Effects and Mechanisms of Patterned Electrical Stimulation of Pudendal Afferents for Bladder Control

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biomedical Engineering in the Graduate School of Duke University

2015
ABSTRACT

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Abstract

Spinal cord injury (SCI) and neurological diseases can cause lower urinary tract (LUT) dysfunction, significantly disrupting normal urine storage (continence) and efficient bladder emptying (micturition). Electrical stimulation of pudendal afferents is a promising technique to treat LUT dysfunction and restore bladder control via stimulation-evoked bladder inhibition or activation. However, innovative approaches are needed, as the voiding efficiencies produced by traditional pudendal afferent stimulation are insufficient for successful clinical translation. The objective of this dissertation was to investigate the effects of novel patterns of electrical stimulation on the size of bladder contractions and voiding efficiencies produced by pudendal afferent stimulation and to explore the neural mechanisms underlying stimulation-evoked reflex bladder control.

This work quantified the magnitude of bladder contractions and voiding efficiency evoked by spatial and temporal patterns of pudendal afferent stimulation in α-chloralose anesthetized cats. Bilateral stimulation of the dorsal genital nerve (DGN) and co-stimulation of the DGN and cranial sensory nerve (CSN) generated significantly larger isovolumetric bladder contractions and increased voiding efficiencies as compared to individual stimulation and distention-evoked voiding. The temporal pattern of DGN stimulation significantly affected the magnitude of evoked bladder
contractions, revealing that the bladder response to pudendal afferent stimulation is dependent on the pattern of stimulation, as well as the frequency.

The effects of intraurethral co-stimulation, combining individual stimulation sites in the proximal and distal urethra, on bladder activation and the electromyographic (EMG) activity of pelvic floor muscles were measured during urodynamics in persons with SCI. The size of stimulation-evoked bladder contractions was dependent on stimulation location and frequency, reflex EMG activity suggested that multiple reflex pathways contributed to bladder activation, and co-stimulation produced larger isovolumetric bladder contractions than single site stimulation.

Pharmacological block of inhibitory neurotransmitters was conducted to identify the mechanism of bladder inhibition evoked by pudendal afferent stimulation in α-chloralose anesthetized cats. Low frequency pudendal afferent stimulation-evoked bladder inhibition was blocked by picrotoxin, revealing that this requires a lumbosacral spinal GABAergic mechanism, and further pharmacological experiments indicated that glycinergic, adrenergic, or opioidergic mechanisms were not necessary for pudendal afferent stimulation evoked inhibition.

A computational model of the pudendo-vesical reflex and was developed based on previous neuroanatomical and electrophysiological studies to evaluate mechanisms underlying the bladder response to pudendal afferent stimulation. The frequency and pattern-dependent effects of pudendal afferent stimulation were determined by changes
in firing rate of spinal interneurons in the model, suggesting that neural network interactions at the lumbosacral level can mediate the bladder response to different frequencies or temporal patterns of pudendal afferent stimulation.

The effects and mechanisms of patterned pudendal afferent stimulation represent a substantial improvement in our understanding of pudendal afferent stimulation and will be valuable for the continued development of novel methodologies of electrical stimulation for bladder control.
Dedication

This dissertation is dedicated to my husband, Kevin, for his unwavering support and love along this journey to a PhD.
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1. Introduction

Part of this chapter was previously published in the Journal of Spinal Cord Medicine and is used with permission (McGee et al. 2015). This article was accepted for publication on January 2, 2015. Another similar review was published as a book chapter in the Encyclopedia of Computational Neuroscience (McGee and Grill 2014a). It was available online on September 29, 2013 and published in print on February 8, 2014.

The lower urinary tract (LUT), including the urinary bladder, urethra, and periurethral striated muscles, serves two important roles: continence, the storage of urine in the bladder, and micturition, efficient voiding of urine from the bladder at an appropriate time. These functions are controlled by neural circuits in the spinal cord, brainstem, and higher centers, and engage the sympathetic (hypogastric nerve), parasympathetic (pelvic nerve), and somatic (pudendal nerve) nervous systems (Beckel and Holstege 2011b). The generalized neural and anatomical connections important in regulation of the LUT, drawn from both human and animal studies, are shown in Figure 1.1. The bladder and urethral sphincter(s) are controlled in a reciprocal manner to accomplish the two primary functions of the LUT. During storage, urine is retained in the bladder because the sympathetic pathway is activated, producing bladder relaxation via adrenergic signaling through the hypogastric nerve, and activation of the somatic pudendal nerve output from Onuf’s nucleus produces coordinated contraction of the
external urethral sphincter (EUS) (de Groat 2006). The initiation of voiding occurs when the parasympathetic pathway is activated, producing contraction of the detrusor muscle in the bladder via cholinergic excitation through the pelvic nerve and the urethral sphincters are relaxed, allowing urine to leave the bladder and flow through the urethra (Andersson and Arner 2004; Beckel and Holstege 2011a).

During bladder filling, afferent fibers in the pelvic and hypogastric nerves deliver information from mechanoreceptors sensitive to pressure and stretch, or increases in tension of the bladder wall, signaling bladder distention to the sacral and lumbar levels of the spinal cord, respectively (Janig and Morrison 1986; Shea et al. 2000; Fowler et al. 2008). The pudendal nerve (PN) also contains afferent nerves; the dorsal genital nerve (dorsal nerve of the penis – DNP) is well documented in humans, and other smaller sensory branches have been identified in animals, such as the cranial sensory nerve (CSN) in cats (Yoo et al. 2008b). These somatic fibers provide sensory input from the pelvic floor, urethra, and external genitalia to the sacral spinal cord and play a role in mediating pudendo-vesical reflexes (Barrington 1931) that can impact continence and micturition by providing negative (guarding reflex) (Mahony et al. 1977) or positive feedback (augmenting reflex) during urine flow in the urethra (Shefchyk and Buss 1998; Shafik et al. 2003) (Figure 1). After entering the dorsal horn, the afferents diverge; some fibers make projections in the dorsal horn to local interneurons and some
send long ascending projections to the periaqueductal gray (PAG) and pontine micturition center (PMC) (Benarroch 2010).

The PMC plays a critical role in the regulation of continence and micturition, and switching circuitry located between the PAG and PMC integrates the ascending afferent signals and descending commands from higher brain centers to direct the transition from continence to micturition that is executed by the PMC (de Groat and Wickens 2013; Griffiths and Fowler 2013). Chemical or electrical stimulation of the PMC produces voiding that is very similar to reflex micturition (Sugaya et al. 1987), indicating that the PMC is a critical center for micturition. Descending signals from the PMC produce excitation of the sacral parasympathetic nucleus (SPN), causing bladder excitation and an increase in bladder pressure, and inhibition of Onuf’s nucleus (the pudendal motor nucleus) to produce relaxation of the external urethral sphincter (EUS), allowing urine to flow (Fowler et al. 2008).

