by IES were dependent on stimulation location, frequency, and intensity. Two distinct reflex pathways were activated by IES to evoke bladder responses, but only the DNP-mediated pathway was present following SCI. This study expanded the stimulation parameters used in previous cat experiments and identified a DNP mediated bladder pathway accessible by stimulation in the penile urethra, suggesting that expanded stimulation parameters in clinical studies will allow for full characterization of the pudendal-to-bladder reflexes that are present in humans. Additionally, IES enables reliable selective activation of pudendal afferent pathways compared to pudendal nerve trunk stimulation.
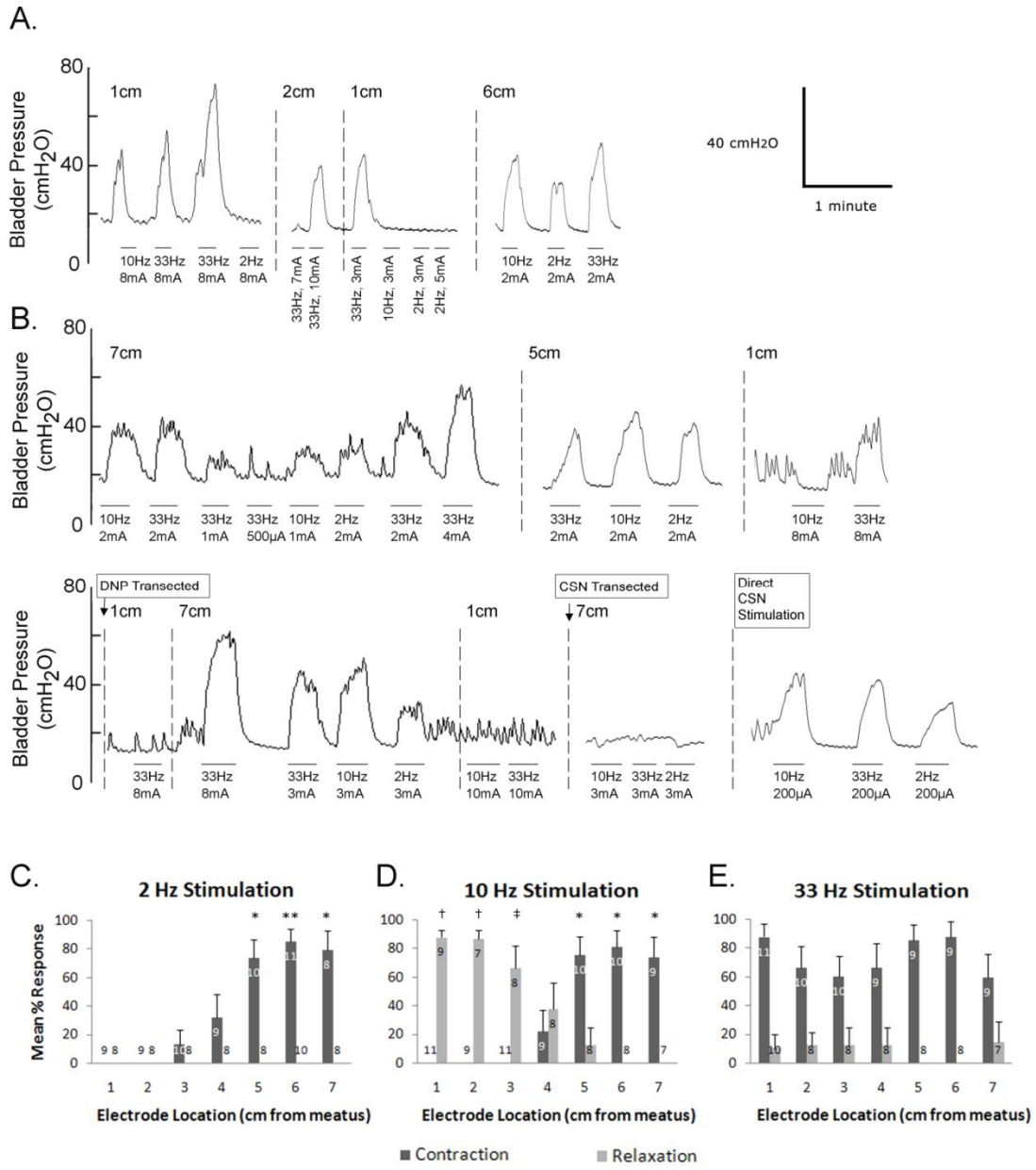
Figure 3.1

Figure 3.1. Bladder responses evoked by intraurethral electrical stimulation.
(A, B) Bladder pressure versus time traces for two cats.   Stimulation periods are
indicated by horizontal bars under the pressure traces, stimulation frequency and intensity
are indicated below each bar, and intraurethral electrode locations are indicated above the
pressure traces. (C-E) Mean percentage of trials (+ standard error) when intraurethral
stimulation at 1-7 cm from the urethral meatus evoked bladder contraction or relaxation.
Numbers in the columns represent the number of cats in which at least 3 stimulation trials
were performed at that stimulation location. (C) Stimulation at 2 Hz evoked bladder
contractions dependent on stimulation location ($p<0.001$, one-way ANOVA). (*)
Stimulation evoked contractions in a significantly greater percentage of trials than
stimulation at 1-3 cm ($p<0.05$, Tukey-Kramer post-hoc comparison). (**) Stimulation
evoked contractions in a significantly greater percentage of trials than stimulation at 1-4
cm ($p<0.05$). (D) Stimulation at 10 Hz evoked bladder contraction or relaxation
dependent on stimulation location ($p<0.001$, one-way ANOVAs for contraction and
relaxation). (*) Stimulation evoked contractions in a significantly greater percentage of
trials than stimulation at 1-4 cm ($p<0.05$, Tukey-Kramer post-hoc comparison). (†)
Stimulation evoked relaxation in a significantly greater percentage of trials than
stimulation at 4-7 cm ($p<0.05$). (‡) Stimulation evoked relaxation in a significantly
greater percentage of trials than stimulation at 5-7 cm ($p<0.05$). (E) Stimulation at 33 Hz
evoked bladder contractions independent of stimulation location ($p=.41$, one-way
ANOVA). Relaxation was evoked in a small number of trials, and the dependence on
location was not statistically significant ($p=.88$, one-way ANOVA).
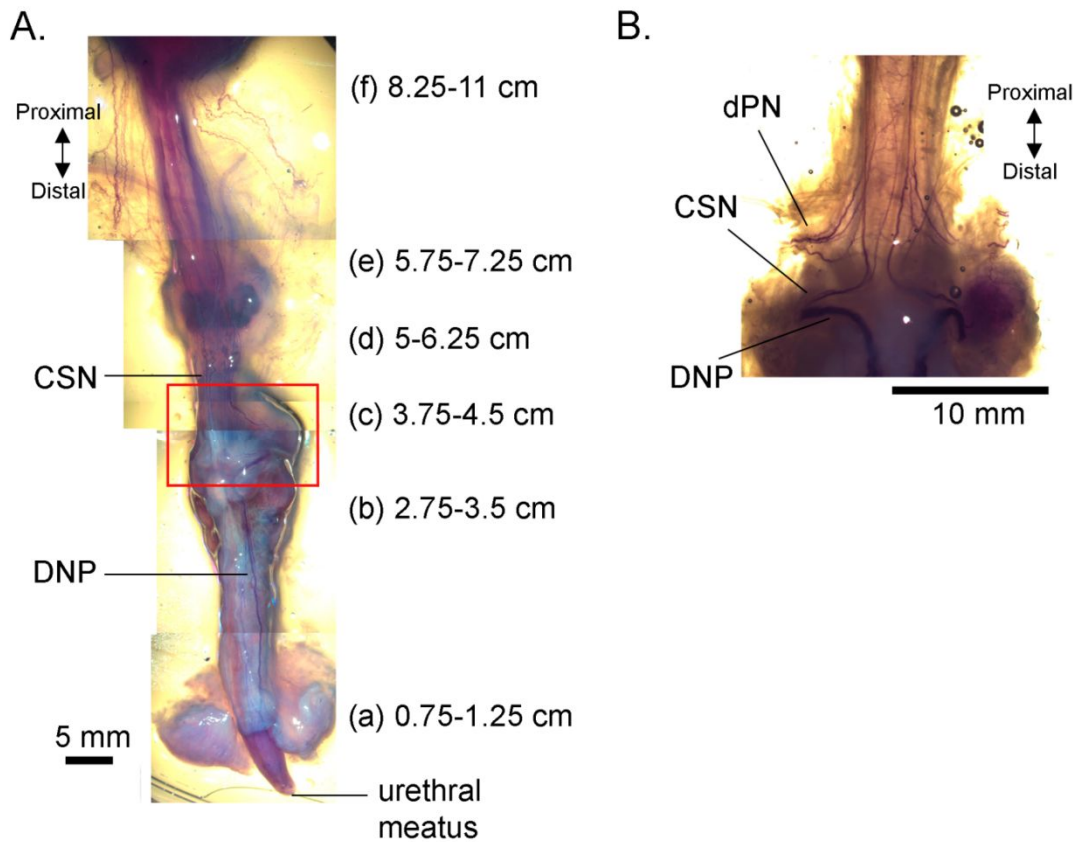
Figure 3.2. Anatomical relationship between intraurethral catheter locations along the male cat urethra and innervation by the cranial sensory (CSN) and dorsal penile (DNP) branches of the pudendal nerve. (A) A ventrolateral view of the lower urinary tract of a male cat with nerves rendered dark purple using Sihler's method (Yoo et al. 2008b). Distance from urethral meatus to several anatomical landmarks, including (a) the base of the glans penis, (b) the base of the penile body, (c) the proximal border of the bulb of the penis, (d) the distal border of the prostate, (e) the proximal border of the prostate, and (f) the bladder neck. All distances are accurate within ±0.5 cm. (B) Image of the ventral side of the membranous urethra and the proximal end of the bulb of the penis (box in (A)), with nerves rendered dark purple by Sihler's method (Yoo et al. 2008b) illustrating the CSN, DNP, and deep perineal (dPN) branches of the pudendal nerve.
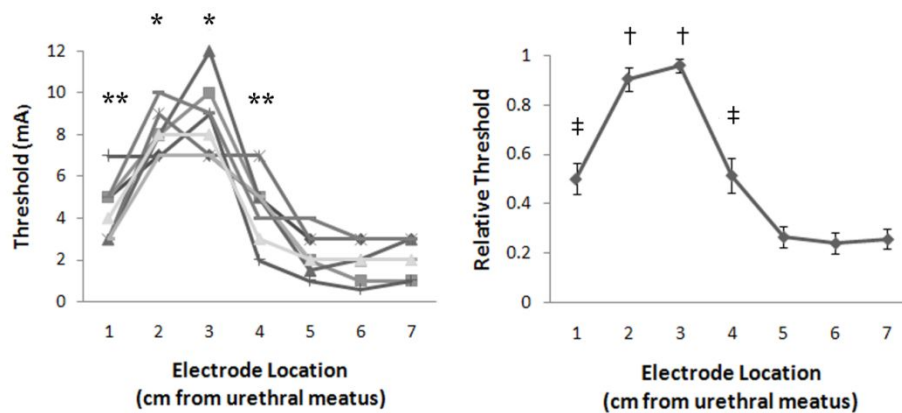
Figure 3.3. Stimulation intensity thresholds for evoking bladder contraction at different intraurethral electrode locations. (A) The stimulation intensity thresholds for 8 cats at 1-7 cm from the urethral meatus were dependent on the electrode location ($p<0.001$, one-way ANOVA). (*) Stimulation thresholds were significantly greater than at 1, 4, 5, 6, and 7 cm ($p<0.05$, Tukey-Kramer post-hoc comparison). (**) Stimulation thresholds were significantly greater than at 6 and 7cm ($p<0.05$). (B) Relative stimulation thresholds, normalized by the maximum threshold in each cat, versus electrode location. The relative intensities were dependent on electrode location ($p<0.001$, one-way ANOVA). (†) Relative thresholds were greater than thresholds at 1 cm and 4-7 cm ($p<0.05$, Tukey-Kramer post-hoc comparison). (‡) Relative thresholds were greater than those at 5-7 cm ($p<0.05$).
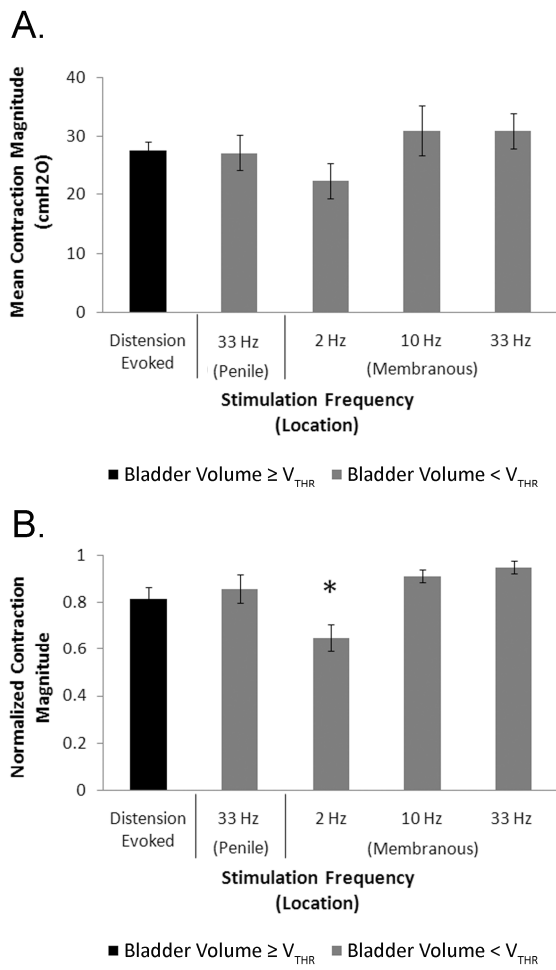
Figure 3.4

Figure 3.4. Magnitude of bladder contractions evoked by intraurethral electrical stimulation (IES) at different stimulation frequencies and locations.
(A) The mean contraction magnitude of distension evoked reflex bladder contractions and contractions evoked by stimulation at 33 Hz in the penile urethra or by 2, 10, or 33 Hz in the membranous urethra. Distension evoked contractions were only observed and measure at bladder volumes greater than V_{THR}, the minimum volume at which distension evoked contractions were observed, while stimulation evoked contractions were measured at volumes between 85 and 100% of V_{THR}. (B) Average normalized magnitudes of distension evoked reflex contractions and IES evoked contractions at different frequencies and locations. The average contraction magnitudes were computed for each cat and normalized by dividing by the largest value. Normalized values were averaged across cats. Contractions evoked by stimulation at 2 Hz were smaller than contractions evoked by 33 Hz stimulation in the penile urethra and 10 and 33 Hz stimulation in the membranous urethra (*$p<0.001$, one-way ANOVA, $p<0.05$, Tukey-Kramer post-hoc comparison), but distension evoked contractions were not significantly different from stimulation evoked contractions at any stimulation parameters. V_{THR} is the minimum volume at which distension evoked contractions were observed, and stimulation evoked contractions were investigated at volumes between 85 and 100% of V_{THR}.
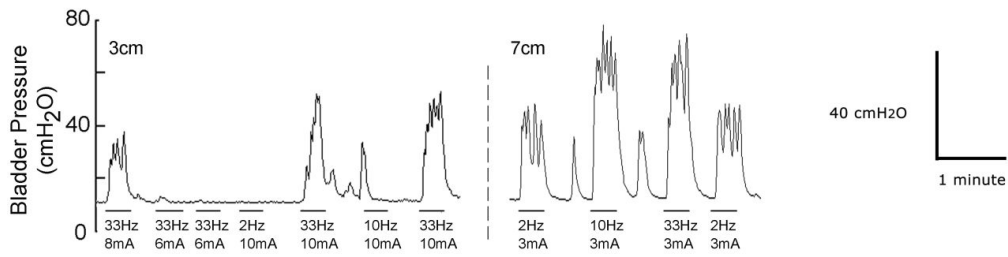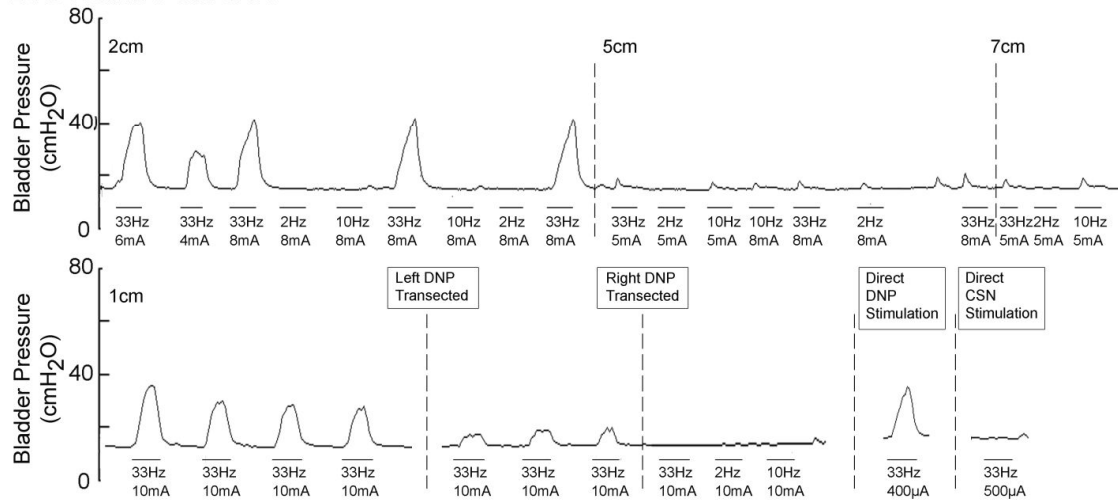
Figure 3.5. Bladder responses evoked by intraurethral electrical stimulation before and after acute spinal cord transection. (A) Bladder pressure versus time traces prior to spinal cord transection. (B) Bladder pressure versus time traces 8 hours post spinal cord transection in the same cat from (A). The horizontal bars below the pressure trace indicate stimulation, the stimulation frequency and intensity are indicated below each bar, and stimulation locations are indicated above the pressure traces.

4. Finite element modeling and in vivo analysis of electrode configurations for selective pudendal afferent fiber stimulation

4.1 Introduction

Pudendal nerve stimulation is a potential means of restoring bladder function to persons with spinal cord injury. Stimulation of sensory (afferent) fibers either in the dorsal penile branch (DNP) or the cranial sensory branch (CSN) of the pudendal nerve can evoke stimulation frequency-dependent contraction or relaxation of the urinary bladder in cats (Chapter 2) (Yoo et al. 2008a). However, the existence of comparable reflexes in persons with spinal cord injury (SCI) remains unclear. In both experimental and clinical settings, intraurethral electrical stimulation (IES) has been utilized as a minimally invasive method to investigate these reflexes. But the activation pattern of multiple nerve pathways (pudendal and pelvic) generated by this approach fail to elucidate the specific sensory nerves responsible for these bladder reflexes. The present study used a finite element model (FEM) of the male cat urethra and *in vivo* measurement to quantify the effects of electrode configuration and position on intraurethral activation of pudendal afferent nerve fibers.

Clinical evaluation of the bladder response to pudendal nerve stimulation is difficult because of the limited access to the pudendal nerve. The pudendal nerve trunk is located in the ischiorectal fossa, where it exhibits a complex and highly-variable branching pattern that results in motor and sensory innervation of the genitalia, urethra, rectum and the pelvic floor (Juenemann et al. 1988; Yang et al. 1998a; Yang et al. 1999; O'Bichere et al. 2000; Schraffordt et al. 2004; Mahakkanukrauh et al. 2005). As a result of this complexity, clinical investigation of specific pudendal afferent fibers has been

difficult. Transcutaneous DNP stimulation in humans can evoke robust bladder

relaxation and promote continence (Wheeler et al. 1992; Kirkham et al. 2001; Horvath et

al. 2009), but this approach is limited to activation of superficial pudendal afferent

branches (the DNP). Percutaneous stimulation can activate the pudendal nerve (Yoo et

al. 2007a; Yoo et al. 2007b), but it is unclear which branches of the nerve are being

activated, and distinct responses are evoked by selective activation of the different

pudendal nerve branches in the cat (Yoo et al. 2008a). IES in the proximal urethra can

evoke bladder contraction in humans (Gustafson et al. 2004), but the conflicting results

between the human and cat (Chapter 3) (Bruns et al. 2009b) IES data suggests a closer

look at the effects of urethral stimulation is necessary.

The goal of this study was to develop a preclinical computer model of IES that

can be used to interpret data and guide design of superior IES electrodes. We developed

three-dimensional (3-D) FEMs to determine the electric potentials generated along the

DNP and CSN by IES. The potentials were used to calculate the second spatial

derivative of the extracellular potential along the nerve fibers (the 'activating function',

AF) for stimulation at different intraurethral locations and using different electrode

configurations. The model and *in vivo* stimulation threshold results provide insight into

the optimal parameters for pudendal afferent fiber activation via IES.

4.2 Methods

4.2.1 Finite element modeling

A 3-D model of the male cat urethra and surrounding structures was developed.

The model spanned from the urethral meatus to 0.5 cm proximal to the prostate (Figure

4.1). Surrounding structures included the prepuce, corpus spongiosum, corpus cavernosum, bulbospongiosus muscle, bulbocavernosus glands, ischiocavernosus muscle, inner urethral muscle, outer urethral muscle, and prostate glands. The dimensions of the structures were determined from urethral cross-sections and gross anatomical observations (Wang et al. 1999; Yoo et al. 2008b). The electrical properties of the tissues were taken from the literature (Table 4.1) and, for simplification, were all modeled as isotropic. The model included a 3.5 Fr intraurethral catheter with different electrode configurations (summarized in Figure 4.2) and was enclosed within a conducting medium.

The model was implemented in COMSOL Multiphysics (version 3.4) and partitioned into mesh elements using the finite element method. The internal tissue boundaries were set so that continuity of current was preserved, and the external boundaries were set to ground (V=0) with the exception of the external boundaries of the prepuce and the adjacent wall of the bounding box which were set to be electrically insulated (current density=0). Increasing the mesh size around the electrode or doubling the bounding box size had minimal effect on the maximum potentials generated at the nerves (<5% change). The electrical input was a 1 mA cathodic regulated current for all simulations, and the model was solved using the conjugate gradient method.

4.2.2 Nerve Modeling

The anatomical courses of the DNP and CSN branches of the pudendal nerve were modeled in Matlab (R2007a, Mathworks) and based on previous anatomical data (Bradley et al. 1973; Martin et al. 1974; Wang et al. 1999; Yoo et al. 2008b). The nerves were represented bilaterally as single trunks, and lateral branches of the DNP were later

included for further examination of DNP activation in the penile urethra. The potentials

generated by IES were exported from the FEM model to Matlab and the potentials along

the nerve paths were determined at 0.1mm increments using interpolation.

4.2.3 The Activating Function

The second spatial derivative of the extracellular potentials, V_e, or the activating

function (AF), was calculated along the modeled paths of the DNP and CSN to estimate

neural activation (Rattay 1989).

$$AF = \frac{\Delta^2 V_e}{\Delta x^2} = \frac{V_e(n-1) - 2 \cdot V_e(n) + V_e(n+1)}{\ell^2} \quad ,$$

where n is the node of interest and $\ell = 0.5mm$ is the internodal length assuming the

modeled nerve fibers were 5μm in diameter (Martin et al. 1974). The maximum AF was

determined for the DNP and CSN bilaterally for each of 5 possible locations of the first

(most distal) node in the fiber. The AF at the termination of the nerve fibers was the first

spatial derivative of the extracellular potential. The resulting maximum AFs were

averaged.

For analysis of the lateral branches of the DNP in the penile urethra, the AFs were

calculated along 5 lateral DNP branches (Figure 4.1) in addition to the DNP trunk. The

branches were initially spaced 3 mm apart (Yoo et al. 2008b) and were located ~1.4 – 2.6

cm from the urethral meatus. The maximum AFs were calculated for each branch for all

electrode configurations (Figure 4.2), and this was repeated for 50 sets of random branch

locations generated by randomly varying the location of each branch within ±1 cm along

the longitudinal axis of the urethra. Maximum branch AFs were ordered from greatest to

smallest for each of the 50 trials. The AFs were averaged across trials based on their rank

(i.e., the maximum AF across the 5 branches was averaged over the 50 trials and so on for the 2nd largest, etc).

4.2.4 Selectivity Analysis

The effects of intraurethral electrode configurations on the ability to activate nerve fibers selectively was quantified by computing both the ratio of the AFs (AFR) under different conditions and the "selectivity", defined as the quotient of a minimum estimate of the maximum AF (mean, $\mu_{AF,1}$, minus one standard deviation, $\sigma_{AF,1}$) generated by one set of stimulation conditions and a maximum estimate of the maximum AF (mean, $\mu_{AF,2}$, plus one standard deviation, $\sigma_{AF,2}$) generated by a second set of stimulation conditions,

$$Selectivity = \frac{\mu_{AF,1} - \sigma_{AF,1}}{\mu_{AF,2} + \sigma_{AF,2}}.$$

An AFR >1.5 combined with a selectivity >1 suggested, conservatively, that activation of a population of fibers under the first set of conditions could be achieved at lower threshold than under the second set of conditions. If the AFR was <1.5 or selectivity was <1, it is likely that variation in nerve location, anatomical dimensions, and other factors would render stimulation thresholds under the two conditions indistinguishable during in vivo IES.

4.2.5 In vivo experiments

Animal care and experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee. Experiments were performed on 13 sexually intact male cats (2.8-4.6 kg) anesthetized with ketamine HCl (35 mg/kg i.m.)

and α-chloralose (65 mg/kg i.v. supplemented at 15 mg/kg as needed). Artificial

respiration maintained the end tidal CO_2 between 3.5 and 4.5 %, IV fluids (lactated

Ringer's solution or saline/5% dextrose/sodium bicarbonate solution) were delivered at

15 cc/kg/hr via a catheter in the cephalic vein, and a thermostatic heating pad was used to

maintain body temperature at ~38º C. Blood pressure was monitored through a catheter

in the carotid artery. A catheter was inserted into the bladder dome and the bladder was

drained externally to maintain an empty bladder.

A 3.5 or 5 Fr catheter modified with platinum electrodes embedded at 2 cm from

the tip was inserted into the urethral meatus. The 3.5 Fr electrode included three 1 mm

rings spaced 3.5 mm apart. The 5 Fr electrode included two 2 mm rings spaced 2 mm

apart. Electrical stimulation (1 Hz) was applied with the catheter electrodes in the urethra

located 1-7 cm from the urethral meatus. Stimulation intensity varied from 0.5-15 mA,

and the intensity threshold to evoke a reflex electromyographic response in the external

anal sphincter (EAS EMG) was measured in 0.5 mA increments.

4.3 Results

4.3.1 Finite element model of intraurethral stimulation

Intraurethral stimulation was applied to electrodes positioned 1-7 cm from the

urethral meatus using the electrode configurations shown in Figure 4.2A. The spatial

distribution of the electric potential varied depending on the electrode configuration.

Stimulation with the short electrode generated the largest voltage gradient along the

urethra, both in maximum value and the volume of tissue that experienced a >1 V change

in potential, while stimulation with the 1 mm bipolar ring electrode generated the

smallest change in potential. The orientation of the short and long electrodes resulted in greater potential changes in the dorsal direction (towards the nerves), whereas the potential changes generated by the ring electrode were more balanced across the dorsal and ventral directions.

4.3.2 Activation threshold depends on electrode location

The maximum value of the AF generated along the DNP and CSN varied depending on the location of stimulation (two-way ANOVA, $p < 0.0001$ for both nerves). The DNP AFs (Figure 4.3A) were significantly greater in the penile urethra (1-4 cm) than in the membranous urethra (5-7 cm), while stimulation in the membranous urethra (5-7 cm) generated significantly greater CSN AFs (Figure 4.3B) than stimulation in the penile urethra (1-4 cm). These results indicate that the DNP is more readily activated by stimulation in the penile urethra while the CSN is more readily activated by stimulation in the membranous urethra, which reflects the proximity of each nerve trunk to the corresponding segment of the urethra.

The maximum AFs, regardless of whether they occurred at the DNP or CSN, also depended on the electrode location (Figure 4.3C, two-way ANOVA, $p < 0.0001$). The maximum AFs for stimulation in the membranous urethra (5-7 cm) were significantly larger than those for stimulation in the penile urethra (1-4 cm). Relative stimulation thresholds were determined by inverting the AFs, and the simulation results suggest that the CSN is activated at lower thresholds by stimulation in the membranous urethra than the thresholds necessary to activate the DNP by stimulation in the penile urethra (Figure 4.3D).

The ability to selectively activate the DNP without coactivation of the CSN (and vice versa) was calculated for each electrode configuration at the different intraurethral locations (Figure 4.4). Selectivity for one nerve was greatest when the electrode was furthest from the other nerve (e.g., DNP selectivity was greatest at 1 cm from the urethral meatus, Figure 4.4A), and selectivity values for all electrode geometries were <1 at 4 cm for the DNP (Figure 4.4A) and CSN (Figure 4.4B). This reveals that at ~4 cm (the proximal portion of the penile bulb) neither the DNP nor the CSN can be activated selectively.

IES with a monopolar ring electrode (1 mm or 2 mm) evoked reflex EMG responses in the EAS in 11 of 11 cats. The threshold to evoke a response with a ring electrode was dependent on the intraurethral location (Figure 4.5, one-way ANOVA, $p < 0.0001$). The threshold intensity was significantly lower in the area of the glans penis (1 cm) and the membranous urethra (5-7 cm) than in the area of the penile body (2-3 cm). The average threshold intensity (Figure 4.5B) revealed a location-intensity relationship similar to that predicted by the finite element model (Figure 4.3D).