Spinal cord injury (SCI) interrupts normal control of bladder function by blocking both the transmission of afferent information to the PAG and PMC and efferent commands to lower spinal levels which modulate the output nuclei of the LUT (Morgan et al. 1991). Without these pathways intact, aberrant reflexes can develop below the spinal cord lesion to produce uncoordinated LUT activity, leading to incontinence and/or urinary retention.
In normal bladder function, spinal reflexes that do not involve the PMC or PAG are well established. For example, the guarding reflex can be initiated by sensory activation of the pudendal nerve following EUS contraction or unexpected urine flow, which inhibits preganglionic sympathetic bladder neurons directly through spinal interneurons and produces continence (Park et al. 1997). Normal pelvic-to-pudendal reflexes can be elicited by a rapid increase in bladder pressure and pelvic afferent activity, leading to increase in pudendal motor output which further contracts the EUS in order to maintain continence (Lin 2004). In cases of LUT dysfunction following SCI, these reflexes may be disrupted, producing undesired contraction of the sphincters, i.e., detrusor-sphincter dyssynergia (DSD). However, lumbosacral spinal mechanisms that remain intact following SCI provide an opportunity for intervention for the restoration of LUT function.

In this review we discuss neurogenic lower urinary tract dysfunction (NLUTD) following spinal cord injury and the development of electrical stimulation as an approach to restore bladder function, including both continence and micturition. Applications of electrical stimulation for control of bladder function following SCI are discussed, spanning from historic clinical approaches to recent pre-clinical studies that may improve the success of electrical stimulation therapy in SCI patients.
1.1 Lower Urinary Tract Dysfunction

1.1.1 Bladder Dysfunction after SCI

Neurological disease and injury can cause significant disruption of both the storage and emptying functions of the LUT. SCI (SCI), specifically, causes LUT dysfunction characterized by neurogenic detrusor overactivity, urinary incontinence, chronic urinary retention (impaired micturition), and detrusor-sphincter dyssynergia (DSD) (Abrams et al. 2002). The location and severity of the SCI affect the degree of bladder dysfunction, from interrupting the communication between sacral and pontine micturition centers, to directly damaging the lumbosacral circuits that control the detrusor and pudendal nerve output (Benevento and Sipski 2002). Spinal cord injuries are classified on a scale by the American Spinal Injury Association (ASIA), where A describes complete spinal transection where no sensory or motor function is preserved and E describes normal spinal cord function (Kirshblum et al. 2011). Bladder dysfunction leads to substantial decreases in quality of life (Anderson 2004) and can cause urinary tract infections, skin breakdown, bladder and kidney damage, and rehospitalization (Van Kerrebroeck et al. 1993b; Ku 2006). Further, bladder dysfunction caused by SCI may change over the course of the injury (Benevento and Sipski 2002) for example changing from an areflexic to an overactive bladder with time following injury (Cruz and Cruz 2011) making bladder management difficult.
Injury to the spinal cord above the lumbosacral level removes the voluntary control of micturition, leading initially to an areflexive bladder and complete urinary retention. However, this is followed by slow development of a sacral spinal reflex mediated by unmyelinated C fibers. This reflex responds to low volume filling and leads to neurogenic detrusor overactivity (NDO). In addition to NDO, high-level spinal cord injuries (upper motor neuron lesions above T12) are more likely to produce detrusor sphincter dyssynergia (DSD) although any cord injury above S2 can result in DSD. Concurrent contraction of the bladder and urethral sphincter (DSD) can limit urinary flow and cause urinary retention; as well, DSD can lead to elevated detrusor pressures, which can put the upper urinary tract at risk of degeneration (Weld and Dmochowski 2000). Autonomic dysreflexia can occur in SCI above T5 but has been reported in patients with lesions as low as T8. This occurs due to a splanchnic outflow from the sympathetic system emanating from T5 to L2 and the absence of inhibition as a result of SCI. Autonomic dysreflexia leads to an exaggerated reaction to any stimuli below the level of SCI such as rectal impaction or rapid bladder filling during a urodynamic test. Symptoms of autonomic dysreflexia include an increase in blood pressure, bradycardia, sweating and headache. Treatment includes removal of the stimulant, i.e., drain the bladder or evacuate the rectum and possibly medical management of the blood pressure. High level SCI was found to increase the risk of autonomic dysreflexia in one
study,(Helkowski et al. 2003) while another found that 48% of patients with complete SCI (ASIA A) above T6 had documented episodes of autonomic dysreflexia (Lindan et al. 1980).

Lower level spinal cord injuries (at S2 and below) produce very different effects on bladder function. Injury to the lower motor neurons classically results in bladder areflexia and low bladder compliance due to damage to the spinal micturition circuits(Weld and Dmochowski 2000) and poor urethral function due to loss of somatic innervation. This type of injury can result in a variety of symptoms, including urinary retention, urinary incontinence, and if low bladder compliance is present, deterioration of kidney function.

The goals of management of NLUTD, including NDO, are protection of the upper urinary tract, improvement of urinary incontinence, restoration of LUT function, and improvement in quality of life (Ku 2006).

Typically, the first line of treatment for NDO includes a combination of anticholinergic drugs. Anticholinergic therapy is frequently prescribed at higher doses for NDO than in overactive bladder (OAB) and this can lead to increased incidence of side effects including dry mouth, blurred vision, constipation, and cognitive changes (Pannek et al. 2013). Although beta-3 adrenoceptor agonists are used in OAB, there is no evidence of effect in patients with NLUTD.
If DSD is present, alpha-blockers, e.g. tamsulosin, may be used to reduce outlet resistance. Baclofen, a skeletal muscle relaxant often used to treat lower extremity spasticity, can decrease spasticity in the pelvic floor muscles but is not typically used for urethral outlet obstruction (Burns et al. 2001; Benevento and Sipski 2002). Intermittent catheterization (IC), on average 4-6 times per day, is the gold standard for patients who are unable to empty their bladder (Pannek et al. 2013). Aseptic or clean IC are feasible alternatives to sterile IC and decrease the risk for urinary tract infection as well as decrease the risk of significant complications seen with indwelling transurethral or suprapubic cystostomy (Pannek et al. 2013). Other methods may be used to initiate voiding, such as bladder compression to expel urine (Credé), voiding by abdominal straining (Valsalva), and triggered reflex voiding. These maneuvers should be avoided in those with DSD as they create high pressures and are potentially hazardous to the upper tracts (Weld et al. 2000; Benevento and Sipski 2002).

If anticholinergic medications prove to be ineffective or poorly tolerated, botulinum toxin type-A injections in the bladder wall are the most effective minimally invasive treatment at this time to reduce NDO (Pannek et al. 2013). The vanilloids, capsaicin and resiniferatoxin, are intravesical treatments that desensitize C-fibers but have limited clinical efficacy compared to botulinum toxin type-A injections into the detrusor (Cruz and Dinis 2007). For patients with DSD, minimally invasive procedures
such as sphincterotomy (Reynard et al. 2003) or chemical deafferentiation of the sphincter using botulinum toxin type-A (Dykstra and Sidi 1990) can be used to reduce bladder outlet resistance. When these less invasive procedures have failed, surgical procedures such as bladder augmentation, posterior rhizotomy with or without sacral anterior root stimulation (SARS) (complete lesions) and neuromodulation (incomplete lesions) are tried. Continent or incontinent diversion is indicated for small, contracted, noncompliant bladders.

1.2 Clinical Applications of Electrical Stimulation for Bladder Control

Different locations have been investigated for application of electrical stimulation to restore functional bladder control, each with varying degrees of success. In the past, electrodes for stimulation to modulate bladder function have been placed on the bladder, skin, peripheral nerves, sacral roots, or spinal cord (Gaunt and Prochazka 2006). Figure 1.2 depicts the neural innervation of the lower urinary tract and common electrode locations for restoration of bladder control. The effectiveness of recently developed applications of electrical stimulation for bladder control has been evaluated in clinical studies in persons with SCI (Van Kerrebroeck et al. 1996; Creasey et al. 2001; Yoo et al. 2007; Lombardi and Del Popolo 2009) although not in randomized, controlled clinical trials. The associated morbidities in patients with SCI adds complexity to the
understanding of how each approach might be used to treat NLUTD, and the optimal, or even suitable, therapy is likely to vary across individuals.

There has been limited clinical success with direct bladder wall stimulation due to problems with concomitant sphincter activation (DSD) caused by reflex activity evoked by activation of pelvic afferents in the bladder, pain, or device failure (Bradley et al. 1963; Hald et al. 1967; Stenberg et al. 1967). Pelvic nerve stimulation, i.e., stimulation of the nerve supply to the bladder, was shown to produce bladder contractions in dogs, but also resulted in co-activation of urethral sphincters (Holmquist and Olin 1968). Pelvic nerve stimulation requires lower amplitudes of stimulation than direct bladder wall stimulation but application in humans is limited due to the difficulty of electrode placement (Hald 1969).

Spinal cord stimulation, particularly intraspinal stimulation for bladder control, has shown promising results in animals (Grill et al. 1999; Tai et al. 2004a; Pikov et al. 2007). However, this technique has not advanced because it is highly invasive, has high complication rates, and it is not clear that it provides benefit over sacral root stimulation (Nashold et al. 1981). Transcutaneous electrical stimulation for bladder control, e.g., surface stimulation of the dorsal nerve of the penis to inhibit bladder activity, targets peripheral nerves through a less invasive approach. But there are challenges of chronic clinical deployment at this location. The technology for electrical stimulation of the
sacral roots and various peripheral nerves continues to evolve with promise for an
effective neural prosthetic to restore NLUTD.

1.2.1 Finetech-Brindley Bladder Control System

Brindley and colleagues developed a system to allow bladder emptying with
sacral anterior root stimulation (SARS) (Brindley 1977) that showed positive results in
SCI patients. The Finetech-Brindley Bladder Control System (branded as VOCARE in the
US) was granted a Humanitarian Device Exemption by the FDA in 1998 for bladder
dysfunction in spinal cord injured patients. This system was the most successful
electrical stimulation devices implanted for bladder control in SCI, increasing bladder
capacity and allowing patients to void efficiently (Van Kerrebroeck et al. 1993a; Creasey
et al. 2001). However, the company that distributed VOCARE in the US, NeuroControl
Corporation (Valley View, OH), went out of business in 2007.

The SARS system targets efferent nerve fibers emerging from the sacral spinal
cord to produce bladder contraction (Brindley 1977) and provide efficient, on-demand
voiding and a significant reduction in residual volumes and urinary tract infections, as
well as bowel emptying (Van Kerrebroeck et al. 1996; Vallès et al. 2009). However,
treatment of NDO and DSD required a concomitant posterior rhizotomy. Transection of
the sacral dorsal roots (posterior rhizotomy) performed in combination with SARS to
increase bladder capacity and compliance and improve voiding efficiency is
effective,(Krasmik et al. 2014) but irreversibly eliminates reflex erection, reflex micturition and any remaining pelvic sensation (Brindley 1994; Gaunt and Prochazka 2006). Brindley reported that out of 12 of the early patients with reflex incontinence, all became fully continent following sacral posterior rhizotomies, and this is now standard practice with SARS implantation (Brindley 1988).

In persons with supra-sacral SCI, SARS coupled with posterior rhizotomy produced voiding volumes greater than 200 mL in 18/21 patients and decreased post-void residual volumes below 50 mL in 15/21 patients (Creasey et al. 2001). SARS also substantially reduced the prevalence of urinary tract infections, reflex incontinence, autonomic dysreflexia, and decreased the frequency of catheterization and anticholinergic drug use in persons with SCI (Creasey et al. 2001). Other studies report that SARS is safe and effective: 28 patients with SCI who were incontinent were completely dry after SARS with posterior rhizotomy (Madersbacher and Fischer 1993). Further, this approach increased the quality of life of persons with SCI,(Vastenholt et al. 2003) and the cost of managing the neurogenic bladder and bowel after SCI was greatly reduced with SARS and posterior rhizotomy, compared to standard treatments (Creasey and Dahlberg 2001).

Although SARS is a very effective approach to restoring bladder control following SCI, through the year 2004 it was implanted in only approximately 2,500
people (Rijkhoff 2004). The limited penetration may be due to the requirement of the irreversible posterior rhizotomy, the complexity of the implant surgery, as well as limited access at selected centers. In addition, the use of Botox and clean intermittent catheterization to manage LUT dysfunction after SCI may have decreased the demand for SARS. For those that are implanted and receive a posterior rhizotomy, bladder control with the device persists for many years (Van Kerrebroeck et al. 1993a; Brindley 1994; Martens and Heesakkers 2011).