4.3.3 Activation threshold depends on electrode geometry

The maximum AFs were also dependent on the electrode configuration (two-way ANOVA, $p < 0.0001$). Intraurethral stimulation with the short monopolar and wide bipolar configurations generated larger AFs than stimulation with the other electrode configurations (Figure 4.3C, $p < 0.05$, post hoc comparisons with Bonferroni correction). Stimulation with 1 mm and 2 mm monopolar ring electrodes evoked significantly larger AFs than stimulation with 1 mm and 2 mm bipolar ring electrodes (Figure 4.6A, $p < 0.05$, post hoc comparisons with Bonferroni correction). Although the 1 mm monopolar

electrode evoked larger AFs than the 2 mm monopolar electrode at all locations except 4 and 5 cm from the meatus, the differences were not significant ($p>0.05$). Similarly, the 2 mm bipolar electrode evoked larger AFs than the 1 mm bipolar electrode at all locations, but the differences were also not significant ($p>0.05$). Stimulation with the 1 mm and 2 mm bipolar ring electrodes evoked significantly smaller AFs than stimulation with all other electrode configurations ($p<0.05$).

Comparison of the selectivity and AFR of the 2 mm monopolar and 2 mm bipolar ring electrodes revealed that bipolar stimulation requires greater stimulation amplitudes to activate pudendal afferent fibers within the penile urethra (1-4 cm, Figure 4.6B), while no difference was predicted for stimulation in the proximal urethra. In vivo, the normalized stimulation thresholds for evoking an EAS response were not significantly different for bipolar ring electrodes and the monopolar ring electrodes (Figure 4.6C, two-way ANOVA, $p=0.17$, n=6 cats). However, the threshold ratios (bipolar electrode threshold divided by monopolar electrode threshold) revealed that thresholds were higher at 2-4 cm for the 2mm bipolar ring electrode (Figure 4.6D), as predicted by the model simulations.

The electrode geometries modeled assume the contact electrodes are oriented in the direction of the nerve trunks and that electrode contact with the urethral lumen is flush. The effects of changing the orientation of the short electrode contact and modifying the diameter of the ring electrode were examined. Rotating the short contact electrode to face ventrally (away from the DNP) reduced the maximum AF by 30% for simulation of IES at 2cm from the meatus. For the ring electrode, reducing the diameter of the stimulation catheter to one-half the diameter of the urethra (and filling the urethral

cavity with urine, σ=1.55 (Suhel et al. 1997)) reduced the maximum AF by 20 and 30 % for stimulation at 2 and 7 cm, respectively.

4.3.4 DNP branch activation depends on electrode geometry

The feline DNP also exhibits lateral branches that project ventrolaterally along the penile body and innervates the urethra and perineum (Figure 4.7A) (Yoo et al. 2008b). AFs generated by stimulation with 12 different electrode configurations (Figure 4.2A,B) positioned 2 cm from the urethral meatus were compared to determine the electrode configurations that could selectively activate these lateral DNP branches, without activating the DNP trunk and vice versa (Figure 4.7). Maximum branch AFs were averaged across trials based on their rank (i.e., the maximum AF across the 5 branches was averaged over the 50 trials and so on for the 2nd largest, etc). AFs were dependent on the stimulation target (p<0.0001, MANOVA). For all electrode configurations, the largest maximum AF generated over all the branches was greater than the maximum AF at the DNP trunk (Figure 4.7B, post hoc paired comparison by single-sided t-test, p<0.001). However, the relative magnitudes of the 2nd-5th maximum branch AFs varied compared to the maximum AF at the DNP trunk. For each electrode configuration, Table 4.2 shows how many branch AFs were greater than the DNP AF based on paired comparisons (single-sided t-test, p<0.001) or selectivity and AFR (branch AF greater than DNP AF if selectivity > 1 and AFR > 1.5). The long, 5-contact, 3-ring, and 5-ring electrode configurations provided the most selective activation of the lateral DNP branches without activation of the DNP trunk, allowing for activation of ~4 branches, or ~0.9 cm of urethral length, while the ring electrode activated the fewest branches at lower thresholds than DNP activation. These results implied that in vivo comparison of DNP

stimulation thresholds with the ring electrode and the 3-ring electrode could provide

evidence of whether activation of pudendal afferent fibers by IES in the penile urethra

occurs at the lateral branches or at the DNP trunk (Figure 4.7C). The selectivity of the

ring electrode compared to the 3-ring electrode (Figure 4.7D) indicated that the ring

electrode stimulation threshold will be lower than the 3-ring electrode only in the case of

DNP trunk activation.

In 7 of 7 cats, stimulation at 2 cm with the 1 mm ring electrode evoked a reflex

EMG response in the EAS at a lower threshold than stimulation with the 3-ring electrode

(Figure 4.8A). The normalized threshold for the ring electrode was 60%±5% of the

normalized threshold for the 3-ring electrode (Figure 4.8B, $p<0.0005$, paired one-sided t-

test), and comparable to the simulation result that the ring electrode DNP trunk activation

threshold is ~67% of the 3-ring electrode DNP trunk activation threshold. Thus, IES

generates activation at the DNP trunk and not via the lateral branches along the urethra.

4.4 Discussion

IES is a minimally invasive method to investigate the bladder responses evoked

by activation of pudendal afferent fibers. However, the complex innervation of the

urethra and surrounding structures makes it unclear what nerve branches are being

activated and how this varies with electrode geometry and location. The results of this

study show that the location and geometry of the electrode with respect to the urethra

both play significant roles in determining the stimulation threshold and selective

activation of the two primary sensory branches (DNP, CSN) of the feline pudendal nerve.

In this case, electrode location appears to be the primary factor in determining selectivity

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of activation. Also, the results indicate IES in the penile urethra activates the DNP trunk and not the lateral branches of the DNP.

This study provides a quantitative analysis of different electrode geometries for intraurethral stimulation, however there are several important limitations. First, the model is a simplified representation of the male feline lower urinary tract with neural innervation by the CSN and DNP. The nerves were represented as single trunks but the DNP has been shown to branch extensively in the area of the glans penis and the CSN typically has a lateral branch along the membranous urethra in addition to the medial branch modeled here (Yoo et al. 2008b). Second, the AF is only an approximation that provides the relative thresholds for nerve activation by electrical stimulation (Warman et al. 1992). Additionally, in vivo the quality of the fit between the urethra and the electrode contacts may vary, altering the current density at the different contacts in the multi-contact electrodes and ultimately affecting the threshold intensity for activation. However, the similarities between the in vivo stimulation intensity thresholds and those predicted by the model demonstrate the simplifications were adequate to support our conclusions.

4.4.1 Stimulation Location

Selective activation of the DNP was best achieved by stimulation in the distal urethra (near the glans) while selective activation of the CSN was best achieved by stimulation in the proximal urethra (near the prostate). Bladder responses evoked by intraurethral activation of pudendal afferent fibers also exhibit different characteristics for stimulation near the glans penis (high frequency [33-40 Hz] excitation; low frequency inhibition [5-10 Hz]) and near the prostate (excitation at all frequencies [2-33 Hz])

(Chapter 3) (Bruns et al. 2009b). Further, these in vivo observations highlight the importance of selective DNP or CSN activation because the bladder response to activation of these nerves is different for different stimulation frequencies and involves different neural pathways (Yoo et al. 2008a). The CSN-bladder reflex involves a supraspinal pathway, while the DNP-bladder reflex involves a spinal pathway (Yoo et al. 2008a).

The innervation of the urethra is spatially distinct (Bradley et al. 1973; Yoo et al. 2008b). IES can activate afferent fibers in the pudendal, pelvic, and hypogastric nerves, and the degree of activation of each nerve is dependent on intraurethral electrode location (Bradley et al. 1973). Innervation of the proximal urethra by autonomic nerve fibers from the pelvic and hypogastric nerves overlap with the somatic innervation by CSN fibers (Bradley et al. 1973; Yoo et al. 2008b), and IES in the proximal urethra may result in coactivation of pudendal and autonomic fibers. A previous study of intraurethral stimulation in the cat found that the pudendal and pelvic nerves were both activated by intraurethral stimulation in the membranous urethra (Bradley et al. 1973). The simulation and in vivo results show that the threshold for pudendal afferent fiber activation for stimulation in the proximal urethra (CSN activation) was lower than the threshold for stimulation in the penile urethra (DNP activation), so future clinical studies should investigate the use of lower amplitude stimuli in the proximal urethra compared to the penile urethra to avoid spillover of activation to neighboring nerves (e.g., autonomic innervations of the proximal urethra). Afferent pudendal nerve and pelvic nerve urethral innervation consists of myelinated A-fibers and unmyelinated C-fibers. However, the myelinated pudendal urethral afferent fibers are larger, potentially consisting of Aα-, Aß-,

and Aδ-fibers, than the myelinated pelvic urethral afferent fibers, primarily Aδ-fibers (Sengupta et al. 1994; Yoshimura et al. 2003). These differences in fiber diameters suggest that it may be possible to limit coactivation of pelvic and hypogastric nerve afferent fibers by minimizing stimulation intensity, but the intensities necessary to evoke responses in vivo were significantly high to suggest coactivation may be difficult to avoid. Pelvic and hypogastric nerves were not modeled here, but should be considered in future work. The inability to distinguish pudendal and autonomic activation in the proximal urethra is of concern for clinical studies investigating the ability to evoke bladder responses via urethral pudendal afferent fiber activation. In a previous study, intraurethral stimulation evoked contractions in persons with spinal cord injuries (Gustafson et al. 2004), but effective stimulation was 2-4 cm from the bladder neck over a broad range of intensities (2-20mA), so the role of activation of pudendal and autonomic nerve fibers in the observed response is unclear. The broad range of intensities may in part result from use of an electrode that is not snug with the urethral lumen, as the effect of catheter diameter on the maximum AF indicates that the presence of urine around the catheter electrode can significantly increase stimulation thresholds.

4.4.2 Electrode Geometry

The different electrode geometries generated different AFs, which suggests that stimulation thresholds would be different for the different electrode geometries (Figure 3D). The short electrode configuration exhibited the lowest stimulation thresholds (determined by the comparing the inverse of the AFs), followed by the ring electrodes (1 and 2 mm), while the bipolar ring electrodes (1 and 2 mm) required higher stimulation intensities to activate the pudendal afferent fibers. The short electrode was directed

dorsally towards the nerve branches, and in practice the orientation of the electrode may be difficult to maintain. Further, the results confirm that improper orientation significantly increases stimulation threshold. The ring electrode would be more consistent even though the thresholds might be slightly higher than the thresholds for the ideally oriented short electrode. Previous studies of intraurethral activation in the cat found no difference between stimulation thresholds for monopolar and bipolar stimulation. However, one study focused on stimulation in the proximal urethra (Gustafson et al. 2004), which our results predicted would not have different thresholds, while the second study compared thresholds in different animals (Bruns et al. 2009b), which are unlikely to be significantly different because of interanimal variability. Further, contact size and spacing between contacts differentially affect the ability to activate pudendal afferent fibers (e.g., increasing contact length decreases AFs but increasing contact spacing increases AFs) so comparison of electrodes of varying lengths and spacing is complicated. In vivo, the thresholds for evoking an EAS response were smaller for monopolar stimulation than bipolar stimulation, but the difference in threshold magnitude was less than that predicted by the model. Anatomical variability may confound this measure in vivo because the location of the stimulation target with respect to the electrodes contributes to threshold differences between monopolar or bipolar stimulation (Stark et al. 1962).

The selectivity between activation of the DNP and the CSN was dependent on electrode geometry. The narrow bipolar electrode had the greatest selectivity, but selectivity values for all electrodes tended to be high near the urethral meatus and the prostate. No electrode geometry exhibited selectivity >1 at 4 cm from the urethral

meatus, suggesting that stimulation in the penile bulb will produce co-activation of the CSN and DNP. In vivo investigations of the bladder response to intraurethral stimulation considered the effect of stimulation location but failed to address the potential for simultaneous excitation of the CSN and DNP (Chapter 3) (Bruns et al. 2009b). Variability in the bladder response to intraurethral stimulation 4 cm from the urethral meatus led this distance to be excluded from quantification in our previous study of intraurethral stimulation in the cat (Chapter 3) , while 4-6 cm was grouped together in another study of IES (Bruns et al. 2009b). Further, in addition to location, IES evokes bladder relaxation or contraction in the male cat dependent on the stimulation frequency (Chapter 3) (Bruns et al. 2009b). Bladder responses evoked by IES in the penile and membranous urethra are abolished by bilateral transection of the DNP and CSN, respectively (Chapter 3), indicating that IES at different intraurethral location is a means of minimally-invasive selective activation of different pudendal afferent branches. A better understanding of IES will enhance our ability to selectively target pudendal afferent branches in clinical investigations.

4.4.3 Functional Significance of Lateral Branches of the DNP

A potential benefit of intraurethral stimulation verses transcutaneous or percutaneous stimulation of the DNP would be selective activation of urethral as opposed to cutaneous DNP fibers. Our results suggest that the range of activation of the lateral branches of the DNP without activation of the DNP trunk varies with electrode geometry. The lateral DNP branches are observed to give off branches that dive towards the urethra (sparsely) (Yoo et al. 2008b), and these urethral offshoots were not modeled, making our estimate of the impact of electrode configuration on urethral activation even more

conservative. Based on these results, both clinical and experimental IES studies (which all utilize a ring electrode) are activating the DNP trunk, not the lateral branches. The 3-ring electrode could be further tested in vivo to determine if selective activation of the lateral DNP branches has any influence on the evoked bladder reflexes.

While intraurethral stimulation is an ideal means of activating urethral nerve fibers in the proximal urethra, percutaneous or transcutaneous stimulation of the DNP (or dorsal clitoral nerve) may be achievable at lower thresholds (Goldman et al. 2008). A previous experiment found that percutaneous, transcutaneous, and intraurethral stimulation (monopolar ring electrode) activated the DNP at 3-5 mA, 10-15 mA, and 15-25 mA, respectively (Yang et al. 1998c). Anatomical observations in humans and cats identified two populations of DNP axons (Chapter 2) (Yang et al. 1998b; Yoo et al. 2008b), those travelling laterally on the penile body to the urethra and those travelling down the penile midline to the glans. If these populations play different roles in the inhibitory and excitatory bladder response to DNP stimulation, use of a 3-ring electrode configuration may be valuable for selective activation of the urethral fiber mediated reflex pathway. DNP activation in humans evokes robust inhibition of the bladder (Wheeler et al. 1992; Kirkham et al. 2001; Horvath et al. 2009), but selective activation of the urethral afferent fibers of the DNP may be necessary to evoke robust excitatory bladder responses in humans. This model relied on the detailed description of the innervation of the cat urethra. A thorough description of the innervation of the human urethra is needed to determine the ideal settings (electrode geometry and location) for clinical investigation of the bladder response to IES evoked selective activation of pudendal afferent branches.

Table 4.1. Electrical properties of the cat urethra model

Tissue type	Specific structures	Conductivity	Source
	Urethral catheter	0.1 nS m^{-1}	(Wei et al. 2005), (Wongsarnpigoon et al. 2008)
Muscle tissue	Bulbospongiosus m., ischiocavernosus m., ischiourethralis m., inner urethral m., outer urethral m.	0.291 S m^{-1}	(Li et al. 2005)
Erectile tissue	Corpus cavernosum, corpus spongiosum	0.6 S m^{-1}	(Gandhi et al. 2001), (Li et al. 2005)
Glands	Bulbocavernosus g., prostate g.	0.4 S m^{-1}	(Gandhi et al. 2001), (Li et al. 2005)
Connective tissue, etc.	Prepuce, bounding box	0.05 S m^{-1}	(Suhel et al. 1997)
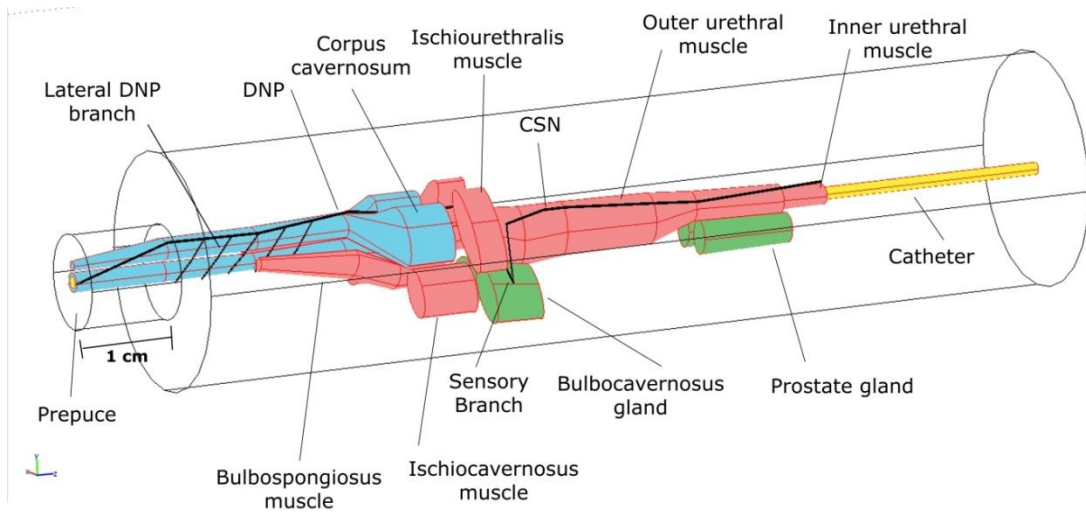
Figure 4.1. Three dimensional model of the male cat urethra and surrounding structures. The sensory branch of the pudendal nerve splits into proximal (CSN) and penile (DNP) branches, but the nerves locations shown were used for calculating the activating function and were not physically included in the finite element model. The model was encased in a cylinder and bounding box (not shown) to allow for finer element sizes around the model components and larger elements near the outer boundaries. Scale = 1 cm at prepuce.
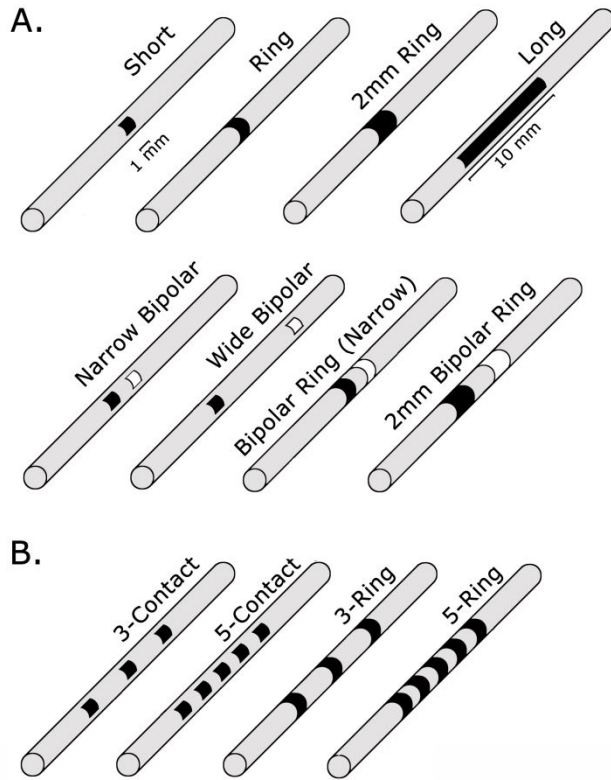
Figure 4.2. Intraurethral catheter electrode configurations. (A) Intraurethral electrical stimulation was simulated at 1-7 cm from the urethral meatus with eight different electrode configurations. (B) Four additional electrode configurations were simulated only at 2 cm from the urethral meatus for inclusion in the DNP branch analysis. In vivo stimulation was done with 1 mm ring, 2 mm ring, 3-ring, and 2 mm bipolar ring electrodes.
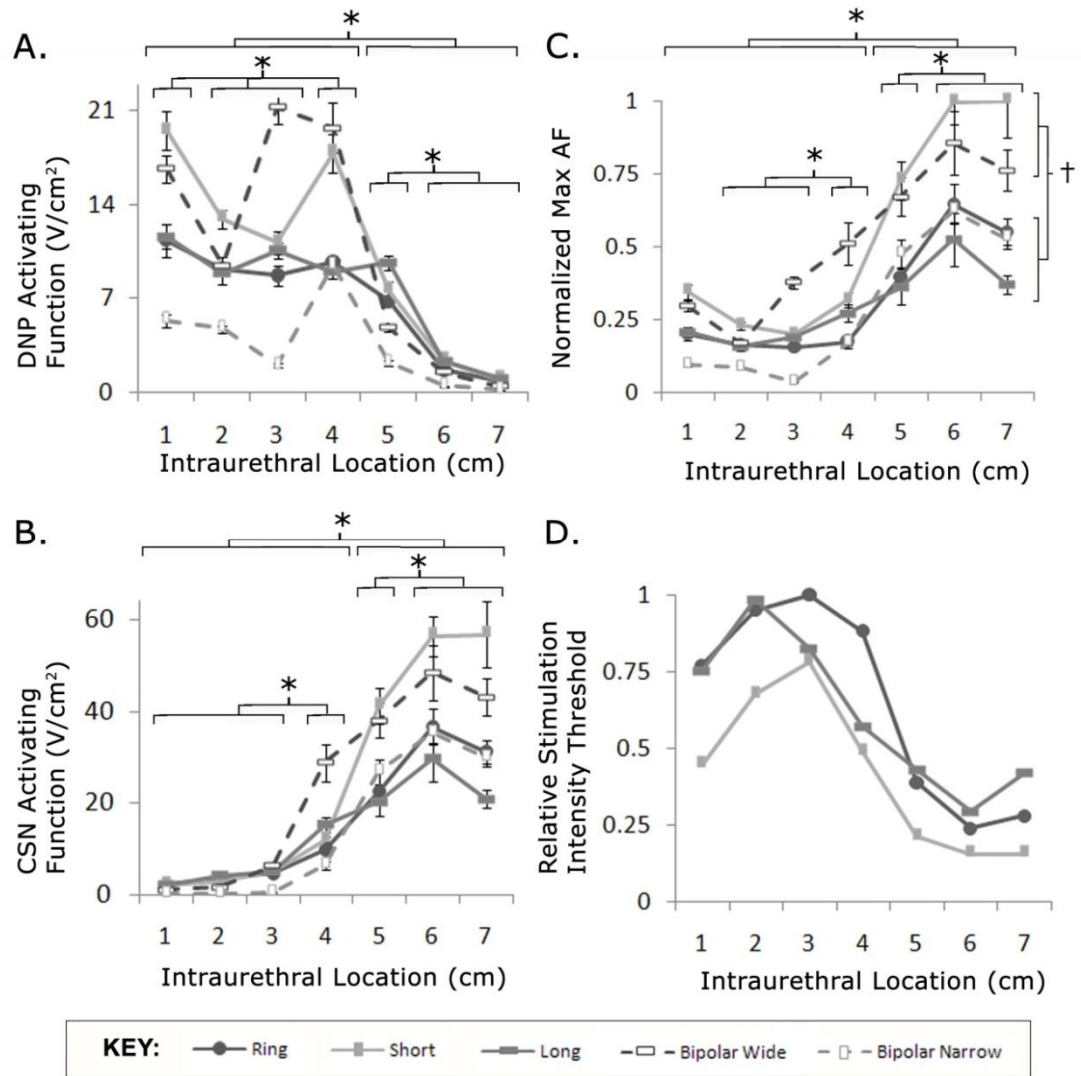
Figure 4.3

Figure 4.3. Maximum activating functions (AFs) evoked along the DNP and CSN by intraurethral stimulation. (A) The maximum AFs evoked along the DNP for intraurethral stimulation 1-7 cm from the urethral meatus. (B) Maximum AFs evoked along the CSN. (C) Maximum AFs evoked at each intraurethral stimulation location for combined DNP and CSN AFs, normalized by dividing by maximum AF over all locations and electrode configurations. (D) Relative stimulation threshold for short, ring, and long electrode configurations. Relative thresholds were determined from the relative values of the inverse of the maximum AFs. (A-C) Maximum AFs were dependent on stimulation location and electrode for the DNP AFs, CSN AFs, and combined DNP and CSN AFs (two-way ANOVA for each, $p < 0.0001$). (*) indicates significant difference between AFs evoked at different locations ($p < 0.05$, post-hoc comparison with Bonferroni correction). (C) (†) indicates significant different between AFs evoked with different electrode configurations ($p < 0.05$, Bonferroni post-hoc comparison).
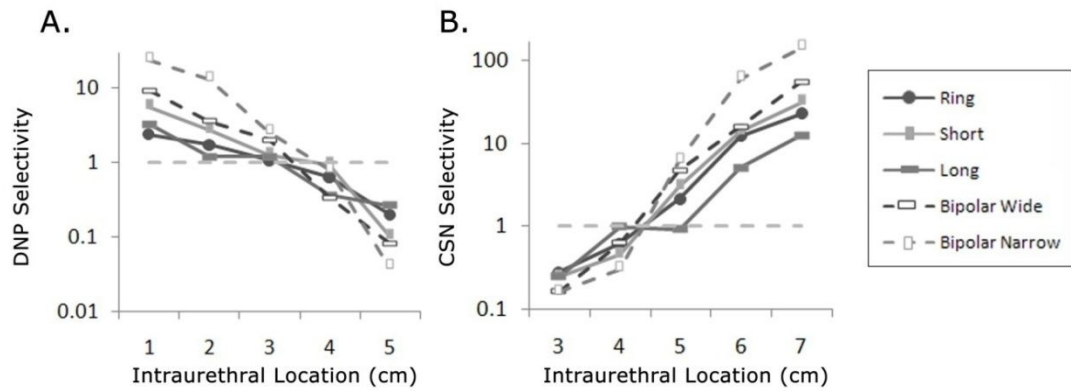
Figure 4.4. Pudendal sensory branch selectivity. (A) DNP selectivity (compared to CSN) as a function of electrode location for 1-5 cm from the urethral meatus. (C) CSN selectivity (compared to DNP) for 3-7 cm from the urethral meatus. (A,B) When selectivity was >1, AFRs were also >1.5.
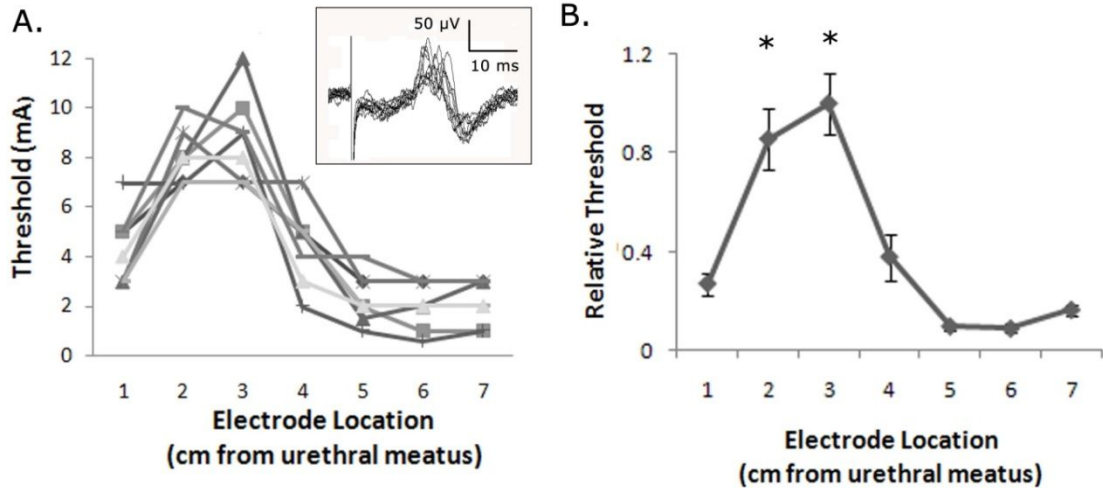
Figure 4.5. In vivo thresholds to evoke reflex EAS activation by intraurethral stimulation. (A) Stimulation intensity thresholds for evoking an EAS reflex response in 11 cats with 1 mm or 2 mm ring electrodes. Inset: Reflex EAS EMG responses evoked by 1 Hz, 6 mA IES at 1 cm from the urethral meatus. (B) Normalized intensity thresholds were dependent on stimulation location ($p < 0.001$, one-way ANOVA), and stimulation at 2 and 3 cm required significantly higher thresholds to evoke an EAS reflex response than stimulation at all other locations ($p < 0.05$, Bonferroni post-hoc comparisons).
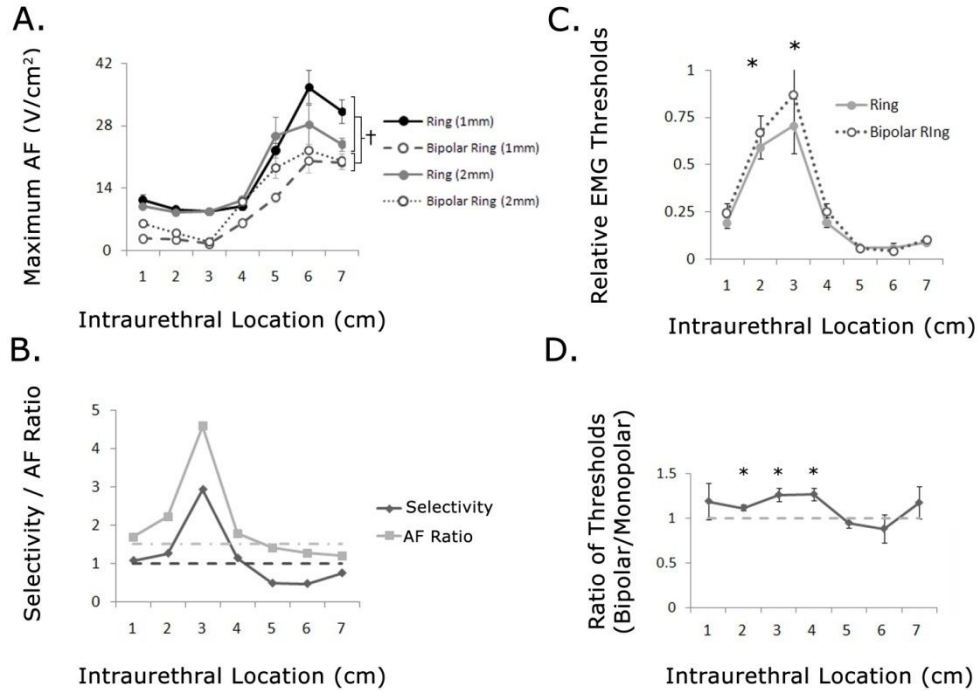
Figure 4.6. Simulation and in vivo comparison of monopolar and bipolar ring electrodes. (A) Maximum activating function (AF) along the DNP or CSN evoked by 1 mm and 2 mm monopolar and bipolar ring electrodes. The monopolar electrode configurations generated larger AFs than the bipolar configurations (†, $p < 0.05$, Bonferroni post-hoc comparison). (B) Selectivity and AF ratio for the 2 mm monopolar ring electrode compared to the 2 mm bipolar ring electrode. (C-D) In vivo stimulation intensity thresholds for evoking EAS EMG reflex responses with 2mm monopolar ring and 2mm bipolar ring electrode configurations. (C) Relative stimulation thresholds were dependent on stimulation location ($p < 0.001$, multi-way ANOVA) but not electrode configuration ($p = 0.26$). Stimulation at 2 and 3 cm required significantly higher thresholds to evoke an EAS reflex response than stimulation at all other locations (*$p < 0.05$, Bonferroni post-hoc comparison). (D) Averaged ratio of EAS threshold for bipolar and monopolar 2mm ring electrodes at different stimulation locations. The ratio of EAS reflex thresholds (bipolar/monopolar) was significantly greater than 1 at 2, 3, and 4 cm from the urethral meatus (*$p < 0.05$, paired one-sided t-tests).

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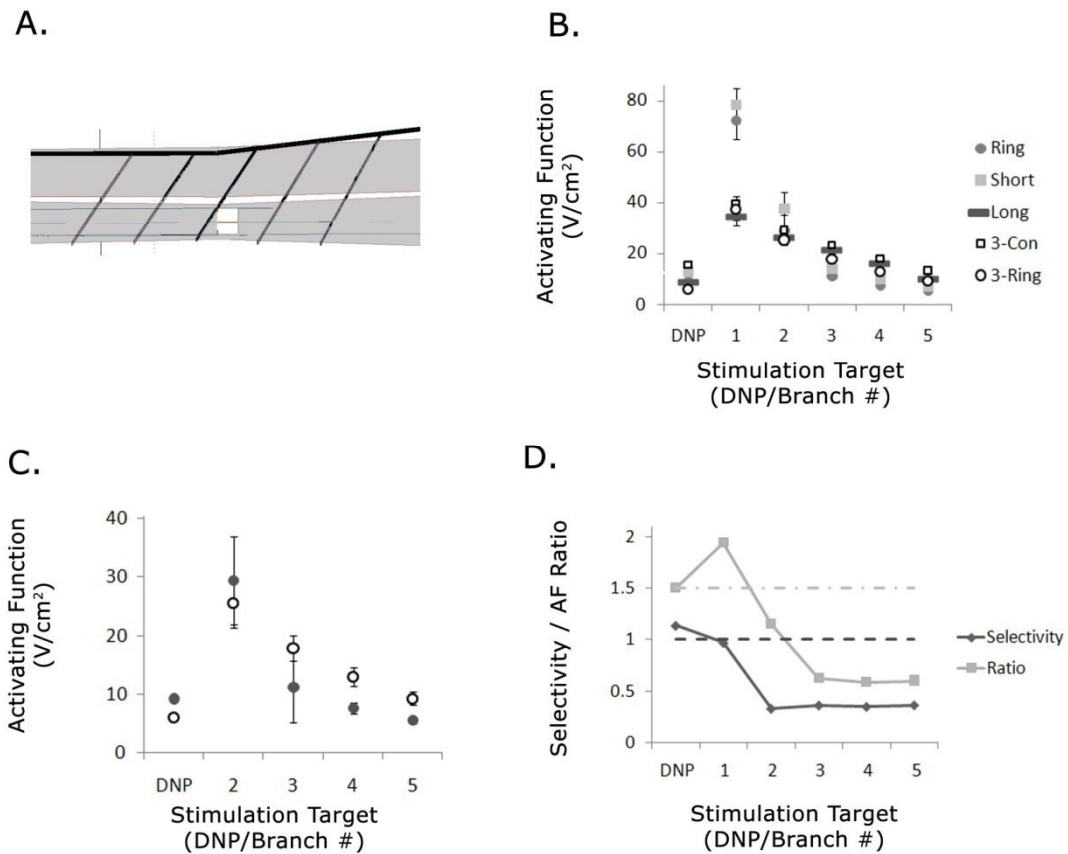
Figure 4.7. Simulation of the effects of electrode geometry on activation of the trunk and lateral branches of the DNP. (A) Lateral view of penile urethra from finite element model with simulated DNP branch locations (Figure 1). Each branch location was randomly varied between ±1 cm along the urethra from the locations shown. (B) Maximum AFs evoked at the DNP trunk and in the DNP branches by stimulation at 2 cm from the urethral meatus. (C) Maximum AFs for the 1 mm ring and 3-ring electrodes only. The maximum branch AF ("Branch 1") was omitted for easier comparison of the AFs evoked by the different electrodes. (D) Selectivity and AF ratio for the ring electrode compare to the 3-ring electrode.

Table 4.2. Number of DNP lateral branches activated before DNP trunk activation determined by statistical significance (t-test) or selectivity & AF ratio

Electrode	p<.001	Selectivity>1, AF Ratio>1.5
Ring	2	1
Short	2	2
Long	4	4
Bipolar Narrow	3	2
Bipolar Wide	3	2
3-Contact	2	2
5-Contact	4	4
3-Ring	4	4
5-Ring	4	4
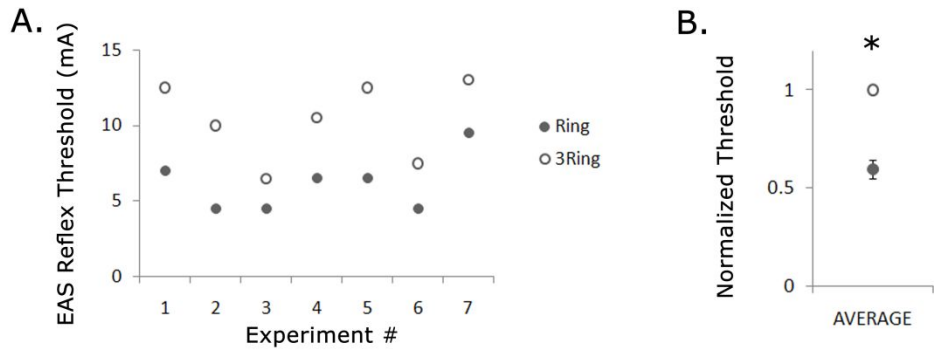
Figure 4.8. In vivo intensity thresholds for ring and 3-ring electrodes at 2cm from the urethral meatus. (A) The stimulation thresholds for evoking an EAS reflex for stimulation at 2cm from the urethral meatus were greater for stimulation with the 3-ring electrode than for stimulation with the ring electrode in 4 of 4 cats. (B) The normalized, average stimulation intensities reveal that stimulation with the ring electrode evoked an EAS reflex at significantly lower threshold than stimulation with the 3-ring electrode (*p<0.0005, paired one-sided t-test).

5. Efferent Pathway

5.1 Introduction

Spinal cord injury (SCI) and other neurological disorders can impair urinary function, leading to incontinence and/or urinary retention. Pudendal nerve stimulation is a potential means of restoring bladder control to persons living with SCI. Activation of pudendal afferent fibers by electrical stimulation evokes bladder excitation and voiding or bladder inhibition and continence dependent on the frequency of stimulation in both cats (Chapter 2) (Boggs et al. 2006a; Boggs et al. 2006b; Tai et al. 2008; Yoo et al. 2008a) and persons with SCI (Wheeler et al. 1992; Kirkham et al. 2001; Yoo et al. 2007b; Horvath et al. 2009). Although previous results suggest that bladder inhibition by pudendal afferent stimulation arises partially from activation of hypogastric efferent fibers and subsequent synaptic and ganglionic inhibition of parasympathetic efferent fibers (Lindström et al. 1983), the mechanisms of bladder activation by pudendal afferent stimulation are not known. The objective of this study was to determine the contribution of sympathetic activity to the reflex activation of the bladder evoked by high frequency stimulation of pudendal afferent fiber.

Activity in the sympathetic hypogastric nerve and parasympathetic pelvic nerve innervating the lower urinary tract coordinates urine storage and voiding (de Groat 1975). Parasympathetic nerve activity increases transiently at the onset of voiding to cause bladder contraction, while tonic sympathetic nerve activity inhibits bladder contraction and promotes urine storage. Sympathetic activity inhibits the bladder via α-adrenergic receptor mediated inhibition at the vesical ganglia and ß-adrenergic receptor mediated direct inhibition of the detrusor muscle. As well, sympathetic activity causes contraction

of the smooth muscles of the bladder neck and urethra via α-adrenergic receptors (de Groat 2006).

Previous data suggest that sympathetic mechanisms contribute to activation of the bladder by pudendal afferent stimulation. The bladder response to pudendal afferent stimulation depends strongly on the stimulation frequency; stimulation at 5-10 Hz inhibits the bladder and improves urine storage, while stimulation at 33-40 Hz excites the bladder and improves urine voiding (Chapter 2) (Boggs et al. 2006b; Tai et al. 2006). Similarly, the hypogastric (i.e., sympathetic) reflex response to pudendal afferent stimulation depends strongly on the stimulation frequency. Low frequency pudendal afferent stimulation evokes robust reflex activation of the hypogastric efferents, which are thought to mediate, in part, bladder inhibition evoked by pudendal afferent stimulation. However, reflex activation of hypogastric efferents decreases as the frequency of pudendal afferent stimulation increases, and stimulation suppresses ongoing intrinsic hypogastric activity (Lindström et al. 1983). The correlation between the stimulation frequency tuning of pudendal afferent evoked bladder activation and pudendal afferent evoked hypogastric efferent suppression suggests that reduction of the tonic inhibitory sympathetic bladder activity (i.e., inhibiting the inhibitor) is a potential mechanism underlying reflex bladder contraction evoked by pudendal afferent stimulation.

Alternatively, bladder contraction evoked by pudendal afferent stimulation may occur due to reflex activation of the parasympathetic bladder efferents (i.e., exciting the exciter). Pudendal afferent stimulation evokes bladder contraction only when the bladder volume is at or above a threshold volume (~70-80% of the volume at which distension

evoked reflex bladder contractions occur) (Chapter 2) (Boggs et al. 2005), and this volume dependence is mediated by a neural rather than a biomechanical mechanism (Boggs et al. 2005). Similarly, the magnitude of the pelvic efferent reflex response evoked by pudendal afferent stimulation increases with increased pelvic afferent activity (bladder pressure) (McMahon et al. 1982a), suggesting that converging pudendal and pelvic afferent activity could drive bladder activation. The similarity between the pelvic afferent (bladder volume) dependence of pudendal afferent evoked pelvic efferent activity and pudendal afferent evoked bladder contractions suggests that convergence of somatic (pudendal) and parasympathetic (pelvic) afferents may underlie pudendal afferent evoked contraction of the bladder.

Determining the role of sympathetic mechanisms in the bladder activation generated by pudendal afferent stimulation may enable development of pudendal nerve stimulation paradigms that optimize stimulation efficacy and provide a basis for establishing selection criteria for appropriate clinical subjects. The effect of selective sympathetic block (by the α-adrenergic antagonist phentolamine and the ß-adrenergic antagonist propranolol) and hypogastric nerve transection on bladder contractions evoked by stimulation of pudendal afferents in the dorsal nerve of the penis (DNP) was investigated in α-chloralose anesthetized male cats. The results suggest that the sympathetic bladder pathway is not critical to DNP stimulation evoked bladder excitation, but that sympathetic tone does play an important role in determining the volume thresholds for stimulation-evoked and distension-evoked bladder contractions.

5.2 Methods

Experiments were performed on 9 sexually intact, male cats weighing 3.3-4.5 kg. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at Duke University. Anesthesia was induced with ketamine HCl (35 mg/kg i.m.) and maintained with α-chloralose (65 mg/kg i.v. supplemented at 15 mg/kg as needed). The end tidal CO_2 was maintained between 3.0 and 4.0 % with artificial respiration. Core body temperature was maintained at ~38ºC with a thermostatic heating pad and blood pressure was monitored through a catheter in the carotid artery. IV fluids (lactated Ringer's solution or saline/5% dextrose/sodium bicarbonate solution) were administered at 15 cc/kg/h through the cephalic vein. The bladder was accessed through a midline abdominal incision. In 6 cats, a 3.5 Fr catheter was inserted into the bladder dome and secured with a purse-string suture, and the catheter was connected to a solid-state pressure transducer (Deltran, Utah Medical) to record the bladder pressure. The catheter was also connected to a syringe for infusion of room temperature saline into the bladder. The abdominal incision was closed in layers. In all cats, a 3.5 or 5 Fr catheter was inserted into the urethra via the urethral meatus to occlude the urethra and prevent bladder voiding. The urethral catheter was connected to a syringe and used for instilling saline into the bladder in the 3 cats without suprapubic catheters. Whether filling was performed through the suprapubic or urethral catheter did not appear to impact the results.

5.2.1 Nerve access and stimulation

An incision was made from the skin around the prepuce to the caudal border of the gracilis muscle. The DNP was dissected free (unilaterally) from the body of the penis

at the proximal end of the penile body, just distal to the bulb of the penis. A monopolar cuff electrode consisting of a platinum contact embedded in a silicone elastomer cuff was placed around the nerve. A subcutaneous needle was inserted in the ipsilateral leg for the return electrode. Stimulation consisted of trains of constant current ($50 - 750$ µA) 100 µs stimulation pulses.

The hypogastric nerves were accessed via a midline abdominal incision and, where indicated, were transected distal to the inferior mesenteric ganglia. The hypogastric nerves were exposed prior to control filling trials to prevent colon manipulation during nerve exposure from affecting the comparison of control and nerve transection trials.

5.2.3 Drug Administration

Drugs were administered intravenously via a catheter in the cephalic vein. Propranolol HCl (Bedford Laboratories), a ß-adrenergic blocking agent, and phentolamine HCl (Sigma-Aldrich), an α-adrenergic blocking agent, were administered at 1 mg/kg and 2 mg/kg respectively (Edvardsen 1968b; Edvardsen 1968a; Koley et al. 1984; Poirier et al. 1988; Danuser et al. 1995). Heart rate and arterial blood pressure were monitored to establish the onset of drug effects. Adrenergic agonists phenylephrine HCl (α-agonist, Parenta Pharmaceuticals) and isoproterenol HCl (ß-agonist, Sigma-Aldrich) were administered intravenously (at 30 µg/kg and 50 µg/kg, respectively) before and after administration of phentolamine and propranolol, respectively, to confirm the effects of the antagonists. Hexamethonium bromide (Sigma-Aldrich) was administered intravenously at 1 mg/kg in 2 cats to investigate the effect of ganglionic block on the bladder contractions evoked by DNP stimulation.

5.2.4 Determining Volume Thresholds

DNP stimulation was delivered intermittently during bladder filling to determine the volume thresholds for stimulation evoked bladder contractions and distension evoked reflex bladder contractions. Starting with an empty bladder, the bladder volume was increased by infusion of a 1 ml bolus of room temperature saline every 1 minute through the urethral or suprapubic catheter. Stimulation at 33 Hz for 20 seconds was applied 20 seconds after injecting each bolus. Stimulation intensity was fixed at two times the threshold for evoking reflex EMG responses in the external anal sphincter (EAS). Trials were ended several boluses after the appearance of robust distension evoked reflex contractions. At least 2 control filling trials preceded trials following drug administration. Volume threshold experiments were initiated 15-20 minutes following drug administration and were not continued beyond 90 minutes following drug administration. At least two hours elapsed following drug administration before establishing new controls for subsequent drug administration or nerve transection. At least 15 minutes with the bladder empty elapsed between consecutive filling trials. Stimulation evoked bladder inhibition was investigated at the end of filling trials in 4 animals by stimulating during distension evoked contractions at 10 Hz with an amplitude equal to 3 times the threshold for evoking reflex EMG in the EAS.

5.2.5 Data Analysis

The bladder response to electrical stimulation was analyzed by comparing the bladder pressure before and during DNP stimulation. The bladder pressure for 3 seconds prior to stimulation onset was defined as the baseline pressure, and a stimulation evoked contraction was determined to have occurred if the bladder pressure increased > 10

cmH$_2$O during the first 8 seconds of stimulation and was maintained until the end of stimulation. Distension evoked reflex contractions were defined as transient rises in bladder pressure (> 10 cmH$_2$O compared to the pre-bolus bladder pressure) occurring between 5 and 20 seconds after injecting a 1 cc bolus. Bladder inhibition was defined as a > 10 cmH$_2$O decrease in bladder pressure within the first 8 seconds of stimulation. The mean and maximum bladder contraction magnitudes were determined for the contraction evoked by stimulation at volumes 1 ml less than the distension evoked contraction volume threshold. Mean stimulation evoked contraction magnitudes were determined by averaging the bladder pressure from the onset of contraction (> 10 cmH$_2$O increase in pressure) until the termination of stimulation. The maximum contraction magnitude was defined as the maximum bladder pressure during stimulation. Distension evoked contraction magnitudes were quantified at volumes 2 ml above the distension evoked threshold volume by calculating the average bladder pressure for the 15 seconds prior to the onset of DNP stimulation.

Experimental trials were grouped with the control trials that preceded those experimental trials, and the volume thresholds were normalized by dividing the threshold volumes by the average of the control distension evoked threshold volumes. The normalized values were compared for statistically significant differences using ANOVA with post-hoc paired comparisons using Bonferroni correction. Contraction magnitudes (mean and maximum) were normalized for each set of control and experimental trials by dividing each magnitude by the average of the control magnitudes. Normalized contraction magnitudes were compared using an unpaired, two-tailed t-test.

Bladder inhibition was quantified by taking the ratio of the average bladder pressure for the final 10 seconds of 10 Hz DNP stimulation and the average pressure for 3 seconds prior to the onset of the distension evoked contraction. Ratios before and after sympathetic block were compared using an unpaired, two-tailed t-test.

All reported values are mean ± standard deviation.

5.3 Results

5.3.1 Volume thresholds for distension-evoked and stimulation-evoked contractions

High-frequency (33 Hz) DNP stimulation evoked bladder contractions in 9 of 9 cats. The bladder volume thresholds for distension evoked reflex contractions (DTV) and DNP stimulation evoked contractions (STV) were determined during bladder filling in 1 ml increments (Figure 5.1A). Stimulation evoked bladder contractions during control filling (in the absence of sympathetic block) were evoked at STVs (mean: 18 ± 9 ml, range: 4-34 ml, n=42 trials) that were 73 ± 12 % of the DTVs (mean ± s.d.: 24 ± 12, range: 7-44 ml). Mean ($30 ± 10$ cmH_2O, n=42 contractions) and maximum ($58 ± 15$ cmH_2O) stimulation evoked contraction magnitudes were determined at bladder volumes 1 ml less than DTV, and mean ($10 ± 5$ cmH_2O) and maximum distension ($42 ± 30$ cmH_2O) evoked bladder contraction magnitudes were determined at bladder volumes 2 ml more than DTV.

5.3.2 Blockade of ß-adrenergic receptors decreased volume thresholds

Bladder filling was performed following administration of the ß-adrenergic antagonist propranolol (1 mg/kg) in 6 cats. Bladder contractions were still evoked by

DNP stimulation and distension following propranolol administration (Figure 5.2A), but volume thresholds were significantly reduced (Figure 5.2B). After propranolol administration, stimulation evoked bladder contractions were evoked at lower volumes (STV: 10 ± 6 ml, range: 2-21 ml, n=19 trials) than under control conditions (STV: 16 ± 11 ml, range: 4-28 ml, n=16 trials), and propranolol similarly decreased threshold volumes for distension evoked contractions (DTV: 18 ± 13 ml, range: 6-41 ml) compared to control (DTV: 22 ± 15 ml, range: 7-47 ml). Thus, after propranolol administration, stimulation evoked bladder contractions were still evoked at volumes less than the threshold for distension evoked reflex contractions (60 ± 18 % of DTV, compared to 67 ± 13 % under control conditions). Stimulation evoked contraction magnitudes after propranolol (mean: 32 ± 12 cmH$_2$O, maximum: 63 ± 19 cmH$_2$O) were similar to those evoked in control conditions (mean: 31 ± 13 cmH$_2$O, maximum: 58 ± 23 cmH$_2$O) (Figure 5.2C). Similarly, distension evoked contraction magnitudes were not affected by propranolol administration (Figure 5.2C). Propranolol administration blocked the transient increase in heart rate evoked by isoproterenol administration, confirming the effectiveness of the drug and dose (n= 3 cats).

5.3.3 Blockade of α-adrenergic receptors increased volume thresholds and decreased contraction magnitudes

Following administration of the α-antagonist phentolamine (2 mg/kg), bladder contractions were still evoked by DNP stimulation and distension in 6 of 6 cats (Figure 5.3A), but bladder volume thresholds were significantly increased (Figure 5.3B). After phentolamine, STVs (mean: 27 ± 5 ml, range: 18-34 ml, n= 13 trials) and DTVs (mean: 33 ± 7 ml, range: 22-45 ml) were increased compared to controls (STVs: 22 ± 5 ml, range:

14-30 ml, n=15 trials; DTVs: 29 ± 7 ml, range: 16-38 ml). DNP stimulation still evoked

bladder contractions at volumes below the DTV (STVs were 82 ± 9 % of DTVs after

phentolamine compared to 79 ± 11 % under control conditions). However, stimulation

evoked contraction magnitudes were smaller after phentolamine (mean: 21 ± 10 cmH_2O,

maximum: 45 ± 27 cmH_2O) compared to control contraction magnitudes (mean: 41 ± 22

cmH_2O, maximum: 54 ± 24 cmH_2O) (Figure 5.3C). The distension evoked contraction

magnitudes following phentolamine were also smaller than under control conditions, but

the difference was not significant (Figure 5.3C). The effectiveness of phentolamine was

verified by its ability to block the increase in arterial pressure induced by phenylephrine

administration (n=3 cats).

5.3.4 Hypogastric nerve transection decreased volume thresholds

Bladder contractions were still evoked by DNP stimulation and distension

following bilateral transection of the hypogastric nerve in 5 of 5 cats (Figure 5.4A), but

threshold bladder volumes were decreased (Figure 5.1B, Figure 5.4B). Hypogastric

nerve transection caused a decrease in STVs (control: 16 ± 10 ml. n=11 trials;

transection: 10 ± 7 ml, n=14 trials) and DTVs (control: 22 ± 12 ml, transection: 14 ± 9

ml), but STVs remained lower (73 ± 11 %) than DTVs after hypogastric transection,

comparable with the relative volume thresholds under control conditions (73 ± 14 %).

Bilateral hypogastric transection did not cause significant changes in the mean (30 ± 17

cmH_2O) or maximum (61 ± 29 cmH_2O) magnitude of stimulation evoked bladder

contractions compared to control contraction magnitudes (mean: 30 ± 12 cmH_2O,

maximum: 58 ± 19 cmH_2O) (Figure 5.4C). The bladder volume thresholds and

stimulation evoked contraction magnitudes observed following hypogastric transection

were not affected by subsequent administration of both propranolol and phentolamine (4

cats) (Figure 5.4B,C).   Distension evoked contraction magnitudes were also not affected

by hypogastric nerve transection or subsequent drug administration (Figure 5.4C).

In 2 of 2 cats, administration of hexamethonium bromide (1 mg/kg) following

bilateral hypogastric nerve transection abolished the bladder contractions evoked by DNP

stimulation (Figure 5.5). The bladder contractions evoked by stimulation returned over

time as the ganglionic block diminished.

5.3.5 Stimulation evoked contraction magnitudes increase with increasing bladder volume

In 3 cats, the mean and maximum contraction magnitudes evoked at increasing

bladder volumes were normalized and scaled to determine the relationship between

contraction magnitude and bladder volume (Figure 5.6).   Regression analysis revealed a

strong, positive correlation between mean or maximum contraction magnitude and

bladder volume. The strong, positive correlation between bladder volume and

contraction magnitude remained following bilateral hypogastric nerve transection.

5.3.6 Bladder inhibition persisted following bilateral hypogastric nerve transection

DNP stimulation at 10 Hz inhibited distension evoked reflex bladder contractions

in 4 of 4 cats. Following hypogastric transection (4 cats) and hypogastric transection plus

administration of phentolamine and propranolol (3 cats), 10 Hz DNP stimulation

continued to evoke bladder inhibition (Figure 5.7A). The reductions in bladder pressure

(relative to baseline) evoked by 10 Hz DNP stimulation following bilateral hypogastric

nerve transection were identical to the reductions evoked under control conditions

(Figure 5.7B).

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5.4 Discussion

The role of sympathetic bladder innervation in the bladder reflexes evoked by DNP stimulation was investigated using a combination of selective pharmacological sympathetic block and sympathetic block via hypogastric nerve transection. The results illustrate that the sympathetic bladder innervation does not play a significant role in the excitatory DNP-bladder reflex, but confirm that the sympathetic bladder innervation plays an important role in determining the micturition volume threshold. These results suggest inhibition of sympathetic bladder inhibition (i.e., inhibiting the inhibitor) is not responsible for bladder contractions evoked by DNP stimulation, and imply that superposition of pelvic afferent and pudendal afferent inputs (driving pelvic efferent activity) is responsible for the excitatory bladder response to DNP stimulation. This is supported by the lower volume threshold for stimulation evoked bladder contractions compared to distension evoked bladder contractions, the block of the DNP-bladder excitatory responses following hexamethonium bromide administration, and the increase in stimulation evoked bladder contraction magnitudes with increasing bladder volume (i.e., increased pelvic afferent activity). These findings suggest that DNP stimulation evokes bladder contractions by increasing pelvic efferent activity (i.e., exciting the exciter).

5.4.1 Limitations

While complete block of adrenergic bladder innervation cannot be guaranteed by administration of adrenergic antagonists, phentolamine and propranolol were administered at doses previously found to be maximally effective on bladder and urethral

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function (Edvardsen 1968b; Edvardsen 1968a; Koley et al. 1984; Poirier et al. 1988; Danuser et al. 1995). Additionally, while the adrenergic antagonists were administered to determine the effect of blocking adrenergic bladder innervation, alternate effects of the drugs may have altered the bladder activity. For example, adrenergic antagonists block sympathetic inhibition of the colon in the cat (Hallerback et al. 1987), and changes in intracolonic pressure can modulate bladder parasympathetic activity (McMahon et al. 1982a).

Bilateral hypogastric nerve transection was also used as an alternate, non-selective means of blocking the sympathetic bladder innervation, and results were similar under surgical and pharmacological block. However, the pelvic nerve also contains a significant sympathetic component (Kuo et al. 1984). While the sympathetic component of the pelvic nerve may be primarily vasomotor (Hamberger et al. 1965), the administration of the adrenergic antagonist subsequent to hypogastric nerve transection ensured substantial, if not virtually complete, block of the sympathetic pathways to the bladder. Even under such conditions, bladder contractions were evoked by pudendal afferent stimulation and distension, albeit at smaller bladder volume thresholds.

5.4.2 ß-adrenergic block

Sympathetic block illustrated the importance of the sympathetic bladder innervation in determining the micturition volume threshold. Blocking the ß-adrenergic bladder pathway resulted in decreased volume thresholds but no change in contraction magnitudes. The reduction in distension evoked volume threshold has been observed previously in the dog (Nishizawa et al. 1986) and the cat (Edvardsen 1968a). However, in the rat propranolol did not alter the micturition threshold (Maggi et al. 1985),

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suggesting the effect of the ß-adrenergic block may differ across species. The results suggest that ß-adrenergic mediated bladder inhibition does not play an important role in the excitatory response to high frequency DNP stimulation but plays a major role in determining the micturition volume threshold.

5.4.3 α-adrenergic block

Blocking the α-adrenergic pathway increased the STVs by ~20 %. Greater increases in the micturition volume threshold following α-adrenergic block were reported in the decerebrate dog (Nishizawa et al. 1986) and conscious rat (Ukimura 1993), while in previous cat studies α-adrenergic block was reported to decrease the micturition volume threshold (Edvardsen 1968a; Espey et al. 1992). However, these studies were performed in pentobarbital anesthetized (Edvardsen 1968a), decerebrate (Edvardsen 1968a), or conscious cats (Espey et al. 1992), while the current studies were conducted under α-chloralose anesthesia. In the cat, α-chloralose affects spinal reflexes (Shimamura et al. 1968) and bladder behavior (Rudy et al. 1991). Additionally, anesthesia with α-chloralose has been observed to reduce spinal reflexes compared to pentobarbital anesthesia (Duggan et al. 1980), suggesting the anesthetic effects may partially explain the conflicting results. Although it is unclear how α-chloralose may affect the DNP-stimulation evoked bladder reflexes, similar reflexes are evoked in conscious, chronic SCI cats (Tai et al. 2008), demonstrating that α-chloralose anesthesia is not critical to evoking these reflexes.

While α-block reduced contraction magnitude, robust contractions were still evoked by pudendal afferent stimulation, suggesting that the α-adrenergic pathway played a non-critical role in the stimulation evoked response. The α-adrenergic

innervation of the vesical ganglia is typically described as inhibitory, but there are two distinct actions of α-adrenergic receptors: α_1-receptor mediated facilitation of ganglionic transmission and α_2-receptor mediated inhibition of ganglionic transmission (Keast et al. 1990; Andersson 1999; de Groat et al. 1999). Additionally, activation of the bladder neck and proximal urethra is mediated by α_1-receptors. In the α-chloralose anesthetized cat (Yoshimura et al. 1990) and urethane anesthetized rat (Yoshiyama et al. 2001), selective α_1-receptor block had an inhibitory effect on distension evoked bladder contractions, suggesting the α-adrenergic effects on contraction magnitude observed in this study may be due to reduced bladder excitability and not block of stimulation evoked sympathetic reflexes. However, phentolamine also decreases bladder neck pressure and suppresses the increase in bladder neck pressure evoked by DNP stimulation (Reitz et al. 2003). These finding suggests two possible explanations for the increase in volume thresholds and decrease in contraction magnitude: loss of facilitation at the vesical ganglia or loss of activation of the bladder neck. However, α_2-block (by the antagonist atipamezole) decreases bladder excitability (increased bladder capacity, residual urine) in conscious rats (Ishizuka et al. 1996), suggesting α_2-receptor block may have contributed to the increase in micturition threshold and decrease in stimulation evoked contraction magnitudes. The decrease in distension evoked contraction magnitudes following phentolamine, while not significant, is consistent with phentolamine decreasing bladder excitability and not specifically impairing the DNP-bladder reflex. Regardless of the mechanism of the α-block effects, the effects of α-block were not evident following non-specific sympathetic block (hypogastric transection with subsequent drug administration),

providing further evidence that high frequency DNP stimulation-evoked modulation of α-adrenergic bladder activity does not contribute significantly to bladder excitation.