1.2.2 Medtronic Interstim®

Sacral nerve stimulation (SNS) with the InterStim® (Medtronic Inc., Minneapolis, MN, USA) was approved by the FDA in 1997 for urge urinary incontinence and in 1999 for urinary retention (Rijkhoff 2004). SNS targets somatic afferent fibers entering the spinal cord and is thought to modulate the micturition reflex (Vodusek et al. 1986) for treatment of urge urinary incontinence and urinary retention (Rijkhoff 2004). Electrode implantation does not require laminectomy and is performed after a period of test stimulation with either a temporary percutaneous electrode or chronic lead placed in the S3 sacral foramen. Following this test period, device implantation occurs only in those patients who have \( \geq 50\% \) improvement in the LUTD symptoms (Bosch and Groen 2000). Long-term improvement of overactive bladder symptoms and urinary retention are achieved with SNS with Interstim®, but these studies were all in non-SCI populations.
(Schmidt et al. 1999; Jonas et al. 2001; Dasgupta et al. 2004; van Kerrebroeck et al. 2007; Chaabane et al. 2011). Even in the non-neurogenic populations, success with InterStim® is highly etiology-dependent, (Comiter 2003; Chaabane et al. 2011) patients with urinary retention arising from specific electromyographic abnormalities of the EUS (i.e., Fowler’s syndrome) were more likely to benefit from InterStim® than those from various etiologies (De Ridder et al. 2007).

There has been limited study of Interstim® in SCI; however, in general, SNS has not been as effective in resolving symptoms of chronic urinary retention or incontinence in those with complete spinal cord lesions (Chartier-Kastler et al. 2001; Kessler et al. 2010). In subjects with neurogenic bladder from complete SCI there was no significant difference in maximal bladder capacity, maximal detrusor pressure, or bladder volume at first uninhibited contraction with acute SNS (Chartier-Kastler et al. 2001). Additionally, one study reported that none of the 5 patients with complete SCI showed any improvement of their incontinence during the SNS test phase (Schurch et al. 2003). SNS may be more effective in incomplete SCI, and some studies show that the device improved continence by increasing cystometric capacity; persons with urinary retention from incomplete SCI saw a significant increase in urinary frequency and voided volume with SNS, while persons with urgency from incomplete SCI saw a significant decrease in the number of incontinent episodes and a significant increase in voided volume up to 5
years post-implant (Lombardi and Del Popolo 2009). In addition, the time before implant after SCI may influence the effectiveness of treatment, as early SNS in individuals with SCI prevented detrusor overactivity and urinary incontinence (Sievert et al. 2010).

### 1.2.3 Percutaneous Tibial Nerve Stimulation

Peripheral nerves are an alternative stimulation target for bladder control following SCI. Stimulation of the tibial nerve, which originates from the L4-S3 lumbosacral plexus as part of the sciatic nerve, has been studied for treatment of OAB. Tibial nerve stimulation with the Urgent® PC Neuromodulation System (Uroplasty, Inc., Minnetonka, MN, USA) received FDA 510(k) clearance in 2007 for the treatment of urinary urgency, urinary frequency, and urge urinary incontinence. Percutaneous tibial nerve stimulation (PTNS) offers a less invasive treatment alternative to SNS, as surgical implantation is not required. PTNS is typically performed via a needle electrode inserted above the ankle. The procedure is office based and stimulation is delivered for 30 minutes once a week for 12 weeks. Randomized controlled studies report significant improvement with OAB symptoms (Peters et al. 2009; Finazzi-Agro et al. 2010; Staskin et al. 2012) however, only prospective non-randomized trials exist to support use in non-obstructive urinary retention (Vandoninck et al. 2003) and few studies have documented the effects of PTNS on NLUTD.
A study of PTNS for bladder dysfunction in 29 patients with various neurological lesions (including 15 patients with SCI) showed that 50% of the population had improvement in either maximum cystometric capacity or volume at first involuntary detrusor contraction (Amarenco et al. 2003). In a study of 70 multiple sclerosis patients, PTNS was well tolerated and produced clinical improvement of OAB symptoms in more than 82% of patients (de Sèze et al. 2011), suggesting that this therapy may be effective in SCI with incomplete lesions. In acute urodynamic tests, PTNS significantly increased maximum cystometric bladder capacity in patients with multiple sclerosis, indicating that PTNS may be an effective approach for clinical treatment of NDO (Kabay et al. 2008). In two persons with SCI, PTNS was used to treat successfully fecal incontinence up to 3 months follow up, pointing to the potential in other applications in SCI population. However, a recent study in animals found that bladder inhibition with PTNS was abolished following acute spinal cord transection, (Xiao et al. 2014b) suggesting that PTNS employs supraspinal pathways and may not be suitable in persons with complete SCI.

1.2.4 Pudendal Nerve Stimulation

Stimulation of the pudendal nerve is another promising technique for the treatment of NLUTD following SCI. Mechanical activation of pudendal afferents, elicited by penile squeeze, inhibits the bladder and quiets existing bladder contractions (Kondo
et al. 1982). Recent clinical studies demonstrate that pudendal nerve stimulation (PNS) can produce both inhibition of bladder contractions, or at different stimulation parameters, bladder activation in persons with SCI (Vodušek et al. 1987; Wheeler et al. 1992; Previnaire et al. 1996; Kirkham et al. 2001b; Gustafson et al. 2003; Lee et al. 2003; Gustafson et al. 2004; Yoo et al. 2007).

The pudendal nerve is a somatic nerve in the pelvic region that originates from the sacral spinal cord at levels S2-S4 in humans (Shafik et al. 1995). Access to the pudendal nerve for electrical stimulation can be made via a trans-gluteal incision (Gustafson et al. 2005) or less-invasive percutaneous or transcutaneous approaches (Gustafson et al. 2003). PNS refers to electrical stimulation of the pudendal nerve, containing both sensory and motor fibers, or electrical stimulation applied to distal branches; for example, the dorsal genital nerve (DGN), a purely afferent branch that transmits sensory information from the urethra and external genitalia.

In one study comparing the effectiveness of SNS with Interstim® to PNS in patients with OAB symptoms, the PN lead produced greater overall reduction in symptoms of urinary frequency and urgency and was chosen as the preferred lead by the majority of participants (Peters et al. 2005). PNS may be an alternative neuromodulation therapy for refractory OAB, particularly in patients who do not respond well to SNS (Sherman and Amundsen 2007; Mashni and Peters 2010; Peters et
al. 2010). However, although a CE mark has been granted in Europe, PNS remains for investigational use only in the United States. Studies are ongoing to evaluate the effectiveness of PNS for bladder control following SCI and to study novel stimulation paradigms for more effective treatment of NLUTD.