5.4.4 Hypogastric transection

Hypogastric nerve transection decreased the DTV, consistent with previous findings (Maggi et al. 1985; Nishizawa et al. 1986; Yoshiyama et al. 2002), and also decreased the STV. The lack of effect of subsequent administration of adrenergic antagonists on the volume thresholds suggests hypogastric transection provided functionally complete block of the sympathetic bladder innervation and eliminates the possibility that sympathetic fibers in the pelvic nerve play a significant role in the excitatory DNP-bladder pathway. Additionally, the decrease in volume thresholds is consistent with the volume threshold changes induced by ß-block but contrary to the changes due to α-block, suggesting that the ß-adrenergic pathway has greater influence over the micturition threshold than the α-adrenergic pathway. As well, the ability to evoke the excitatory DNP-bladder reflex following hypogastric transection also rules out any role of non-adrenergic hypogastric bladder pathways (Creed 1979) in the excitatory DNP-bladder reflex.

Low frequency DNP stimulation inhibited the urinary bladder as previously reported (Chapter 2) (Tai et al. 2008). Inhibition of distension evoked contractions still occurred after hypogastric transection and subsequent adrenergic antagonist administration, and the inhibition was comparable to inhibition evoked prior to hypogastric transection. Previously it was reported that hypogastric transection reduced bladder inhibition evoked by pudendal nerve stimulation, but inhibition comparable to control conditions was evoked when the stimulation intensity was increased (Tai et al.

2006). These results suggest that the significant reflex pathways for bladder contraction and inhibition evoked by DNP stimulation are located in the sacral spinal cord, and they do not engage substantially adrenergic mechanisms.

5.4.5 The Genitovesical Reflex

While previous results suggest a role of the sympathetic and parasympathetic efferent bladder pathways in the inhibitory genitovesical reflex (Lindström et al. 1983; Tai et al. 2006), our finding suggest that convergence of somatic (pudendal) and parasympathetic (pelvic) inputs is a much more significant pathway for stimulation evoked excitation and inhibition. Additionally, while sympathetic block alters the volume thresholds it does not abolish the ability to evoke bladder contractions at volume thresholds lower than those for distension evoked responses. This suggests that the excitatory genitovesical reflex results from converging spinal inputs from genital and pelvic afferents, providing increased excitatory input to pathways that activate preganglionic parasympathetic bladder fibers. Pelvic and pudendal afferents converge on interneurons in the sacral spinal cord (Honda 1985; Coonan et al. 1999), providing a substrate for the excitatory genitovesical reflex. Additionally, the relationship observed for contraction magnitude and bladder volume supports convergence of pudendal and pelvic afferents as the source of the excitatory DNP-bladder reflex. The pudendal-bladder reflex is not limited by the length-tension properties of the bladder, but rather by a neural mechanism (Boggs et al. 2005). The positive correlation between contraction magnitude and bladder volume provides evidence that increasing the level of pelvic afferent input drives increased pelvic efferent activity, implying convergence of the pudendal afferent and pelvic afferent fibers in the sacral spinal cord.

The role of parasympathetic pelvic nerve activity in the excitatory DNP-bladder reflex is further supported by the finding that the reflex is abolished by the nicotinic ganglionic blocker hexamethonium bromide. This illustrates that pelvic afferent activity in the absence of pelvic efferent bladder activity is not a sufficient substrate for the excitatory DNP-bladder reflex, suggesting the pelvic efferent pathway is critical. Additionally, the frequency dependence of the DNP-bladder reflex (Chapter 2) suggests the presence of a complex sacral spinal mechanism. Stimulation of pudendal afferents has been observed to evoke reflex discharges in pelvic efferent fibers when delivered on a pedestal of pelvic afferent activity (McMahon et al. 1982a). However, 33 Hz DNP stimulation does not evoke excitation simply by evoking this pudendal-pelvic reflex as evidenced by the inhibitory effect of 10 Hz DNP stimulation. If a simple pudendal-pelvic reflex were involved, 10 Hz stimulation following sympathectomy would not inhibit pelvic efferent activity.

Computer simulation of the convergence of pudendal and pelvic afferents in the sacral spinal cord revealed that inhibitory and excitatory post-synaptic potentials generated in sacral interneurons by pelvic and high frequency (33 Hz) pudendal afferent activation can drive increased pelvic efferent activity while superposition of pelvic afferent activity and low frequency (10 Hz) pudendal afferent activation can inhibit pelvic efferent activity (Appendix C). The simulations also suggested a mechanism for the dependence on adequate levels of background pelvic afferent activity (i.e., the bladder volume dependence).

5.4.6 Clinical Implications

The finding that excitatory DNP-bladder reflexes can be evoked following sympathetic block provides insight into selection of appropriate clinical candidates. Injuries to the lumbar spinal cord that may affect the sympathetic bladder pathways should not affect the ability to evoke the DNP-bladder reflex. An intact sacral spinal cord and intact pelvic and pudendal nerves are necessary to evoke the DNP-bladder reflex, so candidates for clinical investigation of the DNP-bladder reflexes should have suprasacral SCIs.

Utilizing stimulation paradigms that optimally activate the pelvic efferent bladder pathway may be critical to evoke effective bladder voiding clinically. Variable patterned stimulation paradigms (e.g., 2 pulses @ 200 Hz repeated at 33 Hz) were found to increase the magnitude of pudendal nerve stimulation evoked bladder contractions compared to continuous 33 Hz stimulation in the cat (Bruns et al. 2008), but it is unclear if variable patterned DNP stimulation can evoke greater bladder voiding efficiency. Biomimetic stimulation (stimulation that mimics the temporal and spatial patterns of the pudendal afferent fiber response to urethral flow or genital stimulation) may be another means of evoking optimal pelvic efferent activity.

5.4.7 Conclusion

The sympathetic bladder pathway does not play a significant role in the excitatory DNP-bladder reflex, but sympathetic bladder innervation is important in determining the micturition threshold volume. This implies that the excitatory DNP-bladder reflex is driven by increased activation of parasympathetic fibers due to convergence of pelvic afferent and pudendal afferent fibers in the sacral spinal cord.

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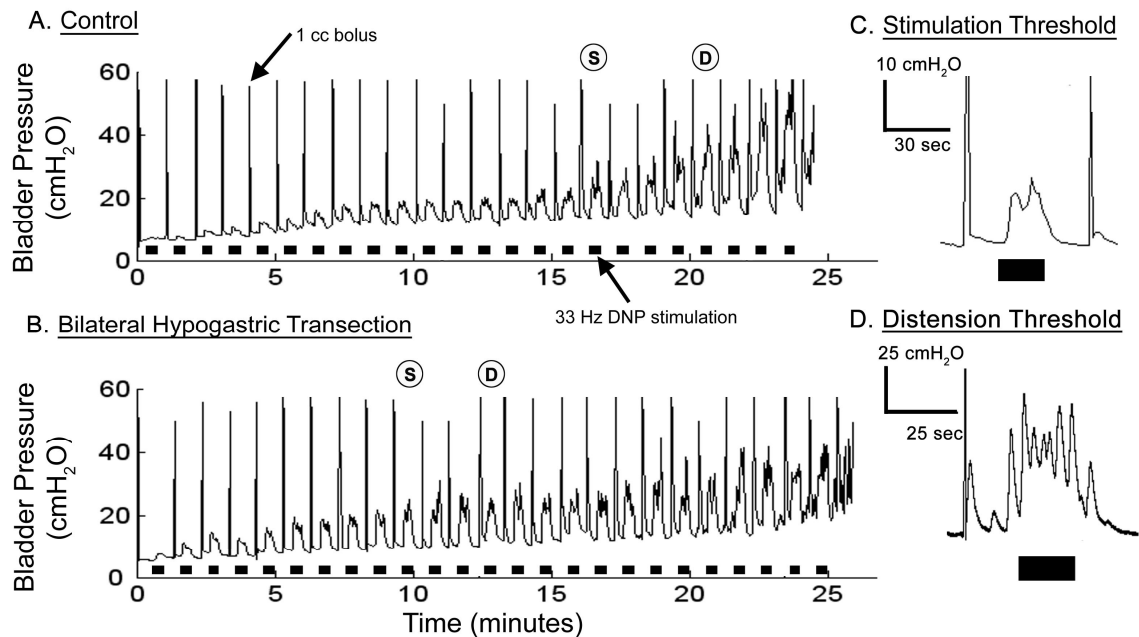
Figure 5.1. Stimulation-evoked and distension-evoked reflex bladder contraction volume thresholds were determined under control and sympathetic block conditions. (A) Control filling trial consisting of a 1 cc bolus of saline every minute with 20 seconds of 33 Hz stimulation of pudendal afferents in the dorsal nerve of the penis (DNP) delivered between boluses. The stimulation evoked contraction volume threshold (Ⓢ) was 17 cc and the distension evoked threshold (Ⓓ) was 21 cc. (B) A filling trial after bilateral hypogastric nerve transection. The stimulation evoked contraction volume thresholds decreased to 10 cc and the distension evoked threshold decreased to 13 cc. (C) An example bladder pressure trace at the stimulation evoked volume threshold. (D) An example bladder pressure trace at the distension evoked volume threshold. (A-D) Black bars under the pressure trace indicate DNP stimulation at 33 Hz and 2x the intensity threshold for evoking a reflex response in the external anal sphincter.
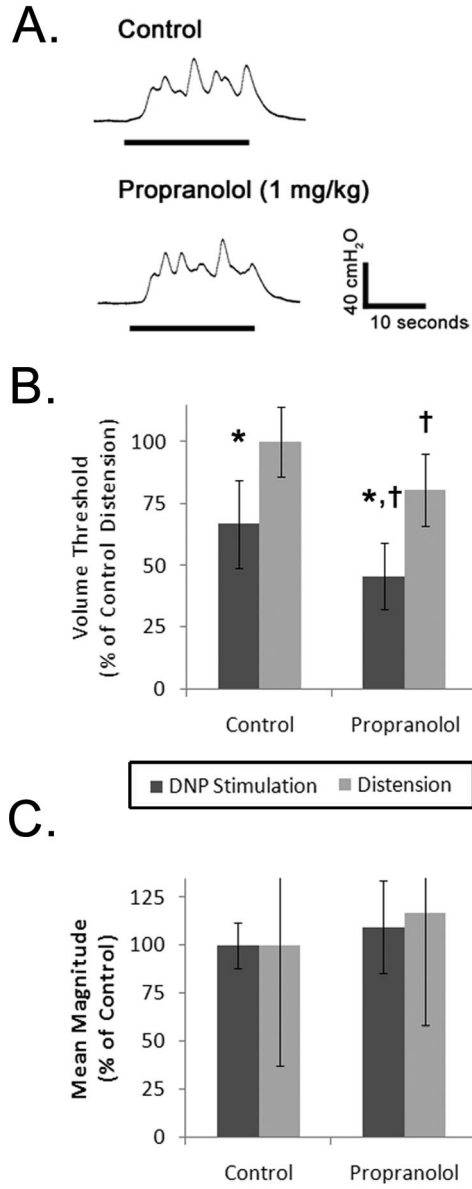
Figure 5.2

Figure 5.2. Effect of propranolol administration on bladder contractions evoked by DNP stimulation or distension. (A) Example bladder responses evoked by 33 Hz DNP stimulation before and after propranolol administration (1 mg/kg). The contractions were evoked during bladder filling trials at bladder volumes 1 cc less than the distension evoked threshold volumes. (B) Normalized volume thresholds before and after propranolol administrations were significantly different. ($p<10^{-14}$, ANOVA, n=35 trials). *, significant difference between stimulation evoked and distension evoked volume thresholds for control trials or for propranolol trials ($p<0.05$, post-hoc Bonferroni comparisons). †, significant difference between control and propranolol stimulation volume thresholds or for distension volume thresholds ($p<0.05$, post-hoc Bonferroni comparisons). (C) Normalized mean contraction magnitudes evoked by stimulation before and after propranolol were not different ($p= 0.16$, t-test, n=35 contractions). Also, distension evoked contraction magnitudes increased slightly following propranolol, but the difference compared to control distension evoked contractions was not significant ($p=0.28$).
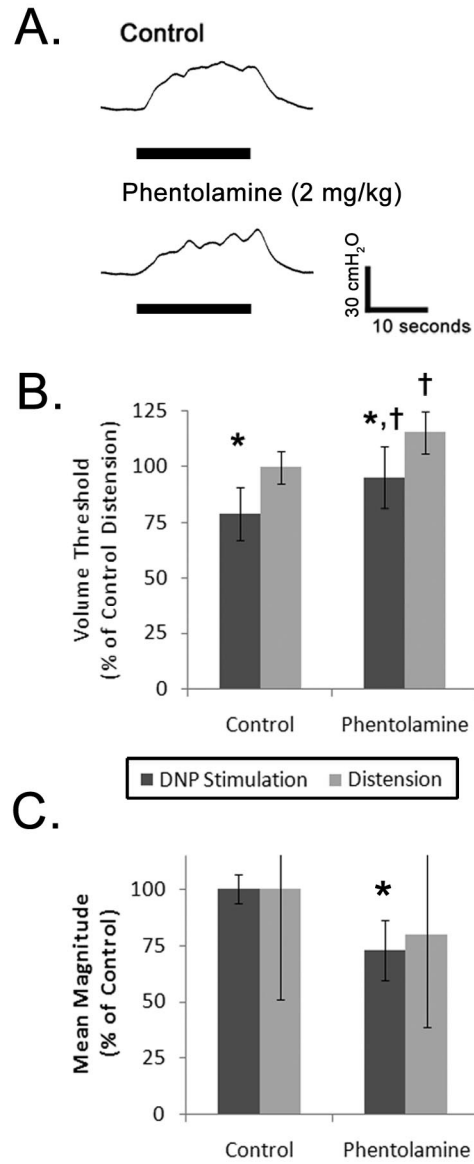
Figure 5.3

Figure 5.3. Effect of phentolamine administration on bladder contractions evoked by DNP stimulation or distension. (A) Contractions evoked at bladder volumes 1 cc less than the distension evoked threshold volumes by 33 Hz DNP stimulation before and after phentolamine (2 mg/kg). (B) Normalized stimulation evoked and distension evoked reflex bladder contraction volume thresholds before and after phentolamine were significantly different ($p < 10^{-8}$, ANOVA, n=28 trials). *, significant difference between stimulation evoked and distension evoked volume thresholds for control trials or for phentolamine trials ($p < 0.05$, post-hoc Bonferroni comparisons). †, significant difference between control and phentolamine stimulation volume thresholds or for distension volume thresholds ($p < 0.05$, post-hoc Bonferroni comparisons). (C) Normalized mean contraction magnitudes evoked by 33 Hz DNP stimulation before and after phentolamine (*$p < 10^{-6}$, t-test, n=28 contractions). Distension evoked contractions decreased after phentolamine, but the difference in relative contraction magnitudes was not significant ($p=0.26$).

A. Control

Hypogastric Transection

20 cmH$_2$O

10 seconds

B.

Volume Threshold (% of Control Distension)

*

*,†

†

*,†

†

Control

Hypogastric Transection

Transection + Antagonists

■ DNP Stimulation   ■ Distension

C.

Mean Magnitude (% of Control)

Control

Hypogastric Transection

Transection + Antagonists

Figure 5.4

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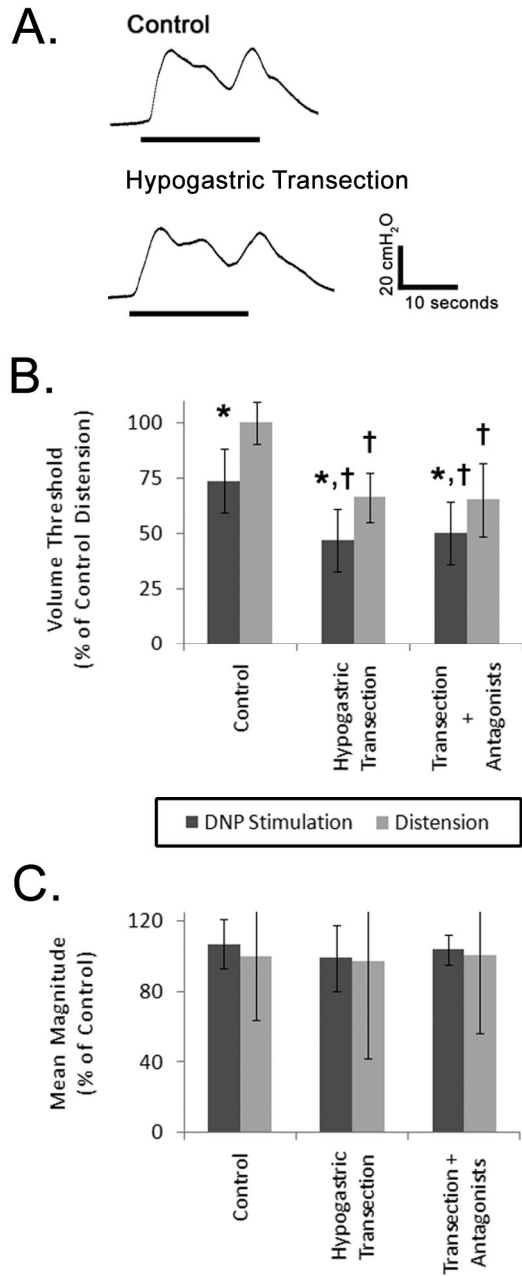
Figure 5.4. Effect of bilateral hypogastric nerve transection and subsequent adrenergic antagonist administration on bladder contractions evoked by DNP stimulation or distension. (A) Contractions evoked by 33 Hz DNP stimulation at a volume 1cc less than the distension evoked threshold volumes before and after bilateral hypogastric nerve transection. (B) Normalized volume thresholds before and after hypogastric nerve transection and after subsequent propranolol and phentolamine administration. Volume thresholds were significantly different ($p < 10^{-12}$, ANOVA, n=36 trials). *, stimulation evoked volume thresholds were significantly less than distension evoked thresholds for the treatment group ($p < 0.05$, post-hoc Bonferroni comparisons). †, significant difference between treatment and control volume thresholds for stimulation thresholds or for distension thresholds ($p < 0.05$, post-hoc Bonferroni comparisons). (C) Normalized mean contraction amplitudes evoked by DNP stimulation before and after hypogastric nerve transection and subsequent drug administration were not significantly different ($p = 0.35$, t-test, n=36 contractions). Normalized distension evoked contraction magnitudes also were not significantly altered by hypogastric nerve transection or subsequent drug administration ($p = 0.96$).
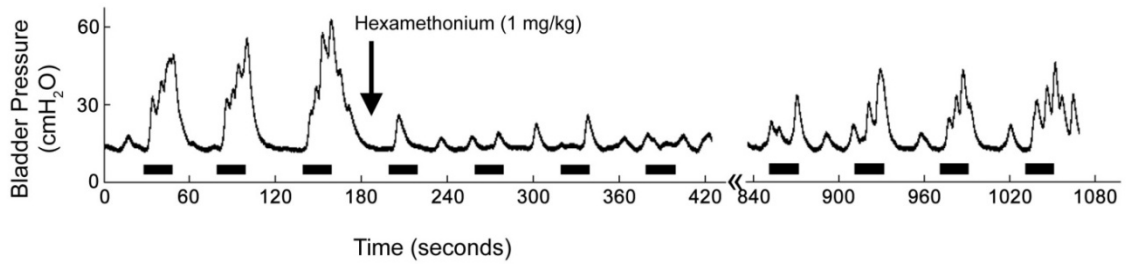
Figure 5.5. Hexamethonium bromide abolished the bladder contractions evoked by DNP stimulation. DNP stimulation (black bars, 33 Hz at 300 µA) evoked bladder contractions following hypogastric nerve transection, but administration of hexamethonium bromide (1 mg/kg) abolished the contractions. The stimulation evoked bladder contractions returned approximately 14 minutes later.

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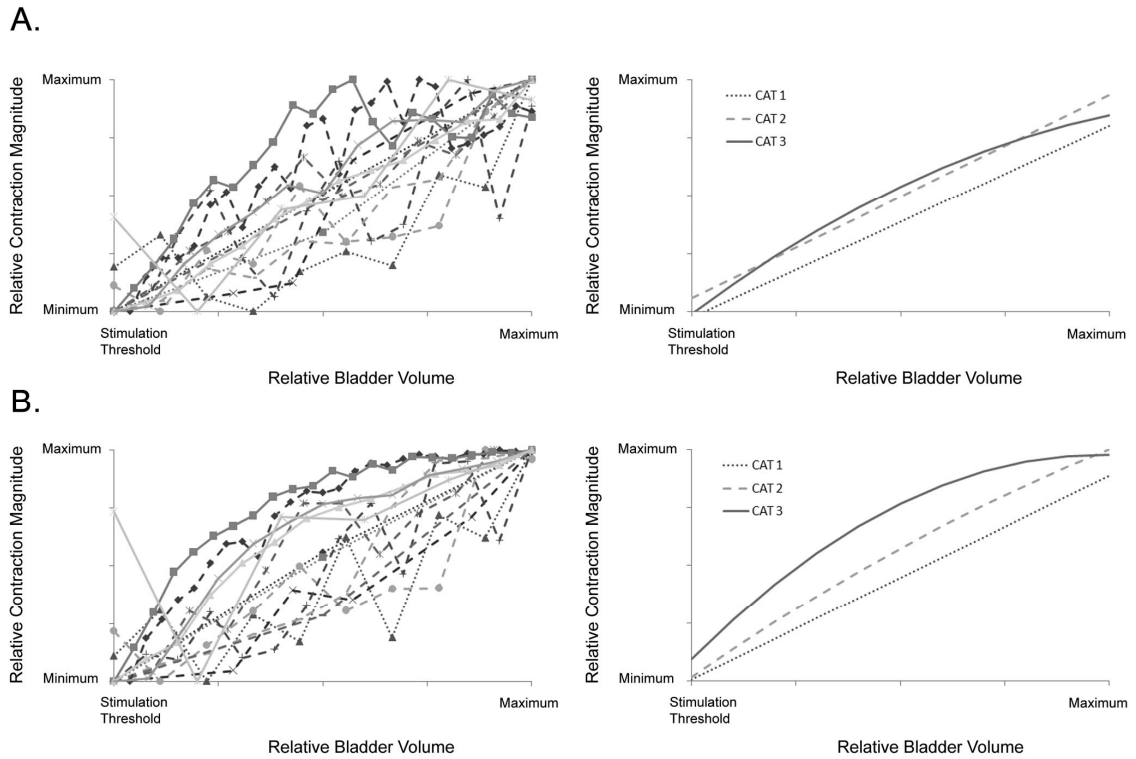
Figure 5.6. The magnitude of bladder contractions evoked by DNP stimulation increased with increasing bladder volume. The magnitudes of DNP stimulation evoked bladder contractions were determined at 1cc intervals between the stimulation evoked contraction volume threshold and 2-5 ml above the distension evoked volume threshold. Values were normalized by scaling between 0 (minimum value) and 1 (maximum value). (A) Left, Normalized mean contraction pressures as a function of normalized bladder volume. Right, Regression lines (linear or quadratic) for the relationship between bladder volume and mean contraction magnitude for each cat. A significant correlation was found between mean contraction magnitude and bladder volume for all 3 cats investigated (p<0.0001, Cat 1: r=0.8518, n=3; Cat 2: r=0.8480, n=7; Cat 3: r=0.8708, n=4). (A) Left, Normalized maximum contraction magnitudes as a function of normalized bladder volume. Right, Regression lines (linear or quadratic) for the relationship between bladder volume and maximum contraction magnitude for each cat. A significant correlation was found between maximum contraction magnitude and bladder volume for all 3 cats investigated (p<0.0001, Cat 1: r=0.8893, n=3; Cat 2: r=0.8687, n=7; Cat 3: r=0.8688, n=4).

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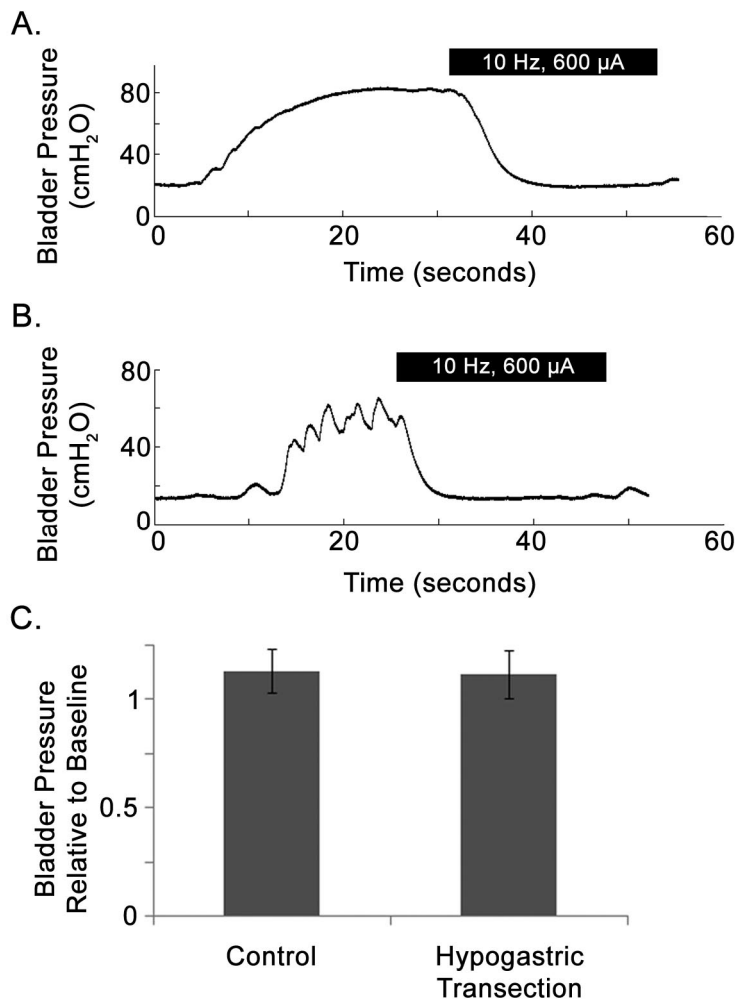
Figure 5.7. Inhibition of distension evoked reflex bladder contractions evoked by 10 Hz DNP stimulation before and after bilateral hypogastric nerve transection. (A) Bladder inhibition was evoked by 10 Hz DNP stimulation under control conditions. (B) After hypogastric transection, 10 Hz DNP stimulation still evoked bladder inhibition. (A-B) Black bars indicate DNP stimulation. (C) The bladder pressures during the last 10 seconds of 10 Hz DNP stimulation were compared to the baseline bladder pressure before the distension evoked contractions. The bladder pressure relative to baseline was not significantly different for DNP stimulation evoked inhibition before and after hypogastric transection (p=0.74, 2-tailed, unpaired t-test, n= 20 trials across 3 cats).

6. Conclusion

Persons with spinal cord injury (SCI) and other neurological disorders may suffer from urinary dysfunction, which decreases their quality of life and leads to serious health complications. Devices that stimulate the sacral nerve roots (Finetech-Brindley) or the sacral nerve (InterStim) are available to persons with SCI for treatment of urinary dysfunction. However, there remains a need for a device that restores control of urinary function as effectively as existing treatments while not requiring sacrifice of residual reflexes or sensation (i.e., no nerve transection(s)).

Pudendal afferent fiber stimulation is a promising approach to restore control of urinary function to individuals with bladder dysfunction resulting from SCI. High frequency (20-40 Hz) pudendal nerve stimulation evokes bladder contractions while low frequency (5-10 Hz) stimulation evokes bladder inhibition (Boggs et al. 2006b). However, the roles of specific pudendal afferent branches in and the mechanisms of the pudendal-bladder reflex responses were unknown. This dissertation aimed to identify and characterize in the preclinical feline model an ideal neural stimulation target for a bladder neuroprosthesis by (1) investigating the ability to evoke bladder contraction and relaxation without concomitant activation of the external urethral sphincter (EUS) by direct dorsal penile nerve (DNP) stimulation, (2) verifying intraurethral stimulation as a valid means of selectively activating the DNP and cranial sensory (CSN) branches of the pudendal nerve and characterizing the evoked bladder responses, and (3) determining the contributions of the sympathetic and parasympathetic bladder pathways in DNP stimulation evoked bladder contraction.

The results illustrated that direct DNP stimulation evoked stimulation frequency dependent excitation and inhibition of the bladder, and that these DNP-bladder reflexes could improve continence and micturition (Chapter 2). Also, it was determined that the bladder response to different pudendal afferent branches (the DNP and CSN) could be characterized via intraurethral stimulation (Chapter 3 & IV), and that the sympathetic bladder pathway is not a critical component of the DNP-bladder reflex (Chapter 5). Additional work described the pudendal innervations of the lower urinary tract in the male and female rat and female cat (Appendix A), simulated a DNP stimulation bladder control neuroprosthesis in the preclinical model (Appendix B), and developed a computer model to simulate the convergence of pudendal and pelvic afferents in the sacral spinal cord to illustrate how activation of these pathways could result in the DNP stimulation evoked bladder responses (Appendix C). These results identify the DNP as an ideal target for a bladder control neuroprosthesis to control urinary function in persons with SCI and provide guidance for future clinical studies.

6.1. Summary of results

6.1.1 Bladder reflexes evoked by DNP stimulation are dependent on stimulation frequency

The first aim of this work was to determine if bladder inhibition and activation could be selectively evoked by DNP stimulation. The results presented in Chapter 2 provided the first extensive characterization of the bladder response to DNP stimulation. Previous work detailed the frequency-dependent bladder inhibition and excitation evoked by stimulation of the compound pudendal nerve (Boggs et al. 2006b), and clinical studies illustrated that DNP stimulation could inhibit the bladder and improve continence

(Vodusek et al. 1986; Wheeler et al. 1992; Previnaire et al. 1996; Kirkham et al. 2001; Hansen et al. 2005). This was the first systematic investigation of the effects of stimulation frequency, and the findings revealed the ability to evoke both bladder excitation and inhibition via DNP stimulation. DNP stimulation at 33 and 40 Hz evoked bladder contraction and resulted in 64±12 % bladder voiding, a significant increase from distension evoked voiding (37±14 %) but well below the voiding evoked by sacral root stimulation (with de-innervation of the urethral sphincter in the cat or dorsal rhizotomy in the human) in the cat (79±17 % (Boggs et al. 2006a)) and clinically (90-96 % (Brindley et al. 1982; Brindley et al. 1986)). Stimulation at 5-10 Hz evoked bladder inhibition and increased continent volumes by 35-77 %, while DNP stimulation has evoked even greater increases in continent volume clinically (22-366 % (Vodusek et al. 1986; Wheeler et al. 1992; Kirkham et al. 2001; Hansen et al. 2005; Fjorback et al. 2006)). Percutaneous stimulation evoked responses similar to those evoked by direct stimulation in the pre-clinical model. This provides a basis for expansion on previous clinical studies of percutaneous DNP stimulation to treat urinary incontinence (Goldman et al. 2008) to full investigation of percutaneous DNP stimulation as a means of evoking both bladder contraction and inhibition. Percutaneous stimulation of the compound pudendal nerve in SCI humans (20-50 Hz) evoked small amplitude or transient bladder contractions (Yoo et al. 2007b), and selective DNP stimulation represents an alternative approach to evoke more robust excitatory responses.

Further, in contrast to compound pudendal nerve stimulation, DNP stimulation does not evoke direct or reflex EUS activation during stimulation evoked bladder contraction (Chapter 2). However, this did not translate into more efficient stimulation

evoked voiding, and continuous DNP stimulation (64% ± 12%, Chapter 2) did not evoke increases in bladder voiding compared to intermittent compound pudendal nerve stimulation (64% ± 14%, (Boggs et al. 2006a)). For clinical acceptance, higher stimulation evoked voiding efficiencies are critical. It is unclear the impact of the anesthesia on DNP-stimulation evoked voiding, as α-chloralose has been reported to decrease voiding efficiency in cats (Rudy et al. 1991). However, less than complete voiding (83% ± 10%) was reported for perigenital stimulation (activation of the dorsal clitoral nerve (DCN), the female homologue to the DNP) in the awake, chronic SCI cat (Tai et al. 2008), suggesting that the presence of α-chloralose alone is not responsible for incomplete voiding.

Bladder inhibition was evoked by low frequency stimulation, but it was unclear if this inhibitory reflex was spinal or supraspinal. The lack of distension evoked bladder contractions in the acute SCI cat (due to loss of the spinal-brainstem-spinal reflex) prevented investigation bladder inhibition by low-frequency DNP stimulation. However, low-frequency pudendal nerve and perigenital stimulation evoked bladder inhibition and increased continent volumes in the chronic SCI cat (Tai et al. 2007b; Tai et al. 2008) demonstrating that the inhibition reflex evoked by low frequency stimulation involves a spinal pathway. As well, genital afferent stimulation at similar frequencies evoked an inhibitory genital-bladder reflex in persons with chronic SCI (Vodusek et al. 1986; Wheeler et al. 1992; Previnaire et al. 1996; Kirkham et al. 2001; Hansen et al. 2005).

These findings identify the DNP as an ideal target for a minimally invasive investigation of pudendal afferent stimulation mediated restoration of control of bladder function to persons with SCIs. We illustrated the potential application of DNP

stimulation in the preclinical model, verifying that autonomous control of DNP stimulation can provide an extended continent period culminating in user-initiated micturition (Appendix B).

6.1.2. Intraurethral stimulation evoked bladder reflexes

The second aim included determining if intraurethral electrical stimulation of pudendal afferents can evoke bladder responses similar to those evoked by direct stimulation of the DNP or CSN. The bladder response to intraurethral stimulation in the cat was characterized at varying stimulation frequencies and locations along the urethra, and the pudendal afferent branches responsible for the evoked bladder reflexes were determined via selective transection (Chapter 3). Previous studies determined that intraurethral stimulation could evoked excitatory bladder responses in the cat and SCI humans (Gustafson et al. 2003; Gustafson et al. 2004), but these studies did not investigate systematically the effect of stimulation location and frequency on the evoked bladder response. The findings presented in Chapter 3 were the first description of the ability to activate selectively two distinct pudendal afferent mediated bladder reflexes via intraurethral stimulation.   Additionally, while other examinations of intraurethral stimulation focused on bladder excitation exclusively (Gustafson et al. 2003; Gustafson et al. 2004; Bruns et al. 2009b), the ability to inhibit the bladder with intraurethral stimulation was investigated, consistent with the goal of developing a neural prosthesis capable of controlling both micturition and continence. Stimulation in the distal (penile) urethra activated the DNP and evoked bladder excitation (33 Hz stimulation) or inhibition (10 Hz stimulation), while stimulation in the membranous urethra activated the CSN and evoked bladder excitation (2, 10, and 33 Hz). These findings were mostly consistent with

the bladder responses evoked by direct DNP and CSN stimulation (Chapter 2) (Yoo et al. 2008a). However, in contrast to direct nerve stimulation where 33 Hz stimulation of the CSN did not evoked bladder contraction (Yoo et al. 2008a), high frequency (33 Hz) intraurethral stimulation of the proximal urethra did evoke bladder contraction. This may be due to laterality effects if selective stimulation of the two CSN branches differs in its ability to evoke bladder contractions because of different frequency characteristics or different degrees of convergence with pelvic afferent fibers. Bilateral activation may also result in bladder contractions evoked by 33 Hz intraurethral stimulation in the membranous urethra if activation of a single CSN is insufficient to drive the reflex to suprathreshold levels but bilateral activation provides sufficient pudendal afferent activity to drive the excitatory reflex. Additional possible explanations include selective activation of a sub-population of CSN fibers, or co-activation of deep perineal nerve fibers or autonomic fibers.

The CSN-bladder response was not evoked in the acute SCI cat, while the DNP-bladder response could be evoked in the acute SCI cat. The finding of two distinct pudendal afferent pathways that modulate bladder function suggests that clinical SCI subjects may respond differently to intraurethral stimulation depending on the level and extent of their injury. The preclinical findings suggest the portion of the SCI population with partial or incomplete injuries may respond to both CSN and DNP activation, while individuals with complete SCIs are likely to respond solely to DNP activation. However, the bladder responses evoked in humans using intraurethral stimulation are not consistent with our findings in the cat. Intraurethral stimulation in humans with complete SCI evoked bladder contractions when stimulation was delivered 2-4 cm from the bladder

neck (Gustafson et al. 2003; Gustafson et al. 2004). In cats, stimulation in the membranous urethra and direct stimulation of the CSN did not evoke contractions following acute SCI. The responses observed clinically imply that either the pudendal-bladder reflexes are different in the cat and human or that activation of the pelvic and/or hypogastric nerves occurred in the human study. Further exploration of the stimulation location and frequency in SCI humans is required to clarify differences in the reflexes in humans and cats. Initial clinical intraurethral stimulation investigations (following our preclinical intraurethral experiments) suggest a DNP-bladder excitatory pathway exists, activated by stimulation in the distal urethra (Yoo et al. 2009), but the excitatory responses evoked are far less robust than the responses evoked in the preclinical model (Chapter 3). Additionally, it was determined that an excitatory CSN-bladder reflex exists in the incomplete and complete SCI human as well (Yoo et al. 2009), in contrast to the finding in the cat that the CSN-bladder reflex requires supraspinal pathways (Chapter 3). These initial clinical results imply the pudendal-bladder reflexes in the chronic SCI human are different than in the acute SCI cat.

The excitatory pudendal-bladder reflex has been ascribed to activation of urethral afferents (Boggs et al. 2005), and recent results suggest that the urethra-bladder reflex mediated by fibers in the CSN is not robust following acute SCI (Yoo et al. 2008a) (Chapter 3). These findings suggest that the CSN mediated reflex is the supraspinal urethra-bladder reflex described by Barrington (Barrington 1941). It is likely that activation of afferents in the DNP that innervate the glans and perineum are responsible for the excitatory pudendal-bladder reflex in acute and chronic SCI cats (Chapter 2) (Tai et al. 2007a). These findings imply that the DNP-bladder reflexes are genitovesical reflexes rather than urethrovesical

reflexes. Additionally, mechanical genital stimulation can excite the bladder in chronic SCI cats (Tai et al. 2008), and examination of the dorsal clitoral nerve (DCN) in the female cat revealed the DCN provided little or no urethral innervations (Appendix A), providing further evidence that the excitatory DGN-bladder reflex is a genitovesical and not a urethrovesical reflex.

The finite element analysis of intraurethral stimulation (Chapter 4) provided additional guidance for clinical investigation of intraurethral stimulation evoked bladder reflexes. A ring electrode similar in diameter to the human urethra was a superior choice for pudendal afferent activation than the current practice of introducing a large Foley catheter (8-16 Fr) in addition to a much smaller (5 Fr) stimulating catheter (Gustafson et al. 2003; Gustafson et al. 2004; Yoo et al. 2009). This is important because use of a larger stimulating catheter increases the likelihood of consistent electrode-urethra contact and also ensures the electrode is oriented towards the target pudendal afferents (Chapter 4). The simulation results also support the in vivo findings (Chapter 3), which imply selective pudendal afferent branch stimulation is most likely at the extremes (anterior and posterior) of the human urethra. Finally, anatomical studies revealed the presence of two populations of DNP fibers (Yang et al. 1998b), those innervating the glans, prepuce, and perineum, and those innervating the urethra. The model results suggests that selective activation of the urethral fibers is possible using a 3-contact ring electrode, while use of a single ring electrode evokes less urethral activation prior to DNP trunk activation (Chapter 4).

Intraurethral stimulation provides the ability to investigate bladder reflexes mediated by multiple pudendal afferent pathways. In addition to guiding clinical

investigation of bladder reflexes evoked via selective intraurethral activation, the results

identify two distinct reflex pathways activated via different pudendal afferent branches.

Finally, the findings that the DNP-mediated bladder activation is a spinal reflex that can

selectively excite or inhibit the bladder, while the CSN is a supraspinal reflex that can

only excite the bladder, supports that the DNP is an appropriate target for a bladder

control neuroprosthesis.

6.1.3. Efferent pathways critical to DNP stimulation evoked bladder reflexes

The third aim of this dissertation was to determine the role of the sympathetic

bladder pathway in the bladder contractions evoked by high frequency DNP stimulation.

The results presented in Chapter 5 clarify the roles of the sympathetic and

parasympathetic bladder pathways in the excitatory and inhibitory bladder reflexes

activated by DNP stimulation. Previous work determined that bladder inhibition evoked

by pudendal afferent stimulation was through simultaneous suppression of pelvic efferent

activity (i.e., inhibiting the exciter) and activation of hypogastric efferent activity (i.e.,

exciting the inhibitor) (Lindström et al. 1983). However, the roles of these autonomic

bladder pathways in the excitatory pudendal-bladder reflex had not been investigated.

The results in Chapter 5 demonstrate using both selective pharmacological blockage and

nerve transection that the sympathetic bladder pathway does not play a significant role in

the excitatory DNP-bladder reflex. Hypogastric nerve transection and selective block of

the ß-adrenergic bladder innervations by propranolol decreased the volume thresholds for

stimulation evoked and distension evoked bladder contraction, but did not impair the

ability to evoke bladder contractions with 33 Hz DNP stimulation or alter the relationship

between the stimulation evoked and distension evoked volume thresholds. Phentolamine

induced α-adrenergic receptor block increased volume thresholds and reduced stimulation evoked contraction magnitudes. However, the lack of effect of hypogastric nerve transection or subsequent administration of phentolamine on contraction magnitude (Chapter 5) and a previous study illustrating that α_1-receptor block had an inhibitory effect on distension evoked bladder contractions (Yoshimura et al. 1990) suggest that α-adrenergic block simply decreased bladder excitability rather than impairing the reflex activation of the bladder by DNP stimulation. This implies that the α-adrenergic sympathetic pathway is not significantly activated or inhibited by DNP stimulation. These results demonstrate that the sympathetic pathway does not contribute significantly to the excitatory DNP-bladder reflex, implying that the pelvic efferent pathway is critical to the excitatory DNP-bladder reflex, likely due to convergence of pudendal and pelvic afferents in the sacral spinal cord.

Convergence of pudendal and pelvic afferent fibers occurs in the sacral spinal cord. Pudendal and pelvic afferents converge in the sacral cord on interneurons that activate preganglionic parasympathetic bladder fibers in the parasympathetic nucleus (de Groat et al. 1969; McMahon et al. 1982a; Honda 1985; Araki et al. 1996; Coonan et al. 1999). Pudendal nerve stimulation evokes reflex discharges in pelvic efferent fibers when coupled with sufficient pelvic afferent activity (McMahon et al. 1982a). However, 33 Hz DNP stimulation does not evoke excitation simply by evoking this pudendal-pelvic reflex as evidenced by the inhibitory effect of 10 Hz DNP stimulation. If a simple pudendal-pelvic reflex were involved, 10 Hz stimulation following sympathectomy would not inhibit pelvic efferent activity.

Recording of the interneurons in the sacral spinal cord revealed that pudendal and pelvic afferent activation evokes responses characterized by both inhibitory and excitatory post-synaptic potentials (PSPs) (de Groat et al. 1982; McMahon et al. 1982a; Araki et al. 1996; Mazières et al. 1997). Simulation of these inhibitory and excitatory PSPs at different rates of pudendal and pelvic afferent activation illustrated how pudendal and pelvic afferent convergence can alter parasympathetic efferent activity, consistent with the observed frequency dependent inhibition and excitation of the bladder evoked by pudendal afferent stimulation (Appendix C).

The importance of these findings is two-fold. First, the results suggest clinical candidates need not have intact sympathetic bladder innervation, and individuals with suprasacral SCIs should have the appropriate intact neural pathways for evoking the DNP-bladder reflexes. Second, future studies that aim to improve the efficacy of DNP stimulation evoked control of continence and micturition may examine how to optimize the stimulation evoked response in the pelvic efferent fibers to the bladder.

6.2. Future studies

6.2.1 Clinical

Extensive investigation of the human pudendal-bladder reflexes evoked by selective pudendal afferent activation is warranted based on the preclinical results described in this dissertation. The finding, in the cat, that DNP stimulation (direct or percutaneous) can evoke bladder inhibition or contraction dependent on stimulation frequency suggests that a more thorough investigation of the bladder response to DNP stimulation in SCI humans is necessary. In humans, DNP stimulation (or stimulation of

the female equivalent, the dorsal clitoral nerve, DCN) has been reported to increase continent bladder volumes by 53-144% (mean values). These studies were designed to investigate bladder inhibition exclusively, and used a limited range of stimulation frequencies, 5-20 Hz (Vodusek et al. 1986; Wheeler et al. 1992; Previnaire et al. 1996; Kirkham et al. 2001; Hansen et al. 2005). Higher stimulation frequencies (33 and 40 Hz) consistently excited the bladder in the cat (Chapter 2). It is unclear if broadening the frequency range of human DNP stimulation will produce bladder excitation. However, recent studies in the rat found that stimulation of the sensory branch of the pudendal nerve (containing the rat DGN) evoked bladder inhibition or excitation depending on stimulation intensity rather than frequency (Peng et al. 2008a). The frequency dependent pathway in the cat suggests a neural mechanism, likely located in the sacral spinal cord, modulates differentially the efferent bladder output dependent on the input frequency from pudendal afferent fibers. The stimulation intensity dependent bladder response in the rat suggests activation of different afferent fiber populations in the pudendal nerve is responsible for evoking bladder excitation or inhibition. While human urinary function is more comparable to cat urinary function, it remains possible that the key to selectively evoking bladder inhibition or excitation via DGN stimulation in humans could be varying stimulation frequency or intensity. The effects of both of these parameters on DGN-evoked bladder responses should be characterized clinically.

The ability to investigate distinct pudendal afferent fiber mediated bladder reflex pathways via intraurethral stimulation indicates that intraurethral stimulation is an ideal clinical method for investigating the bladder responses evoked by selective activation of pudendal afferent branches. FEM analysis (Chapter 4) illustrated that use of a ring

electrode that is comparable in diameter to the urethra should be considered rather than mimicking previous clinical intraurethral stimulation methods (Gustafson et al. 2003; Gustafson et al. 2004). Also, if direct DNP stimulation results suggest the ability to evoke bladder excitation or inhibition is dependent on stimulation amplitude rather than frequency, selective activation of the subpopulation of DNP urethral fibers should be investigated using a multi-ring electrode.

The frequency characteristics of the DNP-bladder pathway (low-frequency inhibition; high-frequency excitation) observed in the intact and acute SCI cat are similar to the frequency characteristics reported for compound pudendal nerve stimulation and perigenital stimulation (dorsal genital nerve (DGN) activation) in the chronic SCI cat (Tai et al. 2006; Tai et al. 2008), providing evidence of the chronic persistence of the frequency dependent DNP-bladder reflexes following the complex neural reorganization due to SCI in the cat (de Groat et al. 1998). However, neural reorganization following SCI induced bladder dysfunction in humans (Steers 2002) may cause significant changes in any urethrovesical or genitovesical reflexes that exist in the intact human. The finding of two distinct pudendal afferent branch mediated bladder reflexes (the supraspinal CSN-bladder reflex and the spinal DNP-bladder reflex) calls for clinical investigation of the impact of the degree of SCI (e.g., complete vs. incomplete) on the pudendal afferent mediated bladder reflexes uncovered by intraurethral stimulation.

The intraurethral results also highlight the need for a detailed description of the human urethral innervation comparable to that recently presented for the rat and cat urethra utilizing the Sihler's method for visualizing nerve fibers in gross specimens (Yoo et al. 2008b) (Appendix A). Understanding the innervation of the cat lower urinary tract

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by pudendal afferent branches was critical to determining the specific pathways associated with intraurethral evoked pudendal-bladder reflexes. While the pudendal anatomy has been described in humans, and branches were identified innervating the genitalia and EUS (Juenemann et al. 1988; O'Bichere et al. 2000; Schraffordt et al. 2004), it is unclear how these branches compare to the pudendal branches of the cat. Specifically, the existence of a human pudendal afferent branch comparable to the CSN branch in the cat needs to be determined. The proximal extent of the human CSN, if it exists, will provide evidence as to whether or not intraurethral stimulation in the region of the bladder neck evoked bladder contraction (Gustafson et al. 2003; Gustafson et al. 2004; Yoo et al. 2009) by activation of pudendal or autonomic nerves.

While DNP stimulation in the intact and acute SCI cat did not evoke EUS activation during stimulation evoked bladder contractions (Chapter 2), perigenital stimulation in the chronic SCI cat does activate the EUS during bladder contraction (Tai et al. 2008). Similarly, DNP stimulation during bladder contraction in persons with normal urinary function does not evoke reflex activation of the EUS but does evoke reflex EUS activation in persons with detrusor hyperreflexia (Dyro et al. 1986) and SCI (Walter et al. 1994). Voiding in the chronic cat by perigenital stimulation was achieved using intermittent stimulation, resulting in post-stimulus voiding (Tai et al. 2008). If detrusor-sphincter dyssynergia (DSD) is triggered by an increase in bladder pressure (i.e., bladder afferent fibers reflexively activating pudendal motorneurons (de Groat et al. 1992)) then intermittent stimulation may not evoke efficient voiding in persons with SCI, consistent with impaired voiding evoked by sacral root stimulation in the absence of a dorsal rhizotomy (Sauerwein 1990). If a robust excitatory DNP-bladder response is

found clinically, subsequent investigation of DNP stimulation evoked bladder voiding should determine the impact of DSD on DNP evoked voiding. If DSD impairs voiding, the pairing of additional therapies (e.g., high frequency nerve block (Tai et al. 2004)) with excitatory DNP stimulation should be investigated.

6.2.2 Pre-clinical

The findings in this dissertation suggest that future preclinical research should focus on optimizing the stimulation evoked voiding efficiencies (minimizing residual urine) and investigating the different stimulation paradigms for autonomous control of bladder function. It is necessary to explore alternative means of improving stimulation evoked bladder voiding (e.g., pharmacotherapy, high frequency stimulation block of EUS activation). Chapter 5 suggests that focusing on stimulation paradigms that increase parasympathetic bladder activation may provide a means of increasing bladder voiding. Variable pattern stimulation has been shown to increase stimulation evoked contraction magnitudes (Bruns et al. 2008), and periurethral stimulation of the proximal urethra suggests variable pattern stimulation can improve voiding (Bruns et al. 2009a). However, urinary neurophysiology provides no basis for the stimulation patterns utilized in this study. Recording studies that characterize the response from the DNP, CSN, and neurons in the dorsal root ganglia could provide a clear picture of the temporal and spatial patterns of pudendal afferent activity evoked by urethral fluid flow and mechanical genital stimulation. The bladder responses evoked by biomimetic stimulation that mimics these natural patterns should be compared to the responses evoked by the stimulation patterns used in this dissertation (continuous, single frequency stimulation).

Alternatively, bilateral pudendal afferent branch stimulation should be investigated in the cat. The results in Chapter 3 show that the magnitude of bladder contractions evoked by DNP stimulation is reduced following unilateral DNP transection in the acute SCI cat, suggesting that bilateral DNP stimulation may evoke larger bladder contractions than unilateral DNP stimulation. Bilateral stimulation of the DNP and CSN (and selective coactivation of these branches at their respective excitatory frequencies) should be investigated to determine the effect of bilateral activation on bladder contraction magnitude, the stimulation evoked contraction volume threshold, bladder voiding efficiency, and residual bladder volume.

The limited investigation of autonomous control of DNP stimulation (Appendix B) suggests that future preclinical studies of autonomous control could provide valuable insight into optimal programming of a clinical pudendal afferent stimulation bladder control neuroprosthesis. The results suggest several issues that must be addressed for optimal device function, including when to transition from bladder inhibition (continence) to bladder excitation (voiding), whether stimulation duration reducing stimulation paradigms (e.g., delayed onset continuous inhibition) can provide equal or better results than continuous inhibition. It has been shown that prolonged inhibitory stimulation (10 Hz genital nerve stimulation) can enhance the micturition reflex (Jiang et al. 1999), suggesting that excitatory DNP stimulation after a prolonged period of inhibitory stimulation may evoke greater voiding than stimulation in the absence of preceding inhibition.

6.3. Conclusion

Selective activation of pudendal afferent branches was investigated by direct and minimally invasive stimulation methods. The results determined that the DNP is an ideal target for a neuroprosthesis to restore control of bladder function in persons with SCI, and that an additional pudendal-bladder pathway exists, mediated by the CSN, that may be relevant in persons with incomplete SCI. This work provides insight for future preclinical and clinical pursuit of pudendal afferent evoked bladder control

Appendix A – Pudendal innervation of the lower urinary tract: a comparative analysis of human, cat, and rat neuroanatomy determined using Sihler's technique

A.1 Introduction

The somatic nervous system provides significant innervation to the lower urinary tract (LUT) via the pudendal nerve. Pudendal afferent activity modulates bladder function in rats (Peng et al. 2008b) and cats (Barrington 1941), and the afferent branch of the urethrovesical reflex observed experimentally in humans is suggested to travel in the pudendal nerves (Shafik et al. 2003a). Additionally, electrical stimulation of specific pudendal nerve branches modulates urinary function in rats (Peng et al. 2008a), cats (Chapter 2) (Yoo et al. 2008a), and humans (Wheeler et al. 1992; Kirkham et al. 2001), but it is unclear the extent to which neural stimulation targets are analogous in the different species. This study investigated the presence of analogous pudendal nerve branches in the rat and cat by utilizing anatomical dissection and Sihler's tissue staining method. The somatic innervation of the major components of the LUT in the rat are compared with previous findings in the cat utilizing the same techniques (Yoo et al. 2008b).

Traditionally, the cat and rat pudendal nerves are thought to divide into separate sensory and motor branches that primarily consist, respectively, of afferent and efferent nerve fibers (Martin et al. 1974; McKenna et al. 1986). Primary targets for a neural prosthesis for bladder control in the male cat include the two branches of the sensory branch of the pudendal nerve; the cranial sensory nerve (CSN) and the dorsal penile nerve (DNP). The CSN innervates the membranous urethra between the prostate and the

penile bulb, and the DNP innervates the penile urethra, glans penis, and perineum. Distinction of these two afferent branches in the cat is important because CSN stimulation modulates bladder behavior solely via a frequency-dependent supraspinal pathway while DNP stimulation can activate a frequency-dependent spinal pathway that modulates bladder function (Chapter 2,3) (Yoo et al. 2008a). Selective activation of these pathways may have different advantages among persons with SCI, and selective activation may avoid coactivation of competing reflexes or reduce side effects by avoiding activation of unrelated (non-urinary) reflexes. However, it is unclear if there exists in rats or humans a pudendal nerve branch analogous to the feline CSN, making cross-species comparisons of stimulation evoked bladder control difficult.

The goal of this study is to compare the somatic innervations of the LUT in the rat and cat to identify, if present, neural stimulation targets in the rat equivalent to the CSN and DNP in cats. While studies have extensively examined the pudendal anatomy in rats (McKenna et al. 1986; Pacheco et al. 1997; Pastelin et al. 2008) and cats (Martin et al. 1974; Mariano et al. 2008; Yoo et al. 2008b), this study of the somatic innervations of the LUT in the rat and cat utilizes a unique staining technique (Sihler's method) to make direct comparisons to anatomical observations in the male cat made utilizing the same staining technique (Yoo et al. 2008b).

A.2 Methods

Somatic innervation of the LUT was investigated in 17 rats (9 male, 8 female) and 4 female cats. This study was approved by the Institutional Animal Care and Use Committee at Duke University. Gross dissection was completed promptly post-mortem.

Tissue specimens were removed to include the LUT from the urethral meatus to the urinary bladder.

A.2.1 Sihler's Method

The specimens were stained following a modified Sihler's method (Wu et al. 1992; Mu et al. 2000; Yoo et al. 2008b) and were rinsed under running tap water between steps (~1 hour per rinse). The approximate length of each steps in the Sihler's stain process varied across species, with the female cat specimens requiring greater duration for each step because of the greater thickness of the cat specimens. Previous reports detailed appropriate means of determining the end of each step in the Sihler's process (Lee et al. 2008). The process included fixation in 10% formalin, maceration by 3% potassium hydroxide, decalcification in Sihler's solution I (1 part glacial acetic acid, 1 part glycerin, 6 parts 1% aqueous chloral hydrate), staining in Sihler's solution II (1 part Ehrlich's hematoxylin, 1 part glycerin, 6 parts 1% aqueous chloral hydrate), destaining in Sihler's solution I, darkening in 0.05 % lithium carbonate solution, and clearing and storage in glycerin.

A.2.2 Analysis

Specimens were viewed through a stereomicroscope using a combination of transmitted and direct reflected light. Digital photomicrographs were obtained during dissection of stained specimens (ProgRes C5 digital camera, JENOPTIK Laser, Optik, Systeme GmbH).

A.3 Results

A.3.1 The sacral plexus of the male rat

The sacral plexus, formed by the lumbosacral trunk and the L_6-S_1 trunk, was

intact in 6 stained male rat specimens (Figure A.1). Both autonomic and somatic nerves

emerged from the L_6-S_1 trunk and were traced from the sacral plexus to the LUT. The

observations of the branches of the sacral trunk were consistent with previous description

(McKenna et al. 1986; Pacheco et al. 1997). The pelvic nerve emerged from the L_6-S_1

trunk proximal to the pudendal nerve and coursed towards the bladder neck, giving off a

projection to the perineal musculature, previously verified by electrophysiology to

innervate the iliococcygeus and pubococcygeus muscles (Pacheco 1989), prior to forming

the major pelvic ganglia (MPG) just lateral to the prostate. The L_6-S_1 trunk continued on

as the pudendal nerve, with a projection to the perineal musculature (the coccygeus

muscle (Pacheco 1989)) prior to bifurcating into the traditionally designated sensory and

motor branches, with the former being significantly larger in diameter than the latter

(Figure A.1). In all 6 specimens, the lumbosacral trunk, before continuing on as the

sciatic nerve, gave off an anastomotic branch to the motor branch of the pudendal nerve,

consistent with previous descriptions of the sacral plexus (Pacheco et al. 1997).

A.3.2 Pudendal Innervation of the LUT in the male rat

The sensory and motor branches of the male rat pudendal nerve projected

ventrally from the sacral plexus and reached the LUT at the dorsorostral border of the

bulb of the penis (Figure A.1). In all 9 male rats the sensory branch was a single nerve

trunk as it emerged from the sacral plexus and when it reached the penile bulb. In 5 of

the 9 specimens, the motor branch also was identified as a single nerve trunk, but in 4

specimens the motor branch consisted of a primary nerve and a thin second nerve. In all 4 specimens this second nerve converged with the primary motor branch at the penile bulb and was indistinguishable from the other motor branch projections to the proximal urethra.