### 1.3 Pre-clinical Studies and Future Translational Opportunities

There is a need for development of methods that improve voiding efficiency and continence control for those with SCI without requiring a dorsal rhizotomy, and a better understanding of the underlying mechanisms of SNS and PNS to improve these therapies, and devise new approaches. Pre-clinical studies in animals provide the opportunity to investigate the potential impact of new techniques of stimulation. A summary of the results of animal studies of pudendal nerve stimulation and pudendal-to-bladder reflexes across species and injury models is shown in Table 1.1.

#### 1.3.1 Pudendal Nerve Stimulation in SCI as an Alternative to Sacral Nerve Stimulation

Peripheral nerve stimulation is an alternative approach to target similar reflexes as sacral nerve stimulation, potentially with more specificity. In contrast to PNS, which targets a particular nerve or nerve branch, SNS with Interstim® targets the entire sacral nerve. The use of this non-selective location results in non-specific stimulation of both afferent and efferent fibers. This ambiguity contributes to the lack of understanding of the mechanisms by which sacral nerve stimulation works and why it remains ineffective.
for treatment of NLUTD after complete SCI. Because SNS is effective for the treatment of non-neurogenic bladder dysfunction and less successful for patients with complete SCI, there is speculation that preserved supraspinal connections are necessary for the positive effects of SNS (Schurch et al. 2003). Many studies have identified that the primary effects of PNS occur through activation of spinal reflexes (Woock et al. 2009a; Xiao et al. 2014b), which remain intact following supra-sacral SCI. This mechanistic difference is further corroborated by studies where patients with SCI experienced better symptom improvement with PNS than SNS (Peters et al. 2005).

Reflex bladder inhibition has been demonstrated by mechanical or transcutaneous electrical stimulation of the perigenital skin in rats, (Sato et al. 1975; Morrison et al. 1995; Hotta et al. 2012) cats (Sato et al. 1977; Thor et al. 1990; Tai et al. 2007b; Tai et al. 2008; Wang et al. 2009; Tai et al. 2011), and humans (Kondo et al. 1982) and is produced by activation of pudendal afferents. Electrical stimulation of the PN or DGN with low frequencies (5-10 Hz) produces reflex bladder inhibition, promoting continence (Boggs et al. 2006b; Tai et al. 2006; Wenzel et al. 2006). Critical for application in SCI, this inhibition persists following spinal cord transection (Tai et al. 2007b; Tai et al. 2011). Additional work established the feasibility of closed-loop continence control (Kirkham et al. 2001a). Conditional PNS, triggered by pudendal nerve activity recorded by electroneurogram, was more effective at inhibiting the onset of bladder contractions
than continuous stimulation (Wenzel et al. 2006). In patients with SCI, increased bladder capacity and decreased storage pressures were produced by event-driven electrical stimulation of the dorsal penile or clitoral nerve triggered by increases in either EMG (Dalmose et al. 2003) or bladder pressure (Hansen et al. 2005).

While low frequency stimulation of pudendal afferents produces reflex bladder inhibition, high frequency stimulation (20-50 Hz) produces bladder excitation in cats (Boggs et al. 2006b; Tai et al. 2007b) and rats (Peng et al. 2008a; Peng et al. 2008b). High frequency stimulation can be used to augment existing bladder contractions or produce robust bladder contractions and voiding when desired (Woock et al. 2008); in many cases contraction can be evoked at lower bladder volumes than distension-evoked activity (Woock et al. 2011). Voiding with intermittent PN stimulation was more effective than distension-evoked voiding or voiding produced by intermittent sacral root stimulation in anesthetized cats and was not limited by stimulation induced bladder-sphincter dyssynergia (Boggs et al. 2006a). Reflex bladder activation with high frequency stimulation of the pudendal nerve has also been demonstrated in humans with SCI (Yoo et al. 2007). The use of pudendal afferent stimulation to produce both bladder inhibition, for continence control, and bladder activation, necessary for efficient voiding, in humans following SCI (Tai et al. 2006; Tai et al. 2007b; Tai et al. 2011), further demonstrates the potential of this approach for the treatment of bladder dysfunction following SCI.
More recent studies differentiated the distal PN branches and evaluated the effects of stimulation of individual nerve branches on activation and inhibition of the bladder (Woock et al. 2008; Yoo et al. 2008b). Selective stimulation of afferent branches of the PN reduced or eliminated activation of efferent pathways to the EUS (Woock et al. 2008; Yoo et al. 2008a), which would otherwise be undesirable for efficient voiding. Selective co-stimulation of PN afferents in multiple sensory branches or bilateral stimulation of DNP evoked significantly larger bladder contractions and improved voiding over individual branch stimulation in cats (McGee and Grill 2014b). Intraurethral electrical stimulation to target the distal branches of the PN produced similar frequency tuning responses to low and high frequency stimulation in cats (Gustafson et al. 2003; Woock et al. 2009a) and evoked bladder contractions in humans with SCI (McGee et al. 2011).

A study of the fascicular anatomy and surgical access of the pudendal nerve in humans found that placement of a nerve cuff on the PN is both anatomically and surgically feasible (Gustafson et al. 2005). This approach could be employed for use with multi-electrode nerve cuffs for selective stimulation of specific fascicles within the pudendal nerve (Kent and Grill 2013), to eliminate dyssynergia produced by direct stimulation or reflex activation of motor fibers innervating the EUS.
Experiments performed in cats further investigated the effects of temporal pattern of stimulation on reflex bladder activation. Variable patterned stimulation resulted in larger evoked reflex bladder contractions and demonstrated an increase in the range of parameters that caused bladder contraction (Bruns et al. 2008). Specifically, bursting patterns of stimulation delivered to the pudendal nerve (Bruns et al. 2008) or in the proximal urethra via intraurethral stimulation (Bruns et al. 2009a; Bruns et al. 2009b) improved bladder excitation and voiding in cats. These animal experiments illustrate the importance of further identification the mechanisms of PNS to produce efficient and effective bladder control following SCI.

1.3.2 Nerve Blocking with Electrical Signals

Application of high frequency (in the kHz range) signals to the PN can block the motor axons to the urethral sphincter and thereby mitigate the effects of DSD (Tai et al. 2004b; Bhadra et al. 2006; Gaunt and Prochazka 2009). Blockade of neural signals with kilohertz frequency stimulation could be used to improve voiding by blocking dyssynergic activity in the PN or efferent activity produced by stimulation of the sacral roots or PN (Boger et al. 2008). This method was inspired by earlier experiments with high frequency stimulation of the PN that produced bladder emptying similar to posterior rhizotomy in chronic SCI dogs, due to EUS fatigue from depletion of the neuromuscular junction following stimulation (Sawan et al. 1996).
High frequency block of the PN coupled with stimulation of sacral nerve or proximal PN in animals produced significant improvements in voiding efficiency compared to sacral nerve stimulation alone (Shaker et al. 1998; Boger et al. 2008). High frequency block of the PN can also be used to reduce EUS spasticity, avoiding the need for a posterior rhizotomy, or be used to reversibly block EUS contractions during bladder contractions to improve voiding (Boger et al. 2008) High frequency block was well tolerated in chronically implanted animals without anesthesia (Gaunt and Prochazka 2009), demonstrating the possible clinical potential of this approach. However, although kilohertz frequency nerve block does not produce acute nerve damage (Tai et al. 2005; Bhadra et al. 2006), the safety and durability of chronic high frequency nerve block remain to be determined (Franke et al. 2014).