Just lateral to the ischiocavernosus muscle, the primary motor branch bifurcated, and a branch continued towards the penile bulb while the other branch coursed caudally (Figure A.2). The caudal branch split again with projections innervating the external anal sphincter (EAS) and the bulbospongiosus muscle. The branch to the penile bulb arrived laterally at the rostral border of the penile bulb and had projections that inserted into the bulb of the penis and innervated the bulbospongiosus muscle and ischiocavernosus muscle. The remaining motor branch at the penile bulb crossed inside (medially) the DNP and coursed along the lateral aspect of the proximal urethra towards the prostate, ramifying into tiny filaments that innervated the external urethral sphincter (EUS) from the penile bulb up towards the prostate (Figure A.3). This motor branch component innervating the proximal urethra provided limited contralateral innervation. While the motor branch appeared to provide limited innervation beyond the caudal border of the prostate, the projections of the motor branch became indistinguishable from autonomic projections from the MPG in the prostatic urethra. The motor branch innervation of the LUT was analogous to that of the rectal perineal nerve in the cat (Yoo et al. 2008b), providing both EAS (inferior rectal branch in the cat) and EUS (deep perineal branch in the cat) innervation.

Unlike in the male cat, the sensory branch in the male rat did not separate into 2 distinct branches as it neared the bulb of the penis (Figure A.2). As it coursed across the

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lateral edge of the penile bulb proximal to the ischiocavernosus muscle, the sensory

branch converged with 3 branches from the major pelvic ganglia. Similar convergence

was not observed in the male cat (Yoo et al. 2008b). The sensory branch also gave off

several tiny branches at this point. One branch appeared to terminate in the urethra at the

proximal edge of the bulb of the penis. Another small projection innervated the

ischiourethralis muscle, which was previously reported to have a motor component

(Pastelin et al. 2008). The sensory branch continued distally along the dorsal side of the

penile body, giving off ventrolateral fibers that innervated the urethra and perineum

before diffusely innervating the glans penis and prepuce (Figure A.4).   Once past the

convergence with the autonomic fibers, the male rat sensory nerve was analogous to the

DNP in the male cat. However, the sensory branch of the male cat contains both the

DNP and the CSN, a branch that innervates the membranous urethra (between the penile

bulb and the prostate), while the sensory branch of the rat did not have a distinct proximal

projection.

A.3.3 Autonomic innervation

The pelvic nerve coursed from the sacral plexus to the MPG, converging with the

hypogastric nerve at the MPG (Figure A.5). Originating from the MPG, nerve

populations projected to the bladder, the prostate and prostatic urethra, and the penis.

The penile projections included the cavernous nerve, which coursed along the

ventrolateral border of the urethra to the base of the penis, innervating the bulbourethral

glands, corpus spongiosum, and penile vasculature (Purinton et al. 1973; Dail et al.

1999). Three additional penile projections, 2 originating directly from the MPG and one

originating from the cavernous nerve just distal to the MPG, traveled superficially along

the ventral and ventrolateral aspects of the post-prostatic urethra and appeared to combine with the DNP at the base of the penis. Similar convergence of autonomic fibers and the DNP was not observed in the male cat (Langley et al. 1896; Yoo et al. 2008b).

A.3.4 Pudendal innervation in the female rat

The LUT innervation in the female rat was similar to the male rat (Figure A.6A). There was a distinct pudendal sensory branch that had convergent inputs from the MPG as it reached the urethra. The sensory branch had 2-3 tiny projections to the urethra just as it reached the LUT then coursed distally, innervating the clitoris, preputial glands, urethra, and vagina. The motor branch, made up of several small fibers, innervated the EAS, EUS, and bulbospongiosus and ischiocavernosus muscles similar to the male rat.

A.3.5 Pudendal innervations in the female cat

The pudendal innervation of the LUT in the female cat is consistent with the male cat (Figure A.6B). The sensory branch split into 2 distinct branches, the dorsal clitoral nerve (DCN) and the CSN. The dorsal clitoral nerve entered in the area of the urethral meatus and ran distally to the clitoris with branches emanating laterally that appeared to innervate the vaginal wall. Unlike the female rat, the cat DCN did not appear to innervate the urethra, which terminated just as the DGN and CSN entered laterally the vicinity of the urethra and vagina. The CSN had tiny projections to the urethral meatus in additional to larger branches that traveled medially and laterally along the proximal urethra. The motor branch split into the EAS branch and a branch that dived below the sensory branches to innervate the EUS, ischiocavernosus muscle, and vaginal wall.

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A.4 Discussion

This study utilized Sihler's technique to examine the somatic innervations of the rat LUT. The identification of the presence and absence of analogous innervation of the rat and cat LUTs provides valuable insight into the role of the pudendal innervation in urinary function in the rat and cat. The innervation of the rat LUT presented here is consisted with previous physiological, histochemical, morphological, and gross anatomical findings (Langworthy 1965; Purinton et al. 1973; McKenna et al. 1986; Pacheco et al. 1989; Pacheco et al. 1997; Dail et al. 1999; Pastelin et al. 2008).

A.4.1 Limitations of the present study

There are several potential limitations of using Sihler's staining method. First, Sihler's method preferentially stains myelinated nerve fibers, but no pudendal nerve branches have been described as primarily unmyelinated (Martin et al. 1974). Also, the stain does not allow for differentiation of sensory and motor fibers, but electrophysiological results in the cat (Yoo et al. 2008b), rat (Pacheco et al. 1989; Pacheco et al. 1997; Pastelin et al. 2008), and human (Yang et al. 1998a; Yang et al. 2000) provide evidence to support the classification of different branches as sensory, motor, or somatomotor. Third, staining becomes less consistent as fibers become extremely fine. However, Sihler's technique is superior to gross dissection because it clearly differentiates myelinated nerve fibers from connective and vasculature tissues and it allows for tracing of tiny filaments beyond the limits of gross dissection.

A.4.2 Comparison in rat and cat

Recent studies have provided conflicting observations regarding the division of the pudendal nerve into sensory and motor branches (Mariano et al. 2008; Pastelin et al.

2008; Yoo et al. 2008b). These findings for the rat are consistent with observations for the cat that the DGN originates from the sensory branch of the pudendal nerve while efferent EUS innervation derives from the motor branch of the pudendal nerve (Yoo et al. 2008b). The finding of a branch to the ischiourethralis muscle is consistent with a previous report of an efferent branch of the sensory branch in the rat to the ischiourethralis muscle (Pastelin et al. 2008). Martin et al noted a small complement of efferent fibers in the cat DNP, and these may innervate the ischiourethralis muscle in the cat. The role of the rat DGN is still quite distinguishable from the motor branch, which innervates the bulbospongiosus, ischiocavernosus, and external urethral and anal sphincter muscles.

A.4.3 The urethrovesical reflex

Urethrovesical feedback plays a critical role in normal micturition. Removal of this feedback impairs voiding in rats(Jung et al. 1999; Peng et al. 2008b), cats (Barrington 1931), and humans (Shafik et al. 2003b). It is unclear whether this mechanism operates through activation of the CSN, DNP, or both. The lack of a CSN analogue in the rat suggests that urethral innervation via the DNP plays a role in the urethrovesical reflex. However, while the dPN is regarded as primarily efferent, it has a significant component of afferent fibers (Martin et al. 1974). The rat motor branch innervation to the EUS could contain sensory fibers whose function is equivalent to the cat CSN. This is unlikely because transection of the sensory branch in the female rat effects EUS EMG, bladder contraction, and bladder voiding (Peng et al. 2008b) in a manner that suggests the urethrovesical reflex travels in the sensory branch in the rat. Additionally, afferent stimulation of the cat dPN does not evoke a bladder response (Yoo

et al. 2008a) providing further evidence that the urethrovesical reflex is contained by the CSN or DNP in the cat and by the DNP ("sensory branch") in the rat. Selective sectioning of the DNP and CSN in the cat during urodynamic evaluation may provide a clearer picture of their respective roles in the feline urethrovesical reflex. As well, recording of activity in the rat sensory branch in response to urine flow following transection of the rat sensory branch distal to the small urethral projections should confirm whether or not those projections include urethral afferents involved in the urethrovesical reflex.

Interestingly, the bladder response to stimulation of the "sensory" innervations of the LUT differs in rats and cats. In cats, the bladder response to DNP stimulation is dependent on stimulation frequency (Chapter 2) , while the rat bladder response to analogous (sensory branch) stimulation is dependent on stimulation amplitude (Peng et al. 2008a). The neuroanatomical differences and differences in the characteristics of stimulation evoked bladder response in the rat and cat suggest the neural pathways mediating pudendal-bladder reflexes are dissimilar. However, the central distribution of pudendal afferents is similar in rats and cats (Ueyama et al. 1985)

Human lower urinary tract innervation shares similarities and dissimilarities with the rat and the cat. While the DCN appears to innervate the distal urethra in the female rat, the lack of urethral innervation by the DCN in the female cat is consistent with the DCN innervation in human females (Vaze et al. 2008). This finding is significant because the urethrovesical reflex is associated with the CSN in the cat, while the DNP may provide urethral feedback in the rat. The lack of urethral innervation by the DCN in humans suggests the urethrovesical reflex in humans is mediated by a pudendal afferent

branch similar to the feline CSN or by autonomic afferents. However, humans have autonomic projections from the MPG that converge on the DGN (Benoit et al. 1999; Yucel et al. 2003) similar to the convergence observed in rats (Purinton et al. 1973; Paick et al. 1993; Colombel et al. 1999; Dail et al. 1999), but this convergence is not present in cats (Langley et al. 1896; Yoo et al. 2008b). Electrophysiology confirms these converging nerves are travelling from the MPG to the DGN (Steers et al. 1988), and these nerves primarily modulate penile vasculature (Dail et al. 1999).

A.4.4 Conclusion

Application of Sihler's method to the rat LUT allowed for demonstration of the pudendal innervations of the LUT. Results reveal that the rat lacks a distinct branch analogous to the CSN in the cat. The functional impact of this anatomical difference is unclear. Extension of this neuroanatomical comparison to humans may provide insight into why DNP stimulation evokes robust bladder contraction or relaxation in the cat (Chapter 2) while evoking robust inhibition (Wheeler et al. 1992) but poor or unreliable excitation (Yoo et al. 2007b) in humans with SCI. Ultimately, selective stimulation of pudendal afferent branches is ideal for optimal investigation of the ability to control the bladder through pudendal nerve stimulation, and understanding the pudendal innervations of the LUT is pivotal for selecting appropriate stimulation target.
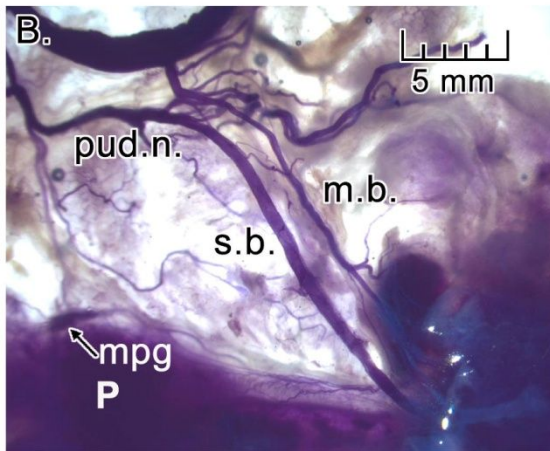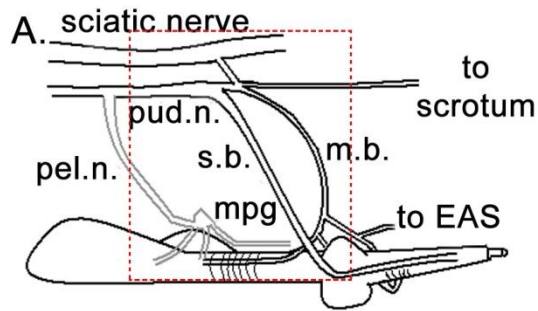
Figure A.1. Sacral plexus in the male rat. (A) Schematic of the sacral plexus and innervation of the lower urinary tract in the male rat. The pelvic nerve (pel.n.) originates from the sacral plexus and courses towards the prostate where it converges with the hypogastric nerve at the major pelvic ganglia (mpg). The pudendal nerve (pud.n.) branches into the sensory branch (s.b.) and motor branch (m.b.). The motor branch divides again, innervating the lower urinary tract and the external anal sphincter (EAS). **(B)** Sihler's stained specimen showing a ventral view of the boxed area in (A), including the pelvic and pudendal nerves. (P=prostate).
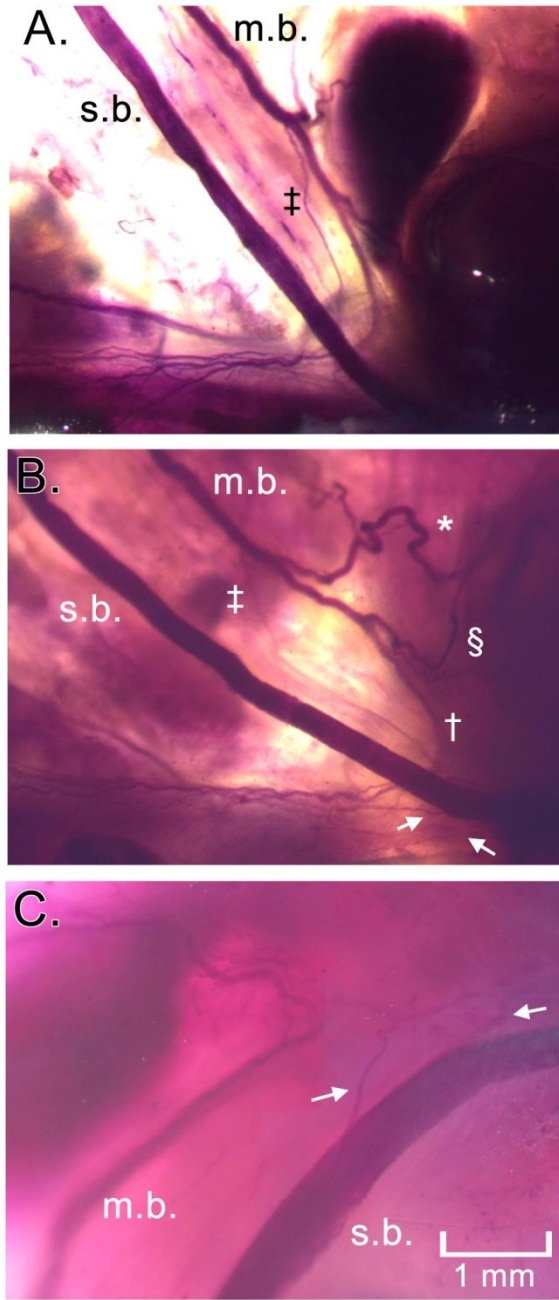
Figure A.2

Figure A.2. Innervation in the area of the penile bulb in the male rat. (A) Ventral view of the sensory (s.b.) and motor (m.b.) branches of the pudendal nerve arriving at the bulb of the penis. The motor branch crosses under the sensory branch and innervates the EUS (†). The bladder is to the left and the penis to the right. (B) Ventral view of the sensory (s.b.) and motor (m.b.) branches arriving at the bulb of the penis in another rat. (*) The motor branch gives off a branch to the EAS. (†) The motor branch crosses under the sensory branch to innervate the EUS (bladder is to the left). Arrows: autonomic fibers from the major pelvic ganglia converging on the sensory branch. (C) Ventral view of the s.b. and m.b. after the branches were dissected in a manner so that they no longer cross one another. Arrows: autonomic fibers from the major pelvic ganglia converging on the sensory branch.
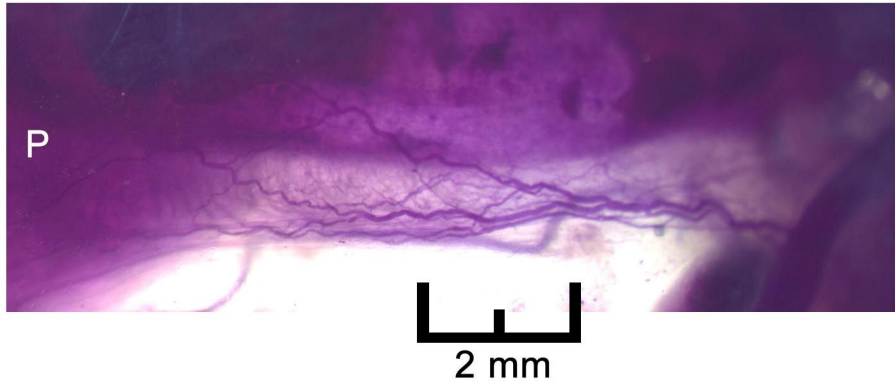
Figure A.3. EUS innervations by the pudendal motor branch in the male rat.
Ventrolateral view of the innervations of the EUS by the motor branch (arrow) of the
pudendal nerve. (P = prostate)
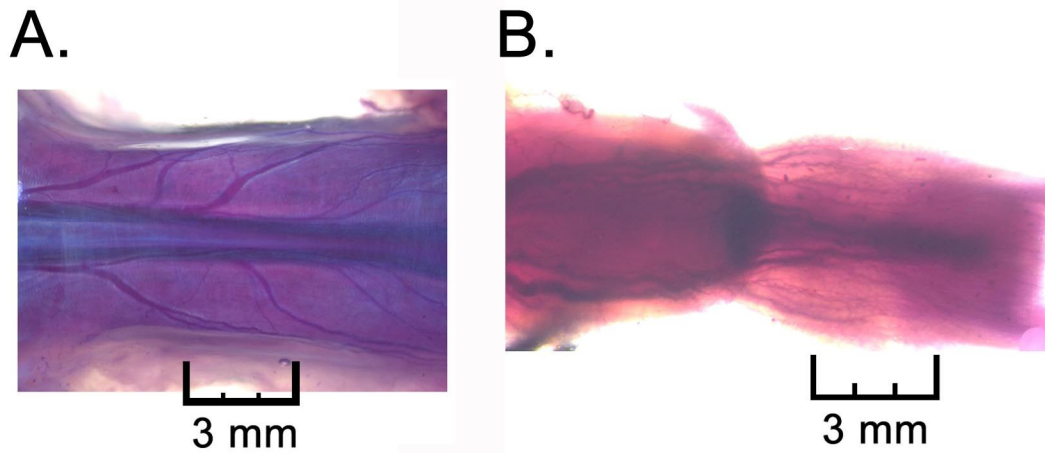
Figure A.4. Penile innervation by the sensory branch of the pudendal nerve in the male rat. (A) Dorsal view of the sensory branch innervating the penile body of the male rat, including branches which project laterally on the penile body (arrow). (B) Dorsal view of the sensory branch innervation of the glans penis in the male rat.
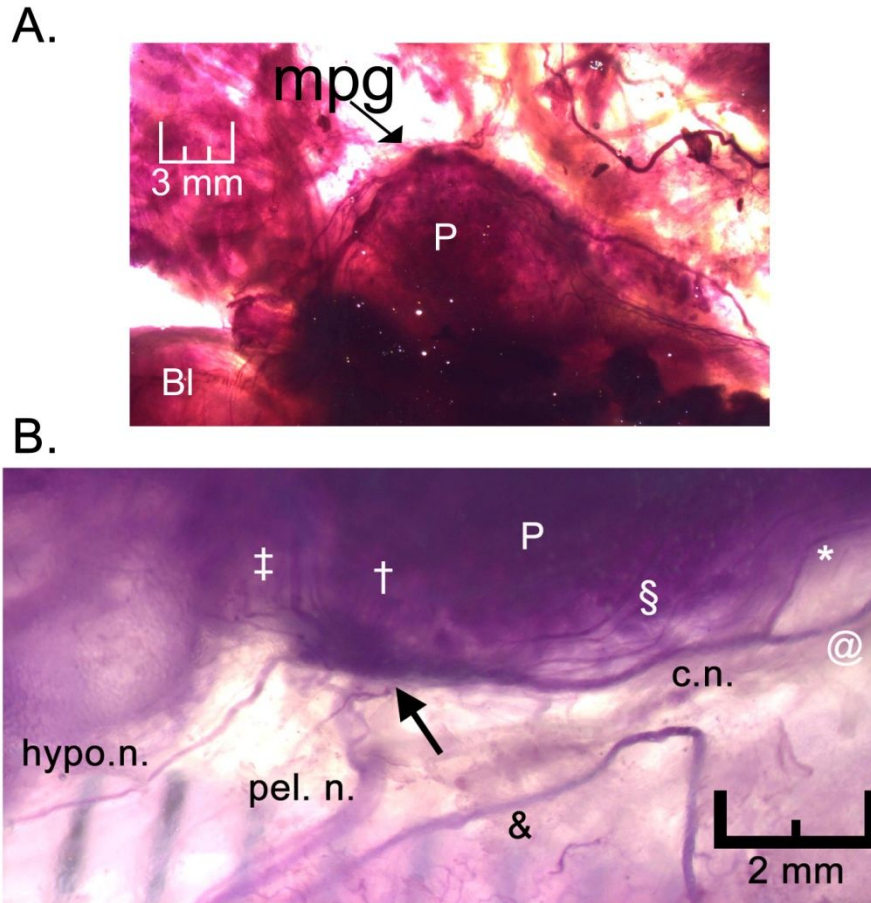
Figure A.5. Autonomic innervation of the male rat lower urinary tract. (A) Ventral view of the major pelvic ganglia (mpg) in a male rat. Branches innervate the bladder (B, *), the prostate (P, †) and the proximal urethra, and the penis (‡) (B) Ventral view of the major pelvic ganglia (arrow) in another male rat. The hypogastric (hypo.n.) and pelvic (pel.n.) nerves supply the major pelvic ganglia. Fibers from the major pelvic ganglia innervate the bladder (‡), the prostate (P) and proximal urethra (†), and the penis. The cavernous nerve (c.n.) projects towards the penile body. A projection from the cavernous nerve (@) and other medial projections from the major pelvic ganglia (§) converge with the DNP at the base of the penis.
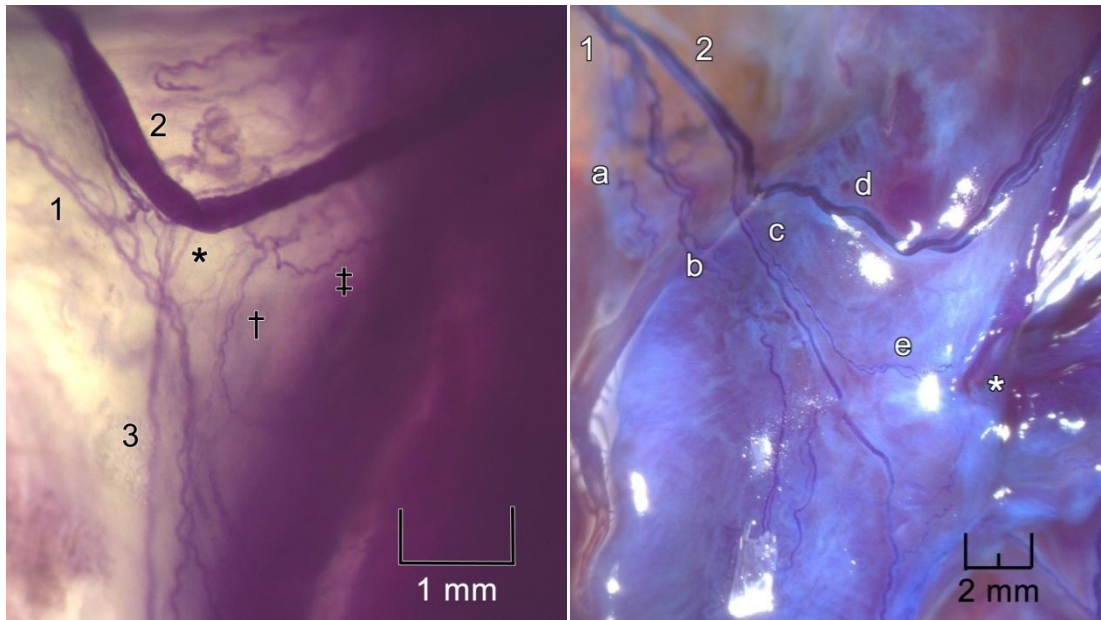
Figure A.6. Pudendal innervations of the female rat and female cat. (A) The motor (1) and sensory (2) branches of the pudendal nerve reaching the LUT in the female rat. The motor branch courses under the sensory branch and innervates the external urethral sphincter. The sensory branch converges (*) with autonomic branches (3,†) from the major pelvic ganglia, gives off a small projection to the urethra (‡), and courses on to the clitoris and distal urethra. (B) The pudendal innervations of the LUT in the female cat shows the motor branch (1) with a projection that goes to the external anal sphincter (a) and also innervating the external urethral sphincter (b). Also, there are two distinct components of the sensory branch (2): the dorsal clitoral nerve (d) which courses distally to the clitoris and vagina, and the cranial sensory nerve (c) which innervates the urethra. The cranial sensory projects proximally along the ventral urethra but also has a small projection to the urethra (e) near the urethral meatus (*).

Appendix B – Autonomous control of bladder continence and voiding by DNP stimulation

B.1 Introduction

Spinal cord injury (SCI) or other neurologic disorder can damage the neural pathways essential to normal urinary function and disrupt control of continence and micturition. Pudendal nerve stimulation is a potential means for restoring control of bladder function to persons with impaired control of continence and/or micturition due to SCI or other neurologic disorder (Gaunt et al. 2006). In this study, we implemented an autonomous system to control bladder function and evaluated serial control of continence and micturition by pudendal afferent stimulation in the anesthetized cat.

Stimulation of pudendal afferents in the dorsal nerve of the penis (DNP) can evoke bladder relaxation and activation, and stimulation can improve bladder continence and voiding (Chapter 2). Stimulation at 20-40 Hz evokes bladder excitation and improves voiding, while stimulation at 5-10 Hz evokes bladder inhibition and improves continence (i.e., increases bladder capacity) (Chapter 2). Additionally, similar frequency dependence and improvements in continence and voiding are observed for pudendal afferent stimulation in the chronic spinalized cat (Tai et al. 2007b). In humans, genital afferent stimulation can improve urinary continence in persons with SCI (Wheeler et al. 1992; Kirkham et al. 2001; Horvath et al. 2009), as well as generate bladder contraction (Gustafson et al. 2004; Yoo et al. 2007b).

Here we developed and implemented a computer-based autonomous control system, and used this system to quantify the efficacy of different stimulation paradigms for control of continence and different stimulation frequencies on the efficacy of bladder

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emptying. Continuous and delayed inhibitory stimulation evoked comparable bladder voiding, but bladder voiding was dependent on excitatory stimulation frequency.

B.2 Materials and Methods

B.2.1 Animal preparation

Experiments were conducted on 5 adult male cats. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at Duke University. Animals were anesthetized with ketamine HCl (35 mg/kg I.M.) and anesthesia was maintained with α-chloralose (65 mg/kg I.V. supplemented at 15 mg/kg as needed). Fluids (lactated Ringer's solution or saline/5% dextrose/sodium bicarbonate solution) were administered I.V. at 15 cc/kg/hr via a catheter in the cephalic vein, blood pressure was monitored via a catheter inserted into the carotid artery, core body temperature was maintained at ~38ºC with a thermostatic heating pad, and end tidal CO_2 was maintained between 3.0 and 4.0 % with artificial respiration. A midline abdominal incision was made to expose the bladder, and a 3.5 or 5 Fr suprapubic catheter was inserted into the bladder dome and secured with a purse-string suture. The abdominal incision was closed in layers. The catheter was connected to a pump for infusion of room temperature saline and also to a pressure transducer (Deltran, Utah Medical) for recording the bladder pressure.

B.2.2 Electrode placement

The DNP was accessed with the animal in a supine position through an incision made between the caudal border of the gracilis muscle and the prepuce. The nerve was dissected free from the body of the penis and a cuff electrode was placed on the nerve.

The cuff electrode consisted of a platinum contact embedded in silicone elastomer. A subcutaneous needle was placed in the ipsilateral leg for the return electrode.

B.2.3 Autonomous control

Computer control of stimulation of the DNP was implemented using a customized LabVIEW (National Instruments) program. The digital voltage signals output from LabVIEW were connected to the stimulating electrodes via voltage to current converters. Stimulation pulses were 100 μs duration at 2x the threshold amplitude to evoke reflex EMG responses in the external anal sphincter, and stimulation frequency was 5-10 Hz for bladder inhibition and 20-40 Hz for bladder excitation.

The bladder was continuously filled at 1 ml/minute with room temperature saline. Randomized control bladder filling trials were run to determine the volume threshold at which loss of continence occurred without stimulation (V_{ns}) and with continuous inhibitory stimulation (V_{cs}). Loss of continence was defined as leakage of urine >1ml or occurrence of a robust distension evoked bladder contraction (>15 cmH$_2$O magnitude for >10 seconds duration).

Autonomous control was implemented by setting the LabVIEW program to switch from inhibitory stimulation parameters (promoting urinary continence) to excitatory stimulation parameters (promoting micturition) at the volume half or three-quarters of the way between V_{ns} and V_{cs}, and this transition volume was denoted V_{ac} (Figure 1).

<div align="center">0.5   0.75</div>

B.2.4 Measurement of voiding

The voiding efficiency was defined as the volume of urine voided divided by the total bladder volume, determined by adding the residual bladder volume (the volume remaining in the bladder when voiding ceases) and the voided volume. For bladder continence, inhibitory stimulation was either continuous starting at the onset of filling (continuous inhibitory stimulation), continuous starting at 50% of V_{ns} (delayed inhibitory stimulation), or intermittent stimulation consisting of 10-20 second trains of stimulation with 10 second periods of no stimulation (duty-cycle inhibitory stimulation). For bladder voiding, excitatory stimulation was applied at 20, 33, or 40 Hz (1 minute on, 10 seconds off).

B.2.5 Data Analysis

Right-tailed, unpaired t-tests were used to determine if the increase in continent volumes for continuous inhibitory stimulation and autonomous control stimulation (compared to no stimulation) were greater than zero. Comparison of voiding efficiencies and residual volumes for control and autonomous control trials were made using one-way ANOVA with post-hoc paired comparisons with Bonferroni correction. Comparison of voiding evoked by excitatory stimulation at different frequencies following continuous or delayed inhibitory stimulation was done by two-way ANOVA with post-hoc comparisons with Bonferroni correction. All reported values for continent volumes, voiding efficiencies, and residual volumes are mean ± standard deviation.