1.3.3 Pathways and Mechanisms of Reflex Bladder Control

The results from these animal experiments illustrate that urethral afferents in the PN play an important role in the micturition reflex and could be utilized to control bladder function, both for inhibition of bladder excitability and to evoke bladder contractions, when needed. However, it is important to understand the mechanisms by which electrical stimulation of PN afferents evokes bladder contractions and voiding through reflex bladder activation for successful clinical translation. Though many of the mechanisms of the pudendo-vesical reflex are unclear, recent work has shed light on the
spinal and supra-spinal circuits engaged in pudendal nerve stimulation for bladder control.

Bilateral transection of the sensory branch of the PN in rats significantly reduced voiding efficiency, indicating that pudendal sensory feedback plays an essential role in efficient bladder emptying (Chen et al. 2012). This indicates that electrical stimulation of PN afferents for reflex bladder control is likely effective because it introduces or mimics activity which acts as a natural feedback to the spinal cord during voiding. In studies of reflex bladder activation with PN afferent stimulation, robust bladder activation was noted to be volume dependent; the ability to evoke bladder contractions required 70-80% of the volume to elicit distension-evoked bladder contractions (Woock et al. 2008). This threshold volume dependence indicated that sensory information from the bladder is also critically involved in the neural circuit reflex processing of electrical stimulation. Sympathetic mechanisms were found to contribute to the volume dependence of activation of the bladder through pudendal afferent stimulation but do not affect the size of robust bladder contractions evoked by the reflex (Woock et al. 2011). This indicated that bladder activation from PN stimulation is a result of convergence of pelvic and pudendal afferents which increase pelvic efferent activity (Woock et al. 2011).

Bladder activation with high frequency DNP stimulation remained intact after acute spinal transection (SCT) (Yoo et al. 2008a). Further, acute SCT experiments
demonstrated that the mechanisms of bladder excitation through electrical stimulation of DNP and CSN differ. Low frequency CSN bladder activation was eliminated with SCT and mediated by a spino-bulbo-spinal or supraspinal reflex (Woock et al. 2008). Because selective stimulation of distal pudendal afferents activates different pathways, stimulation of the pudendal nerve or higher levels, such as the sacral roots, likely activates multiple pathways simultaneously. Identifying these mechanisms is critical to further our understanding of normal neural bladder control and determine which may remain intact after disease or injury.

Several types of computer models that describe the neural control of the LUT have been published in the past; however most of these fail to model neural activity with sufficient detail or fail to include the contributions by pudendal afferents.

Hosein and Griffiths (1990) produced a quantitative computer simulation of the mechanical LUT control system, modeling changes in bladder volume, pressure, and flow rate while qualitatively demonstrating potential neural control signals. Bastiaanssen and colleagues (1996) developed a biomechanical neuromuscular model of the bladder that incorporated neuroanatomy, physiology, and muscle mechanisms and could respond to input signals from an artificial neural network. Control theory and functional considerations to describe a system for control of the micturition cycle were described in another model (Kinder et al. 1999). A series of models describing potential
LUT control pathways was presented by van Duin et al. (2000), they evaluated different networks of neural pathways, represented by nodes, in response to changes in afferent bladder signals and demonstrated that normal lower urinary tract behavior could be simulated. A more recent model of the PAG/PMC in the brainstem showed successful recreation of micturition, but did not include pudendal afferents or purely spinal reflex pathways (de Groat and Wickens 2013).

A model of frequency-dependent reflex selection in the spinal cord, as is suggested to occur with pudendal afferent stimulation (Boggs et al. 2006b), revealed that frequency-dependent network connections may enable selection of specific reflex pathways based on incoming neural codes (Jilge et al. 2004). Similar approaches could be applied for further investigation of the sacral neural network that controls bladder response to pudendal afferent stimulation. A functional neural network model of the reflexes which govern LUT control would be a significant advancement for the understanding of how neuromodulation can be applied to treat various etiologies of bladder dysfunction.

1.3.4 Coupling Neuromodulation and Pharmacological Therapy for Improved Treatment

Given that a large number of neurochemicals mediate bladder control by electrical stimulation, it is possible that pharmacological interventions could augment the effects of electrical stimulation. However, successful development and optimization
of new therapeutic approaches requires understanding the underlying mechanisms of action of electrical stimulation.

Bladder inhibition with low frequency PNS was preserved following hypogastric nerve transection and administration of α- and β-adrenergic antagonists (Woock et al. 2011), and recent work demonstrates that this inhibition is mediated by GABAergic activity in the lumbosacral spinal cord (McGee et al. 2014). Opioids were also found to contribute to the inhibitory pudendal-to-bladder reflex in cats (Chen et al. 2010). In similar experiments, opioids were found to play a differential role in inhibition of nociceptive and non-nociceptive bladder contractions by tibial nerve stimulation (Tai et al. 2012). Other experiments have demonstrated that metabotropic glutamate 5 receptors (Larson et al. 2011; Mally et al. 2013), and serotoninergic receptors (Matsuta et al. 2013), which may interact with opioid mechanisms, are involved in bladder inhibition by PNS and that the effects are primarily mediated by spinal, not supraspinal, processes (Xiao et al. 2014b). Convergence of pudendal and pelvic afferents to increase pelvic efferent activity at the sacral level is likely responsible for bladder excitation with PNS (Woock et al. 2011). A combination of electrical stimulation and pharmacological treatment may improve treatment of bladder dysfunction in persons with SCI; for example, coupling electrical stimulation for bladder inhibition and α-adrenoceptor antagonists may improve continence by increasing bladder capacity (Andersson et al. 1999). Intrathecal
baclofen, a GABA\textsubscript{B} agonist, has been used to treat urethral sphincter spasticity (Nanninga et al. 1989) and could be coupled with electrical stimulation to reduce NDO and urinary incontinence. Intrathecal administration of opioid agonists inhibited contractions and reduced DSD in spinal cord injured animal studies and could be used to boost the effects of bladder inhibition with PNS (Yokoyama et al. 2004). Lastly, benzodiazepines, acting as an agonist of the effects of GABA\textsubscript{A}, could enhance the effects of GABA\textsubscript{A}-mediated PNS bladder inhibition (McGee et al. 2014; Xiao et al. 2014a).