B.3 Results

We implemented autonomous control of stimulation of the dorsal nerve of the penis (DNP) to control bladder continence and bladder voiding in 4 of 5 α-chloralose anesthetized adult male cats (Figure 1). In one cat, the difference between bladder capacity during filling with no stimulation (, 18 ± 3 ml, n= 3 trials) and bladder capacity during filling with continuous inhibitory stimulation (, 21 ± 2 ml, n=3 trials) was not sufficient for autonomous control. In the remaining 4 cats, the difference between   and   was at least 5 ml (range: 5 – 11 ml). Continuous low frequency (5-10 Hz) stimulation resulted in a significant increase in continent bladder volume of 65 ± 40 % (range: 28-116 % , n= 28 trials across 4 cats) compared to   (Figure 2).

The transition volume for switching from inhibitory DNP stimulation to excitatory stimulation was investigated in 2 cats. Switching to excitatory stimulation was programmed at   plus either 50 % or 75 % of   . Inhibitory stimulation prevented loss of continence up to the transition volume in 100 % of the filling trials in which the transition volume was set at   50%   , but inhibitory stimulation prevented loss of continence in only 54 % of the trials in which the transition volume was   75%   (Figure 3). The subsequent autonomous control trials investigating excitatory stimulation frequencies and inhibitory stimulation paradigms were performed with the transition volume at   50%   .

Continent and voided volumes were improved by stimulation of pudendal afferents. Autonomously controlled DNP stimulation increased continent volumes by 35 ± 18 % (range: 17-54%, n = 3-6 trials each in 4 cats) (Figure 2). Autonomous control stimulation (33 Hz excitatory stimulation) increased voiding efficiency (65 ± 13 %, n=26

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trials across 4 cats) compared to distension evoked voiding following either control filling (26 ± 18 %, n=12) or distension evoked voiding following continuous inhibitory stimulation (35 ± 24 %, n=14) (Figure 4A). Autonomous control stimulation (33 Hz excitatory stimulation) decreased residual volumes (9 ± 3 ml) compared to distension evoked voiding following filling with continuous inhibitory stimulation (15 ± 6 ml) and distension evoked voiding following filling with no stimulation (15 ± 5 ml) (Figure 4 B).

Voiding was investigated at different excitatory stimulation frequencies in 3 cats. Stimulation at 33 and 40 Hz resulted in greater voiding efficiency (63 ± 13 %, n= 14 trials and 61 ± 23 %, n= 11 trials) than 20 Hz stimulation (41 ± 22 % n=10 trials) (Figure 5 A). The post-void residual volumes (Figure 5B) were lower for 33 and 40Hz stimulation (10 ± 3 ml and 8 ± 3 ml) than for 20Hz stimulation (14 ± 7 ml).

The effect of the length of preceding inhibitory stimulation on voiding efficiency was examined by comparing voiding after inhibitory stimulation initiated at the onset of bladder filling or initiated at 50% of the distension evoked (no stimulation) volume threshold. The voiding efficiencies and residual volumes following these two different inhibitory stimulation paradigms were similar at each excitatory stimulation frequency (Figure 6). Compared to continuous inhibitory stimulation, delayed inhibitory stimulation reduced inhibitory stimulation duration by 33 ± 8 % (n = 35 trials across 3 cats). Duty cycle inhibitory stimulation (10-20 seconds on, 10 seconds off) was investigated as an alternative means of inhibitory stimulation, but duty cycle inhibitory stimulation failed to increase continent bladder volumes compared to no stimulation (2 cats), as distension evoked bladder contractions and voiding occurred during the periods between stimulation.

B.4 Discussion

Autonomous control of bladder function was implemented in the cat. The results demonstrate the feasibility of autonomous bladder control using programmed DNP stimulation and illustrate that the stimulation paradigm can impact bladder continence and voiding.

DNP stimulation improved urinary continence and voiding. Low frequency stimulation (5-10 Hz) improved continence, consistent with previous studies of pudendal afferent stimulation (Wenzel et al. 2006; Tai et al. 2007b; Woock et al. 2008). High frequency stimulation (33-40 Hz) improved bladder voiding consistent with previous studies (Boggs et al. 2006a; Tai et al. 2007b; Woock et al. 2008; Yoo et al. 2008a) However, this study was the first investigation of automated transition from inhibitory stimulation parameters to excitatory stimulation parameters. It was determined that the transition volume is important because the variability in continent bladder volumes requires a transition volume significantly lower than the bladder capacity for continuous inhibitory stimulation. In the cat, transition at 50 % avoided loss of continence before switching (i.e., incontinence) while voiding at 75 % was less reliable. Bladder volumes are much larger in humans, so difference in continent volumes may allow transition at volumes closer to the bladder capacity in the presence of continuous inhibitory stimulation. The transition volume is an important parameter for serially controlling continence and micturition.

Bladder voiding and residual volumes were dependent on the excitatory stimulation frequency. Stimulation at 33 and 40 Hz evoked greater voiding and lower residual volumes than stimulation at 20 Hz. These results suggest that excitatory

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stimulation frequency can affect the efficacy of a bladder control neural prosthesis. Voiding efficiencies for stimulation at 33 and 40 Hz were comparable to voiding previously reported for DNP stimulation (Chapter II). Optimal parameters for bladder voiding may vary from individual to individual, as observed for bladder voiding evoked by sacral root stimulation (Brindley et al. 1982; Brindley et al. 1986).

Delayed and continuous inhibitory stimulation did not impact subsequent bladder voiding efficiency or residual volumes. Delayed inhibitory stimulation reduced inhibitory stimulation time by 33 ± 8 %. However, conditional stimulation (delivered only when the bladder is contracting) reduced stimulation time by 67 % while increasing continent volume (Wenzel et al. 2006), and conditional stimulation has been shown to improve continent volumes in humans (Kirkham et al. 2001; Dalmose et al. 2003; Hansen et al. 2005; Horvath et al. 2009). Despite the superior reduction in stimulation duration for conditional stimulation, delayed inhibitory stimulation can reduce stimulation duration without requiring feedback signals from recordings of the bladder pressure (Kirkham et al. 2001; Hansen et al. 2005) or external anal sphincter muscle activity (Horvath et al. 2009). User-controlled stimulation is an effective method of delivering inhibitory pudendal afferent stimulation to increasing bladder capacity while minimizing stimulation duration , but the user must have sufficient hand and arm function and also be able to sense bladder contraction (Lee et al. 2002).

This study illustrated that the bladder could be controlled autonomously via DNP stimulation. It highlighted the potential impact of the transition volume, excitatory stimulation frequency, and inhibitory stimulation paradigm in the implementation of a neural prosthesis to control bladder function via DNP stimulation.
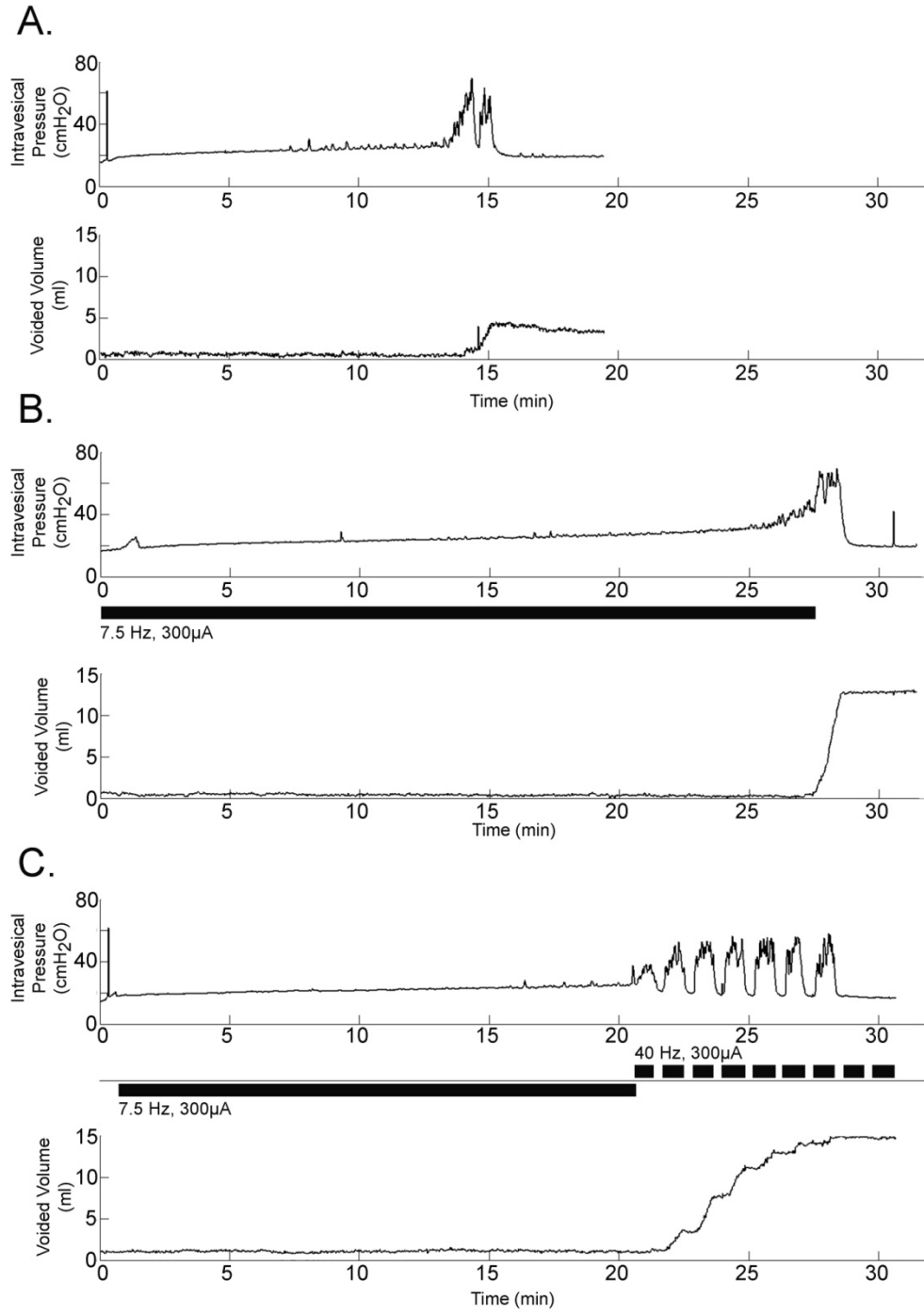
Figure B.1

Figure B.1. Autonomous control of bladder continence and emptying by pudendal afferent stimulation in the anesthetized cat. (A-C) The upper trace shows bladder pressure as a function of time during bladder filling at 1 ml /minute. The lower trace shows the total voided volume over time, and DNP stimulation is indicated by black bars in between the upper and lower traces. (A) Control bladder filling with no stimulation. Continence was lost at 14 ml of infused volume, and 4.5 ml was voided by distension-evoked reflex micturition. The residual bladder volume was 14.5 ml, and the voiding efficiency was 24%. (B) Bladder filling with continuous low frequency (inhibitory) DNP stimulation. Continence was lost at 28 ml of infused volume. 13.5 ml was voided by distension-evoked reflex micturition. The residual bladder volume was 16 ml, and the voiding efficiency was 45%. (C) Bladder filling with autonomous control of excitatory (upper) and inhibitory (lower) stimulation (bars are divided by a horizontal line). Stimulation was switched from inhibitory to excitatory parameters at 20 ml of infused volume. 15 ml was voided by 40 Hz stimulation. The residual bladder volume was 9 ml, and the voiding efficiency was 62%.
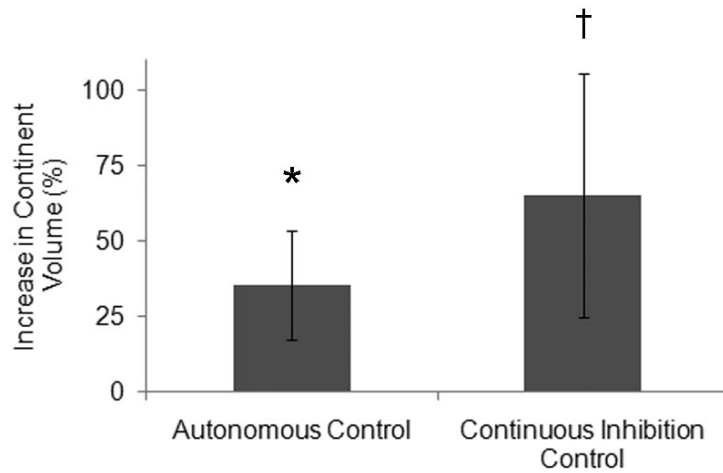
Figure B.2. Increase in continent bladder volume compared to filling with no stimulation. The bladder capacity was increased by continuous inhibitory DNP stimulation compared to no stimulation (†, p<0.05, n= 28 trials across 4 cats). Autonomous control stimulation with transition from inhibitory to excitatory DNP stimulation at 50 % of the difference between control bladder capacity during filling with and without inhibitory stimulation also increased bladder capacity compared to filling with no stimulation (*, p< 0.05, n= 36 trials across 4 cats).
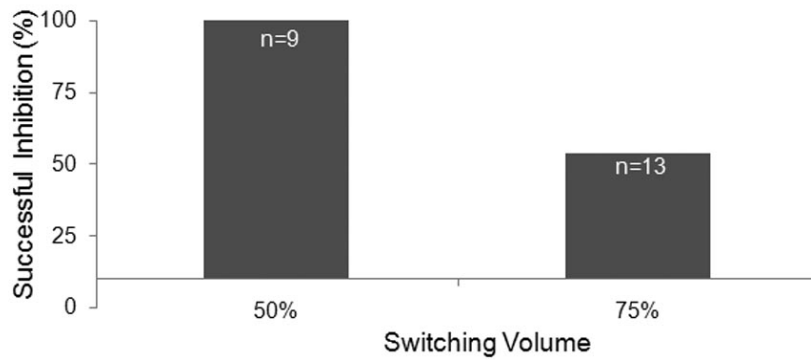
Figure B.3. The reliability of stimulation evoked inhibition was dependent on the transition volume. Switching from inhibitory to excitatory DNP stimulation was initiated at 50 % or 75 % of the continent volume increase from filling with no stimulation to filling with continuous inhibitory stimulation. Switching at the 50 % volume resulted in continence maintained throughout inhibitory stimulation in all trials, while switching at the 75 % volume resulted in successful inhibition in slightly over 50 % of the trials (n= 22 trials in 2 cats).
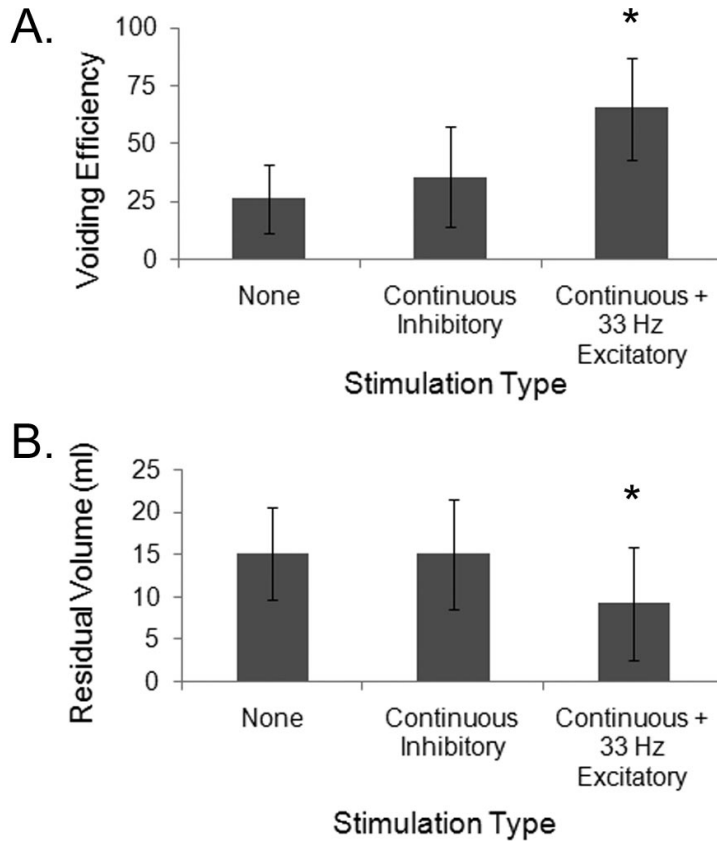
Figure B.4. Bladder voiding was improved by autonomous control. (A) Voiding efficiencies for bladder filling trials with no stimulation, continuous inhibitory stimulation, and autonomous control stimulation were dependent on the stimulation type ($p<10^{-9}$, ANOVA, n=52 trials across 4 cats). Compared to control filling trials (with no excitatory stimulation), autonomous control stimulation trials (33 Hz excitatory stimulation) increased bladder voiding (*, $p< 0.05$, post-hoc paired comparison with Bonferroni correction). (B) Residual bladder volumes were dependent on stimulation type ($p<10^{-6}$). Autonomous control stimulation trials (33 Hz excitatory stimulation) decreased residual volume (*, $p< 0.05$, post-hoc paired comparison with Bonferroni correction).
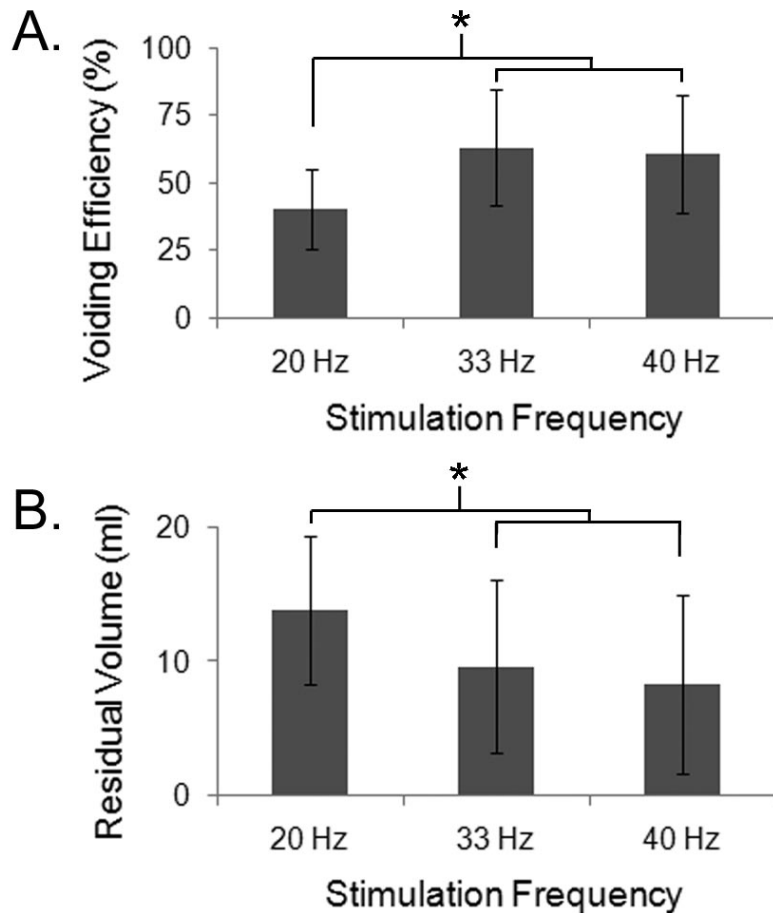
Figure B.5. Bladder voiding at different excitatory stimulation frequencies.
(A) Voiding efficiency was dependent on excitatory stimulation frequency ($p<10^{-9}$, ANOVA, n=35 trials across 3 cats). 33 and 40 Hz stimulation evoked greater bladder voiding than 20 Hz stimulation (*, $p< 0.05$ post-hoc paired comparison with Bonferroni correction). (B) Residual bladder volume was dependent on excitatory stimulation frequency ($p<10^{-6}$). Residuals volumes were less for stimulation at 33 and 40 Hz than for stimulation at 20 Hz (*, $p< 0.015$, post-hoc paired comparison with Bonferroni correction).

A.

B.
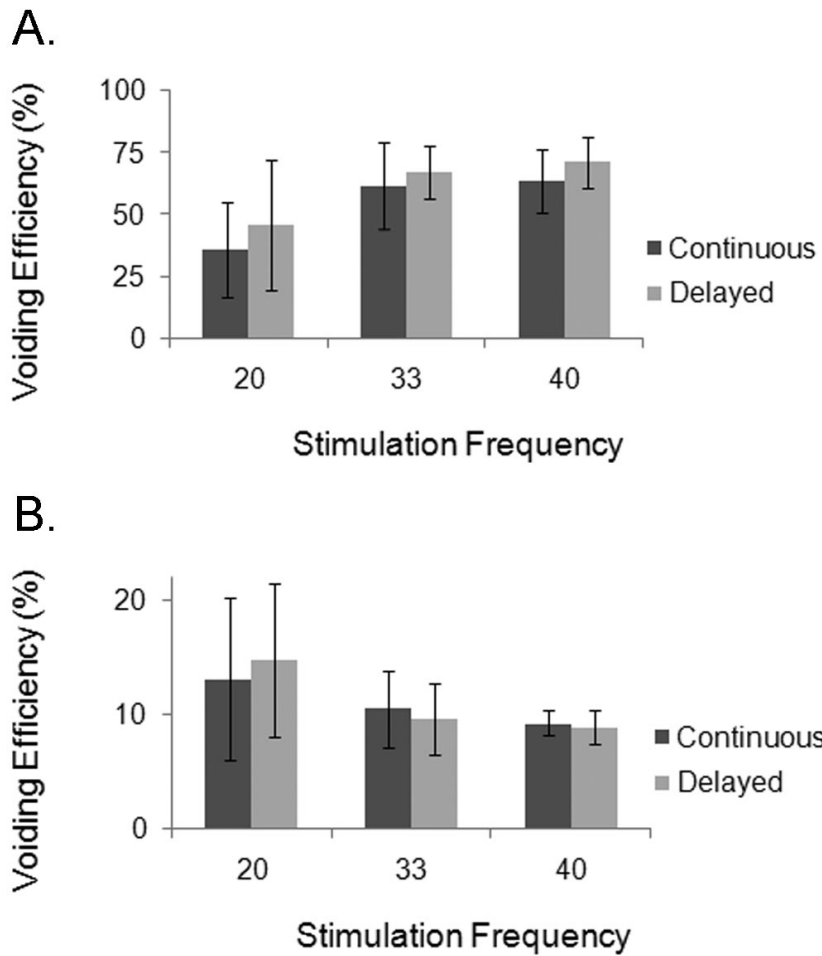
Figure B.6. Bladder voiding at different stimulation frequencies following continuous and delayed inhibitory stimulation. (A) Voiding efficiency was dependent on stimulation frequency ($p < 0.001$, two-way ANOVA, n= 35 trials across 3 cats), but did not depend on the inhibitory stimulation paradigm ($p = 0.17$). (B) Residual bladder volume was dependent on excitatory stimulation frequency ($p < 0.0005$), but residuals for continuous and delayed inhibitory stimulation were similar at each frequency ($p = 0.28$).

Appendix C – Computer simulation of the convergence of pudendal and pelvic afferents illustrates a potential mechanism of the frequency dependent pudendal afferent activation evoked bladder responses

C.1 Introduction

Pudendal afferent fiber (PudA) activation is a potential means of restoring control of bladder function to persons with spinal cord injury (SCI). Activation of pudendal genital afferent fibers at 33-40 Hz evokes bladder excitation and increases voiding efficiency, while activation at 5-10 Hz evokes bladder inhibition and improves continence (Chapter 2). Bladder behavior is modulated by autonomic bladder innervation, but recent results imply that only the parasympathetic bladder innervation (travelling in the pelvic nerve) is critical to bladder excitation and inhibition evoked by genital afferent fiber stimulation (Chapter 5). The objective of this study was to develop a computer model of the convergence of PudAs and pelvic afferent fibers (PelAs) in the sacral spinal cord to investigate the frequency dependence of the bladder response to PudA activation.

Bladder filling activates PelAs (Barrington 1931; de Groat et al. 1969; Habler et al. 1993), and above a volume threshold these afferents activate parasympathetic preganglionic neurons (PGNs) that provide efferent excitatory input to the bladder via the pelvic nerve. The activation of PGNs by PelAs is polysynaptic and relies on a spinobulbospinal pathway between the sacral spinal cord and the pontine micturition center. PudA activation can influence PGN activity through a supraspinal pathway (Barrington 1931) and a spinal pathway (de Groat 1975) . Barrington identified a spinobulbospinal reflex by which activation of urethral afferents in the pudendal nerve

augmented bladder contractions and bladder voiding (Barrington 1931). However, de Groat observed that bladder contractions can be elicited by genital afferent activation of a spinal reflex in neonatal and chronic spinal cord injured cats (de Groat 1975).

Electrophysiological recordings have characterized the response of sacral interneurons (INTs) and PGNs to electrical stimulation of PelAs and PudAs (de Groat et al. 1969; de Groat 1978; McMahon et al. 1982a; McMahon et al. 1982b; McMahon et al. 1982c; Araki et al. 1996; Araki et al. 1997; Mazières et al. 1997; Lu et al. 2000). These recordings illustrate that PudA activation can evoke both depression and facilitation of synaptic inputs to PGNs. The electrophysiological recordings also included chronic spinal cord injured (SCI) cats (de Groat et al. 1969), which is of particular interest because electrical stimulation of PudAs is a potential means of restoring control of bladder function to individuals with SCI (Yoo et al. 2007b).

Based on the response of INTs and PGNs to PelA and PudA stimulation and the bladder responses to pudendal nerve stimulation, models of the micturition pathway in α-chloralose anesthetized cats and chronic SCI cats were simulated using integrate and fire neurons in Matlab. The results provide evidence that the convergence of pudendal and pelvic afferent fibers on sacral interneurons provides an appropriate substrate for the frequency dependent bladder responses evoked by PudA activation in the presence of tonic PelA activity.

C.2 Methods

A model of the micturition related neural elements in the sacral spinal cord, including the convergent input from PudAs, was developed for the α-chloralose

anesthetized intact cat and for the chronic SCI cat (Figure 1).

For simulation in Matlab to investigate the modulation of pelvic nerve transmission by pudendal nerve activity, it was only necessary to simulate as integrate and fire neurons the components of the pathway with multiple inputs or more complex synaptic mechanisms. A sacral interneuron (INT) and a PGN were modeled as integrate and fire neurons. PelA activity and PudA activity were the inputs to the model. When bladder volume is above a minimum threshold, PelAs have been reported to fire in bursts lasting 0.5-2.5 seconds at frequencies within the bursts of 12-60 Hz (de Groat et al. 1982). The intraburst firing rate varied across PGNs but did not change with increasing bladder pressure for a single PGN (de Groat et al. 1982). The effects of PudA stimulation on bladder activity have focused on PudA stimulation at 1-40 Hz (Boggs et al. 2005; Tai et al. 2007b). Spike trains were generated to represent the desired firing behavior for these inputs. The pelvic afferent input was modeled as a Poisson spike train with the average frequency of 12, 36, or 60 Hz. While PGNs fire at these rates, pelvic afferents fire more slowly, but convergence of multiple pelvic afferent inputs (modeled here as a single input for simplicity) may produce the higher PGN firing rates. The pudendal afferent activity was modeled as a regular spike train, and the addition of jitter in the pudendal afferent spike train was observed to have negligible effects on the PGN output (data not included). While the proposed model of the neural pathway is fairly complex, simulation was simplified by generating the output of several neurons simply by adding a delay to the spike times from the PelA or PudA spike times (Table I).

The models investigated here represent experimental conditions for α-chloralose anesthetized adult cats. In the absence of tonic inhibition from cortical neurons (due to α-

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chloralose anesthesia), the supraspinal neurons in the pontine micturition center do not alter the neural activity from PelAs (de Groat et al. 1982).

Integrate and fire neurons were simulated in Matlab. Several parameter values were determined from previous studies (Table II) while others were determined by simulation of the neurons (see Results). The membrane voltage, V, was updated at each time step, dt, by $V = V + dV$, where

$$dV = dt * \frac{1}{\tau_n}(V - V_{rest} + I \cdot R).$$

For this equation, R was the input resistance, τ_n was the membrane time constant, V_{rest} was the resting membrane potential, and I was the synaptic current. When $V \geq V_{thresh}$, an action potential was determined to have occurred and $V = V_{spike}$. Another spike could not occur until after the refractory period. I was determined to be the net current of all the inputs to a particular neuron.

$$I = \sum I_{syn},$$

where I_{syn} was the current due to a single input. I_{syn} depended on the synaptic conductance, g_{syn}, and the synaptic reversal potential, V_{syn}:

$$V_{syn} \cdot I_{syn} = g_{syn} \cdot (V - V_{sny}).$$

The synaptic conductance, g_{syn}, for an input was

$$g_{sny} = \overline{g_{sny}} \cdot (1 + S_{exc/inh}) \cdot (\frac{t - t_{spike}}{\tau_{syn}}) \cdot \exp(-\frac{t - t_{spike} - \tau_{syn}}{\tau_{syn}}),$$

where $\overline{g_{sny}}$ was the maximum conductance in the absence of facilitation or depression, $S_{exc/inh}$ was a value that represents the spike-triggered facilitation of

excitatory inputs or depression of inhibitory inputs, t was the current time, t_{spike} was the

time the last spike occurred, and τ_{syn} was the synaptic conductance time constant. The

synaptic plasticity constant had the same magnitude as a function of time for excitatory

and inhibitory synapses, but to simulate facilitation in excitatory synapses the constant

was positive, while depression was simulated in inhibitory synapses by making the

constant negative.

$$S_{exc} = (\frac{t - t_{spike}}{\tau_s}) \cdot \exp(-\frac{t - t_{spike} - \tau_{syn}}{\tau_s}), \, S_{inh} = -(\frac{t - t_{spike}}{\tau_s}) \cdot \exp(-\frac{t - t_{spike} - \tau_{syn}}{\tau_s})$$

where τ_s is the time constant of the spike triggered facilitation/depression.

For analysis of PGN firing evoked by PelA and PudA inputs, the mean PGN

firing rate was computed for simulation with both PelA and PudA inputs and for

simulation with only PelA inputs. The percent change in firing rate was computed to

determine the effect of the presence of the PudA input on PGN activity.

C.3 Results

C.3.1 Determining $\overline{g_{sny}}$ and τ_s

The characterization in previous studies of the synaptic transmission between

various neural components of the micturition pathway was used to determine the

parameters $\overline{g_{sny}}$ and τ_s.

Because PudA activation at 33 Hz was reported as the optimal frequency for

evoking bladder contractions (Chapter 2), the value of τ_s was initially fixed at

$\tau_s = \frac{1}{33Hz}$ while determining suitable $\overline{g_{sny}}$ values for the different synaptic inputs.

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These values were determined by examining the effects of paired-pulse stimulation and setting the parameter values so the responses of the INT and PGN neurons to pelvic afferent and pudendal afferent stimulation were consistent with in vivo observations (Figure C.2) (de Groat et al. 1969).

Synaptic conductances were also chosen in order for paired pulse stimulation of the interneuron (in the absence of other inputs) to not elicit a spike in the PGN at interpulse intervals less than 50ms (20Hz). For the intact spinal model neurons, behavior similar to that observed in vivo was obtained at maximum synaptic conductance values of 50 nS for inhibitory inputs and 13-30 nS for excitatory inputs, comparable to previously reported values (Lee et al. 2005).

The change in PGN activity was found to depend on the PudA activation rate as well as the value of τ_s (Figure C.3). The value of τ_s did not affect the general effect (increase or decrease) on PGN activity of PudA activation at 10 Hz or 33 Hz, but 20 Hz PudA activation increased or decreased PGN activity dependent on τ_s.

C.3.2 Model Simulation

To examine the effect of PudA activity on simulated PGN output, 3 second simulation trials were run in which the PelA burst occurred from 0.5-2.5 seconds and PudA activity was simulated from 1-2 seconds. This allowed for the activity during PudA activation to be compared with the baseline PelA activity before and after stimulation. PelA activity was simulated at 12, 36, and 60 Hz, a sampling of the lowest, middle, and highest intraburst frequencies observed across PelAs (de Groat et al. 1982).

The effect of PudA activation on PGN output was apparent from raster plots of the activity in PelA, PudA, INT, and PGN (Figure C.4). PudA activation at 33 Hz

appeared to increase PGN firing and PudA activation at 10Hz appeared to decrease PGN firing.

The firing rates of the PGN response to PelA input were compared in the absence and presence of PudA activation. The PGN response to PelA and PudA activation depended on PelA activation frequency and PudA activation frequency (Figure C.5). The increases in PGN activity during 33Hz PudA activation are different for different rates of PelA activity (Figure C.6).

Similar results were obtained simulating the chronic SCI cat pathway model (Figure C.7). However, the decrease in PGN activity evoked by 10 Hz PudA activation does not appear as robust in the chronic SCI cat.

C.4 Discussion

The neural pathways associated with micturition were modeled for the α-chloralose anesthetized cat and the chronic SCI cat. PelA and PudA fiber activity were simulated to determine the effect of pudendal nerve stimulation on PGN activity. The results suggest that the excitatory and inhibitory post-synaptic potentials evoked by convergent pudendal and pelvic afferents can result in PGN activity consistent with the frequency dependence of the DNP-bladder reflexes.

Major contributors to PGN activity were excluded (e.g. colonic afferent activity (Floyd et al. 1982; McMahon et al. 1982a) for the sake of simplicity in this model. First, the simulations here provide a snapshot of the PGN response to PudA activation. In reality, PGN activity drives bladder contractions and the changes in bladder pressure modulate pelvic afferent activity. If PudA activation increased or decreased PGN to a

degree that altered the state of the bladder (generating or inhibiting a contraction) then the change in bladder activity would change the PelA activity. Including this feedback loop may provide more insight into the development and time-course of excitatory and inhibitory bladder responses to PudA activation. Another important missing piece of the micturition pathway is recurrent inhibition of PGN activity. PGN activation has been found to inhibit sacral interneurons that transmit PelA activity to the PGN (Mazieres et al. 2006). This recurrent inhibition has been proposed to have frequency dependent behavior and would likely influence the simulation results. It is also worth noting that it is difficult to determine the effect of the results observed here. It may be that large increases or decreases in firing rate are necessary to alter bladder function, or very small changes across many, many PGNs could account for the PudA elicit excitation and inhibition of the bladder detrusor muscle. In chronic SCI cats it was observed that the inhibitory effect of PudA stimulation increased with increasing stimulation intensity, suggesting that the degree of inhibition depends at least partly on the extent of activation (Tai et al. 2006).

Increases in PGN activity during 33Hz PudA activation and decreases in PGN activity during 10Hz PudA activation were observed for simulations of models of the α-chloralose anesthetized cat and the chronic SCI cat. The degree of the modulation of PGN activity by PudA activity depended on PelA activation rate, PudA firing rate, and the specific conductance and spike-triggered time constants of the model. At low and medium levels of PelA activation (12 and 36 Hz), PudA activation at 33 Hz evoked large increases in PGN activity, but when PelA were activated at 60 Hz PudA activation had little effect on the PGN output. Contrary to 33 Hz PudA activation, 10 Hz pudendal

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afferent activation resulted in greater inhibition of PGN activity when PelA activity was 60 Hz than when PelA activity was 12 Hz, most likely due to the greater probability of overlapping PudA and PelA post-synaptic potentials at higher rates of PelA activity.

These simulations suggest that the frequency characteristics of the bladder response to PudA stimulation may be due to the time constant associated with facilitation and depression of synaptic connections in the micturition pathway (Figure 5). Variability observed in the response of the PGN when the time constant was changed was nothing more severe than the animal to animal variability in the bladder response to PudA stimulation. There appears to be variation in the optimal frequencies for excitation and inhibition from animal to animal, but the range remains relatively small (20-40Hz and 3-10 Hz for excitation and inhibition, respectively) (Chapter 2). Interestingly, whether 20 Hz PudA activation increased or decreased PGN activity was dependent on the value of τ_s. In vivo, the bladder response to 20 Hz DNP stimulation varied across cats, consistent with the theory that the variability in the frequency dependent mechanism may cause variation in the frequency response at 20 Hz.

While the simulations results provide evidence the PudA stimulation can increase or decrease PGN activity dependent on PelA activity and PudA stimulation frequency, it also may provide insight into the volume mechanism of the pudendal-bladder excitatory reflex. If stimulation can increase the PGN bursting activity, and increasing the bladder volume increases the frequency of PGN bursts (de Groat et al. 1982), then it is likely that bladder contractions occur when the combination of PGN intraburst activity and burst frequency reach a critical level. The results suggest PudA stimulation can increase the intraburst activity experienced by the sacral interneuron, reducing the threshold burst

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frequency for bladder contraction. Alternatively, the bursting nature of the PGNs results from an inhibitory mechanism, possibly recurrent inhibition (de Groat et al. 1982), and DNP stimulation may evoke bladder contractions by inhibiting this inhibitory mechanism and increasing the bursting frequency of PGNs.

A major question that arises from these simulations is if the bladder response to stimulation of PudA in the intact, α-chloralose anesthetized cat and the response in the chronic SCI cat originate from the activation of the same excitatory pathway. The models proposed here suggest that before SCI the excitation relies on a relay through the pontine micturition center, but after SCI the excitation is a purely spinal reflex. It is unlikely the spinal reflex can be accessed in intact cats because descending serotonergic inhibitory inputs from the raphe nucleus must be blocked or severed for the spinal excitatory reflex to be accessed by PudA activation (Thor et al. 1990). However, the inhibition of PGN activity by 10 Hz PudA activation in the model is not as robust as expected, suggesting further neural reorganization following SCI may contribute to the sacral spinal cord inhibitory mechanism.

Based on these simulation results it appears that a plausible mechanism for the bladder response to PudA stimulation is the paired facilitation and depression of excitatory and inhibitory synapses in the sacral spinal cord micturition pathway.

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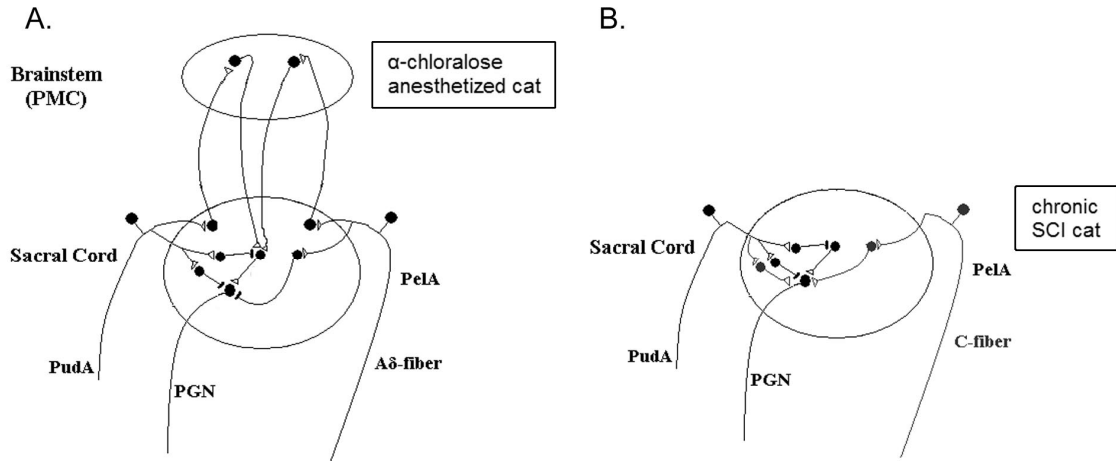
Figure C.1. Neural pathways associated with micturition. **(A)** Micturition pathway in the α-chloralose anesthetized cat. The activation of PGNs relies heavily on the involvement of spinobulbospinal connections between the sacral spinal cord and the pontine micturition center (PMC). Pathways involving sacral interneurons were simplified as disynaptic connections even though some may be polysynaptic. **(B)** Micturition pathway in the chronic SCI cat. The supraspinal excitatory inputs in the α-chloralose anesthetized cat are replaced by spinal excitatory inputs unmasked by the loss of supraspinal inhibition of these spinal excitatory pathways (Thor et al. 1990). A c-fiber pelvic afferent fiber pathway emerges in the chronic SCI cat. **(A, B)** Excitatory synaptic inputs are designated by projections ended in open triangles. Inhibitory synaptic inputs are designated by projections ending in solid bars.

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Table C.1. Synaptic Input Delays

Input Source	Via	Input Target (E/I)	Delay (ms)	Reference
PudA	PONS	INT (**E**)	90	(McMahon et al. 1982a; Mazières et al.
PelA	PONS	INT (**E**)	80	(de Groat et al. 1982)
PudA	Sacral Interneurons	INT (**I**)	3	(Araki et al. 1996)
PelA	Sacral Interneurons	PGN (**I**)	5	(de Groat et al. 1969)
PudA	Sacral Interneurons	PGN (**I**)	30	(Mazières et al. 1997)
INT	direct	PGN (**E**)	2.1	(Araki et al. 1996)
E-excitatory synaptic input, **I**-inhibitory synaptic input				

Table C.2. Integrate and Fire Neuron Parameters

Parameter	Value		Ref
	INT	PGN	
Input resistance	40MΩ	25MΩ	(Lee et al. 2005)
tau, neuron	10ms	6.5ms	(Lee et al. 2005)
Vrest	-65mV	-50mV	(Lee et al. 2005)
Vspike	20mV	20mV	
Vthresh	-49mV	-34mV	
refractory period	2ms	2ms	
Vsyn,i	-75mV	-75mV	(Brette et al. 2005)
Vsyn,e	0mV	0mV	(Brette et al. 2005)
tau,i	10.5ms	10.5ms	(Brette et al. 2005)
tau,e	2.3ms	2.3ms	(Brette et al. 2005)
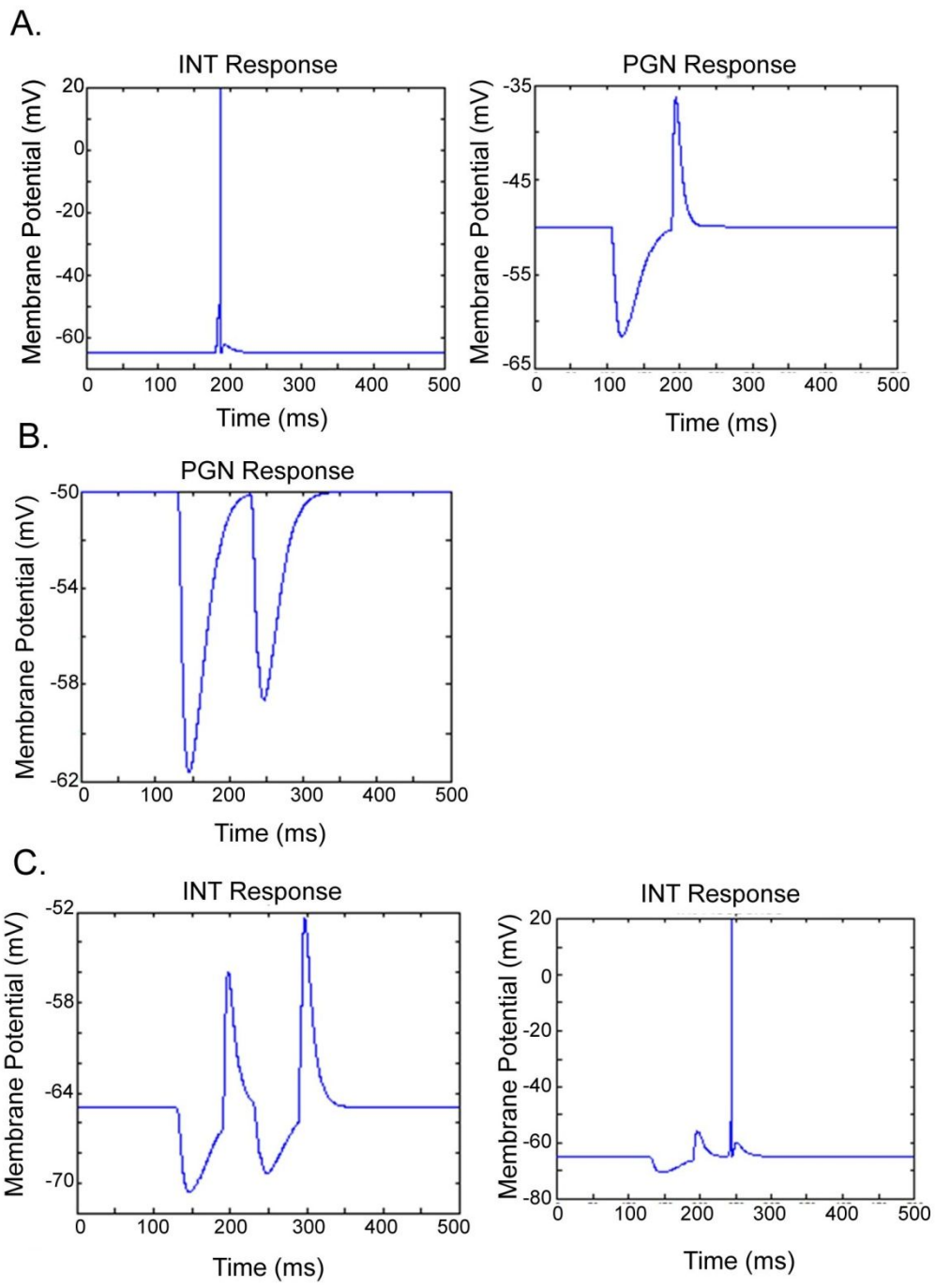
Figure C.2

Figure C.2. Responses evoked in the PGN and INT by single and paired pulses from the PudAs and PelAs. (A) Single pulse PelA activity initiates an action potential in the INT (left) but not the PGN (right). The PGN response shows an early inhibitory post-synaptic potential and late excitatory post-synaptic potential similar to that described in vivo (de Groat et al. 1969). **(B)** Paired pulse stimulation of the PudA at 10 Hz evoked IPSPs in the PGN. **(C)** Paired pulse stimulation of the PudA at 10Hz produced biphasic responses in the INT but no action potential (left). For stimulation at 20 Hz, the second pulse elicits a spike in the INT (right).
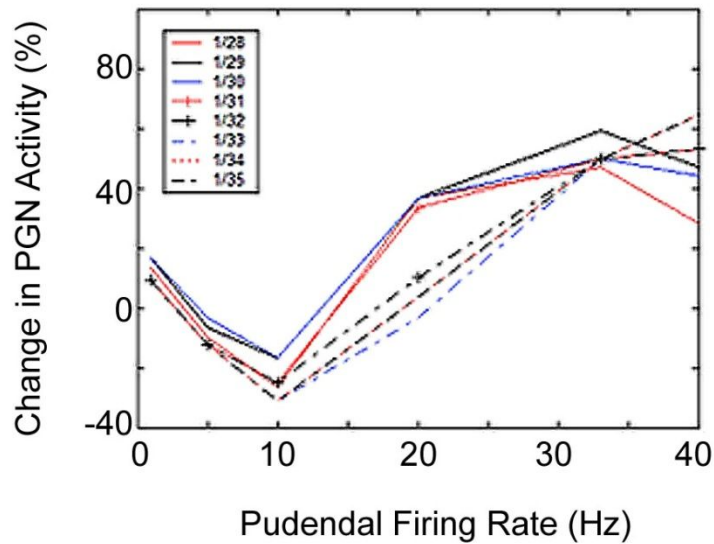
Figure C.3. The effect of the spike-triggered time constant on the change in average PGN firing due to various rates of PudA activation. The change in PGN activity due to PudA activation was dependent on the spike-triggered time constant (time constant of spike triggered facilitation or depression of synaptic conductance) and PudA activation rate. However, at all the spike-triggered time constant values examined there was still a reduction in PGN activity at 10Hz PudA activation and an increase in PGN activity at 33Hz PudA activation, but the effect of 20 Hz PudA activation (reduction or increase in PGN activity) was dependent on the spike-triggered time constant.
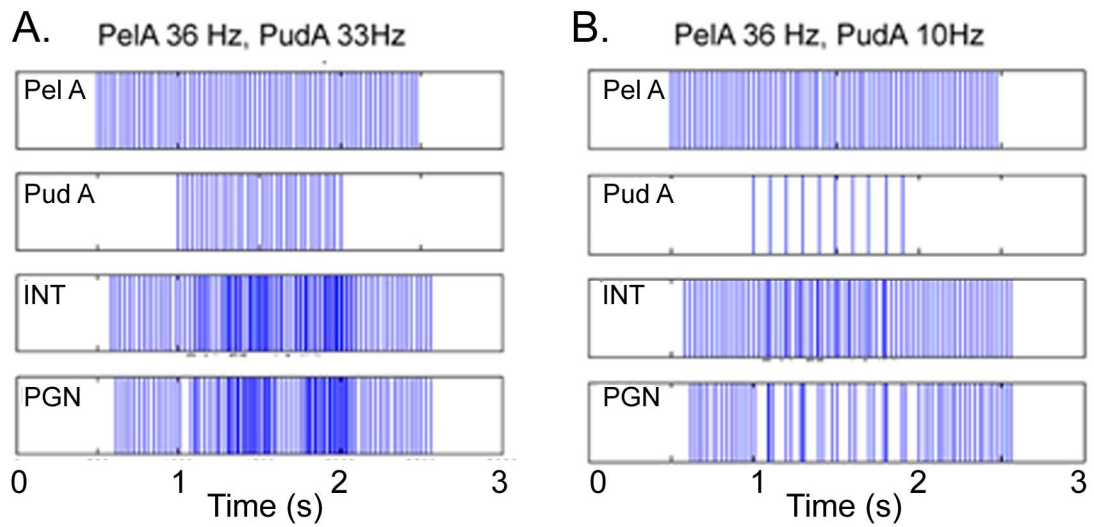
Figure C.4. Raster plots of the neural firing in PelA, PudA, INT, and PGN.
The raster plots show the generated PelA and PudA spike trains and the simulated
activity of the INT and PGN when the PelA and PudA spike trains are used as inputs to
the simulated network. **(A)** The PelA is active at 36Hz and the PudA at 33Hz. The
increase in INT and PGN firing during PudA activation is apparent in this plot. **(B)** The
PelA is active at 36Hz and the PudA at 10Hz. The decrease in PGN firing during PudA
activation is visible in this plot.

12 Hz Pelvic Afferent Firing
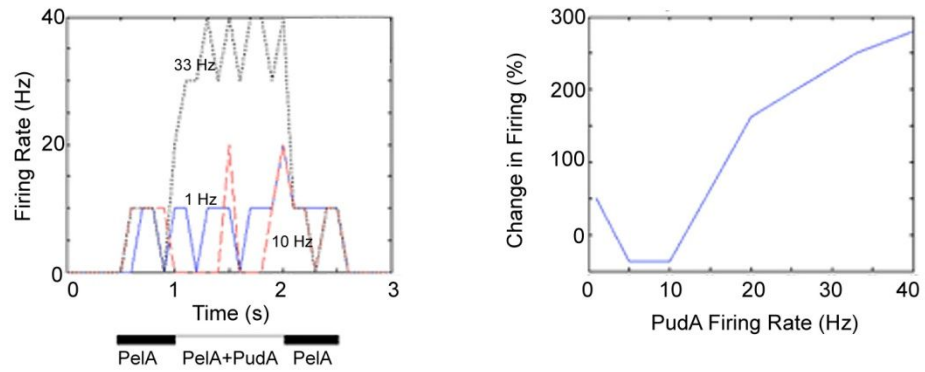
36 Hz Pelvic Afferent Firing
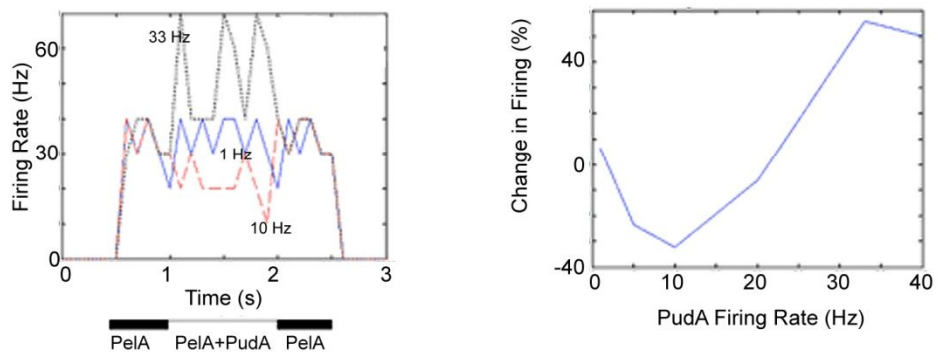
60 Hz Pelvic Afferent Firing
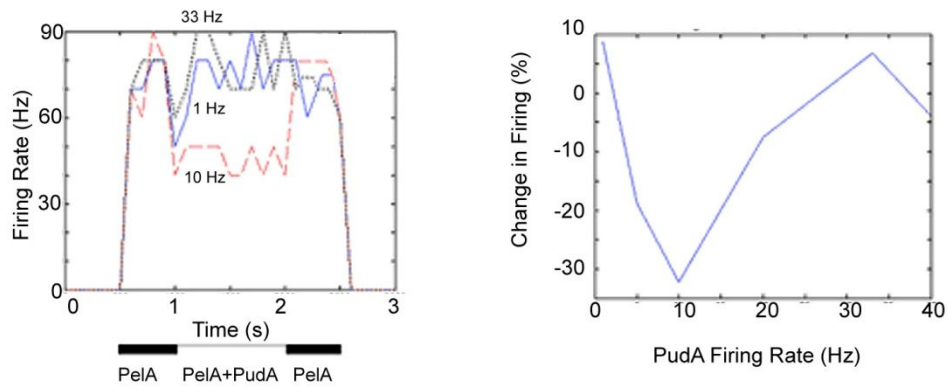
Figure C.5

Figure C.5. PGN response to PelA activation at 12, 36, and 60Hz for PudA activation at 1- 40 Hz. The left column of plots shows the PGN firing rate for PudA activation at 1, 10, and 33 Hz for the different PelA activation rates. The horizontal bars under the plots indicate when the PelA activity was present (black bar) and when the PelA activity and PudA activity were present simultaneously (white bar). The plots in the right column show the percent change in average PGN firing rate during PudA and PelA stimulation compared to during only PelA activation. It can be seen that regardless of the PelA input rate, average PGN activity appeared to decrease and increase at 10 and 33 Hz PudA activation respectively. However, the proportion change varies depending on the PelA activation rate. Inhibition of PGN activity by 10Hz PudA activation was greatest for PelA activation at 60Hz.
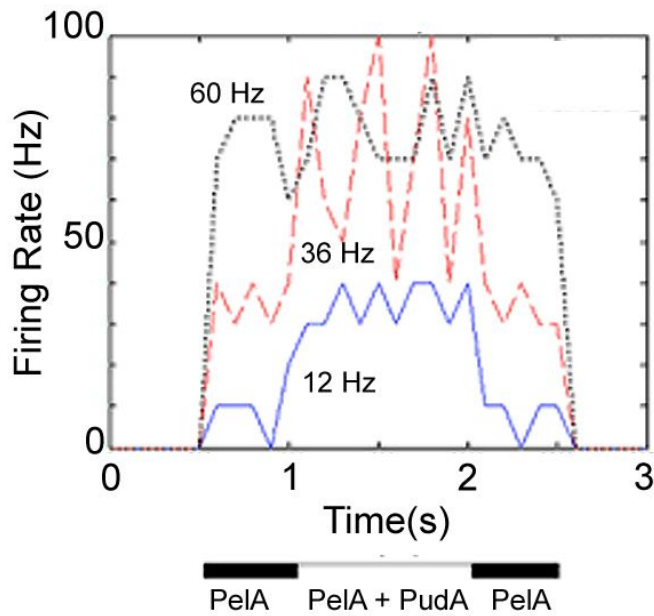
Figure C.6. The firing rate of PGN activity during PudA activation at 33Hz.
The increase in PGN activity is obvious for PudA activation during 12 and 36 Hz PelA activation, but the change is not as evident during 60Hz PelA activation. The horizontal bar under the plots indicates when the PelA activity was present (black bar) and when the PelA activity and PudA activity were present simultaneously (white bar).
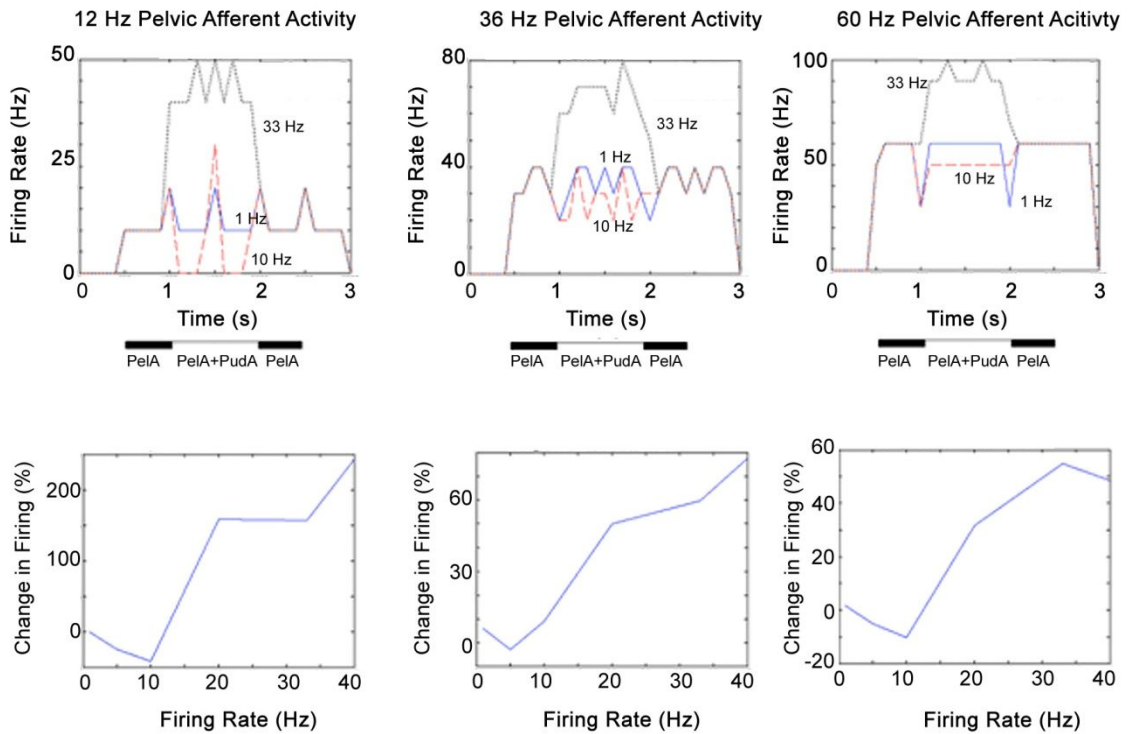
Figure C.7. PGN response to PelA and PudA activity in the chronic SCI cat model. The left column represents PelA activity at 12Hz. A large increase in PGN activity is observed when PudA is active at 33Hz. The middle column shows PGN activity when PelA activity at 36Hz. Again there is an increase in PGN firing when PudA is active at 33H, but there does not appear to be a substantial decrease in firing when PudA is active at 10Hz. Increased and decreased firing is observed for PudA activity at 33 and 10 Hz, respectively, when PelA is firing at 60Hz. The horizontal bar under the plots indicates when the PelA activity was present (black bar) and when the PelA activity and PudA activity were present simultaneously (white bar).

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Biography

John Patrick Woock was born in Louisville, KY on October 21, 1982. He studied biomedical engineering at Washington University in St. Louis where he graduated summa cum laude in May of 2005. During his undergraduate studies, John worked with Dr. Scott Whittemore and Dr. Richard Benton in the Lab of Neurosurgery and Transplantation at the Kentucky Spinal Cord Injury Research Center. For his efforts in the classroom and on the football field at Washington University in St. Louis, John was awarded an NCAA Postgraduate Scholarship and a National Football Foundation postgraduate fellowship. While pursuing his Ph.D in biomedical engineering at Duke University, John was awarded a National Science Foundation Graduate Research Fellowship. His graduate work with Dr. Warren Grill focused on characterizing bladder reflexes evoked by electrical stimulation of peripheral nerves that may provide a substrate for the development of a neural prosthesis to control bladder function in persons with spinal cord injuries.

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