1.4 Significance

Understanding the specific neural circuits and neurotransmitters involved will drive the development of new stimulation paradigms for NLUTD in SCI and reveal additional opportunities for pharmacological intervention. Stimulation of the pudendal nerve is a promising approach to restore control of both continence and micturition in SCI, and continued work in this area will reveal how the effects of PNS compare to other stimulation modalities.

The selective stimulation of peripheral afferents may allow more precise control of bladder function, particularly with the selective stimulation of reflex pathways. Novel stimulation techniques, such as peripheral optogenetics (Towne et al. 2013; Iyer et al. 2014), or the use of light to stimulate selectively genetically-targeted neurons, may enable improved control of bladder activity. Further development of novel applications
of temporal patterns of stimulation, including high frequency conduction block, will drive progress towards additional therapies for bladder control following SCI. Additionally, peripheral nerves allow for electrode implantation via minimally invasive surgical approaches. The optimal solution for restoration of bladder control after SCI may encompass a combination of efficient, targeted electrical stimulation, possibly at multiple locations, and pharmacological treatment to enhance symptom control. Pudendal nerve stimulation remains a suitable alternative in cases where other treatments are not well tolerated or unsuccessful and future work in this area will fully reveal the functionality of PN stimulation in comparison to other stimulation modalities for the restoration of bladder function.

1.5 Specific Aims

The objective of this work was to study the effects of patterned electrical stimulation of pudendal afferents and elucidate the mechanisms of the pudendo-vesical reflex for control of bladder emptying (micturition). This is part of our larger long-term goal of developing a neural prosthetic - an implanted medical device - to restore continence and micturition in persons with neurological disorders, such as SCI. We quantified the efficacy of novel spatial patterns of electrical stimulation to generate bladder contractions and produce bladder emptying in an animal model and in persons with SCI, measured the effects of novel temporal patterns of stimulation on bladder
activation, identified neurotransmitters critically involved in pudendal afferent stimulation-evoked bladder inhibition, and explored the network mechanisms of the spinal pudendo-vesical reflex with a computational model.

### 1.5.1 Aim 1 – Spatial Patterns of Pudendal Afferent Stimulation

Pudendal afferent stimulation evokes robust bladder contractions and generates voiding, but the voiding efficiencies produced by stimulation are insufficient for clinical translation. Incomplete bladder emptying results in undesirable residual bladder volumes, which lead to infection and urinary frequency. The first aim was to improve voiding efficiency by concurrently stimulating multiple pudendal afferent pathways. The hypothesis was that selective co-stimulation, either bilateral dorsal genital nerve (dorsal nerve of the penis, DNP) stimulation or stimulation of both CSN (cranial urethral sensory nerve) and DNP, would evoke larger reflex bladder contractions and higher voiding efficiencies than stimulation of any single pathway alone. Producing lower residual volumes from efficient voiding is imperative for eventual clinical application of pudendal afferent stimulation for restoration of bladder function.

#### 1.5.1.1 Pre-clinical Evaluation of Selective Co-stimulation of Pudendal Afferents (Aim 1A)

The objective of these experiments was to quantify bladder contraction pressure and voiding evoked by bilateral electrical stimulation of the DNP, an afferent (sensory) terminal branch of the pudendal nerve. We measured bladder contractions and
emptying in response to unilateral and bilateral DNP stimulation in α-chloralose anesthetized cats. Our hypothesis was that bilateral DNP stimulation would evoke larger bladder contractions and more complete bladder emptying than unilateral DNP stimulation. In separate animals, we also investigated the effectiveness of co-stimulation of two separate afferent branches of the PN, the DNP and CSN, at evoking bladder contractions and voiding. We measured bladder contraction amplitude and voiding efficiency in response to co-stimulation and individual stimulation of CSN and DNP. The hypothesis was that co-stimulation of the CSN and DNP would enhance bladder contraction pressures and create more efficient bladder emptying than individual stimulation through only one of the reflex pathways. We found that co-stimulation of afferent pathways generated significantly larger isovolumetric bladder contractions and significantly improved voiding efficiency. Co-stimulation of pudendal afferents also suppressed dyssynergic activity in the external anal sphincter produced by low frequency individual stimulation. The decreased threshold volumes required for reflex bladder activation and increased VEs with co-stimulation support the use of stimulation of multiple afferent pathways to improve voiding efficiency.

1.5.1.2 Intraurethral Co-stimulation of Pudendal Afferents in Persons with SCI (Aim 1B)

The objective of these experiments was to translate the co-stimulation experiments from the animal model and to determine whether co-stimulation is effective
in persons with SCI. Previous studies showed that electrical stimulation of the proximal urethra and distal urethra in persons with SCI evoked reflex bladder contractions and closely paralleled the results seen in cats (Yoo et al. 2008a; Yoo et al. 2011). We measured the effects of different frequencies of intraurethral stimulation at different locations on bladder activation and the electromyographic (EMG) activity of pelvic floor muscles during urodynamics. The hypothesis was that co-stimulation of the proximal and distal urethra would generate larger bladder contractions than individual stimulation and produce voiding efficiencies greater than control, distension-evoked voiding. The size of reflex bladder contractions evoked with stimulation was dependent on stimulation location and frequency, demonstrating that methods to improve outcomes in pre-clinical experiments may be valid in the clinical setting. However, reflex EMG activity suggested that multiple reflex pathways contributed to bladder activation.

1.5.2 Aim 2 – Temporal Patterns of Pudendal Afferent Stimulation

The effects of pudendal afferent stimulation on reflex bladder activation are known to be dependent on the stimulation frequency; high frequency DNP stimulation evokes robust bladder contractions, but low frequency stimulation does not evoke contractions and can inhibit existing bladder contractions. The objective of this aim was to determine if temporal patterns of stimulation evoke larger bladder contractions than constant frequency stimulation. We quantified the effects of novel temporal patterns of
stimulation on the size of evoked bladder contractions in anesthetized cats. Several of the temporal patterns were inspired by neural recordings, including decreasing frequency ramp patterns, which mimic accommodation seen in neural firing patterns, and random patterns. The hypotheses were that that temporal pattern of stimulation affects bladder contraction magnitude and that non-regular stimulation would evoke larger bladder contractions. The results show that while the size of bladder contractions was indeed dependent on the temporal pattern of pudendal afferent stimulation, no pattern significantly increased the size bladder contractions compared to regular frequency stimulation.

1.5.3 Aim 3 – Neural Mechanisms of the Spinal Pudendo-vesical Reflex

Although the pudendo-vesical reflex and its physiological properties are well established, there is limited understanding of the specific neural mechanisms that mediate this reflex. The objective of this aim was to better identify the neural network mechanisms underlying the pudendo-vesical reflex. Animal experiments were performed to identify the neurotransmitters responsible for bladder inhibition by pudendal afferent stimulation and a computational model of the neural network was developed to allow for exploration of the mechanisms underlying the bladder response to pudendal afferent stimulation.
1.5.3.1 Pharmacological Blockade of Pudendal Afferent Stimulation-Evoked Inhibition (Aim 3A)

Low frequency electrical stimulation of pudendal afferents inhibits bladder contractions and increase bladder capacity. Recent results suggested that pudendal afferent stimulation-evoked bladder inhibition was not mediated by sympathetic bladder efferents in the hypogastric nerve generating α-adrenergic receptor mediated inhibition at the vesical ganglia and / or β-adrenergic receptor mediated direct inhibition of the detrusor muscle. The objective of these experiments was to identify the neurochemical process primarily responsible for bladder inhibition with low frequency pudendal afferent stimulation. The hypothesis was that blockade of the neurochemical mechanism responsible for stimulation-evoked bladder inhibition would prevent a reduction in bladder pressure caused by low frequency pudendal afferent stimulation.

We investigated several inhibitory neurotransmitters that may be necessary for stimulation evoked inhibition, including sympathetic, adrenergic, glycinergic, opioidergic, and GABAergic mechanisms. The results identified a lumbosacral spinal GABAergic mechanism is necessary for bladder inhibition evoked by pudendal afferent stimulation.

1.5.3.2 Computational Modeling of the Spinal Pudendo-vesical Reflex (Aim 3B)

Although the input-output properties of the pudendo-vesical reflex have been characterized empirically, there is limited understanding of the underlying neural
network mechanisms that mediate the reflexes governing the effects of PN stimulation on bladder function. The objective of this work was to develop and validate a biophysically-motivated model of the neural network underlying the pudendo-vesical reflex. The hypothesis was that the properties and features of reflex bladder activation and inhibition are the result of a network-mediated phenomenon, which can be replicated in a simple neural network model. We implemented and validated a neural network architecture based on previous neuroanatomical and electrophysiological studies. Using synaptically-connected integrate and fire model neurons, we created a network model with realistic spiking behavior. The frequency and pattern-dependent effects of pudendal afferent stimulation were determined by changes in firing rate of spinal interneurons, suggesting that neural network interactions at the lumbosacral level can mediate the bladder response to different frequencies or temporal patterns of pudendal afferent stimulation. The model demonstrates the critical features of the pudendo-vesical reflex and may prove useful to guide development of novel, more effective electrical stimulation techniques for bladder control.
Figure 1.1: Neural and anatomical connections of normal lower urinary tract control.
Figure 1.1: Neural and anatomical connections of normal lower urinary tract control.

Afferents from the bladder and pelvic floor enter the spinal cord at the thoracic and sacral levels where they send ascending projections or synapse with local neurons. Descending modulation from the periaqueductal gray (PAG) and pontine micturition center (PMC) coordinates bladder and sphincter activity by controlling the output of the preganglionic sympathetic nucleus (PSN), sacral parasympathetic nucleus (SPN), and Onuf’s nucleus. Abbreviations: cranial sensory nerve (CSN), dorsal nerve of the penis (DNP), external anal sphincter (EAS).
Figure 1.2: Locations targeted for restoration of bladder control using electrical stimulation.
Figure 1.2: Locations targeted for restoration of bladder control using electrical stimulation.

Anatomical locations of electrodes used for electrical stimulation for bladder function are shown: sacral anterior root stimulation (SARS), sacral nerve stimulation (SNS), pudendal nerve stimulation (PNS), percutaneous tibial nerve stimulation (PTNS), transcutaneous electrical nerve stimulation (TENS), and high frequency nerve block (HFNB). PNS, TENS, and PTNS can be performed with minimally invasive techniques. SARS typically requires a posterior rhizotomy. HFNB combined with PNS or SNS can eliminate unwanted contractions of the external urethral sphincter and produce efficient voiding. Following spinal cord injury (SCI), connections to the brain and higher order spinal circuits are lost. Abbreviations: cranial sensory neuron (CSN), dorsal nerve of the penis (DNP), Onuf’s nucleus (ON), and sacral parasympathetic nucleus (SPN)
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<th>Stimulation Mode</th>
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<td>Mechanical Stimulation</td>
<td>Perigenital Skin</td>
<td>Inhibition of micturition contractions(^{16,17}) &amp; Excitation at empty(^{20}) *</td>
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<td>Bladder inhibition with penile squeeze (^{12})</td>
</tr>
<tr>
<td></td>
<td>Perigenital Skin</td>
<td>Inhibition (^{23})</td>
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<td></td>
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<td>N/A *</td>
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<td></td>
<td></td>
<td>Bladder inhibition (5-7 Hz)(^{10}) &amp; Excitation (20 Hz)(^{10})</td>
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<td>Excitation (33 Hz)(^{7}) &amp; Inhibition (0.5-5 Hz) (^{24}) *</td>
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<td></td>
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<td>Inhibition (3 Hz) (^{3}) &amp; Excitation (20 Hz) (^{3})</td>
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<td></td>
<td></td>
<td>Reduction in voiding dysfunction (^{13})</td>
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<tr>
<td>Electrical Stimulation</td>
<td>Compound Pudendal Nerve (PN)</td>
<td>N/A</td>
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<td></td>
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<td>N/A *</td>
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<td></td>
<td></td>
<td>Excitation (1-40 Hz) (^{6}) &amp; Inhibition (10 Hz) (^{4})</td>
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<td></td>
<td>Excitation (33 Hz)(^{7}) &amp; Inhibition (0.5-5 Hz) (^{24}) *</td>
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<td>Inhibition (5-7 Hz) (^{2,22}) &amp; Excitation (20-40 Hz) (^{2,22})</td>
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<td></td>
<td>Reduction in incontinent episodes and urgency (^{11})</td>
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<tr>
<td></td>
<td>Dorsal Genital Nerve (DGN), Distal Urethra</td>
<td>Excitation (1, 20 Hz) (^{19})</td>
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<td>Excitation (2, 20, 50 Hz) (^{18})</td>
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<td>Excitation (20-40 Hz) (^{6,8}) &amp; Inhibition (5-10 Hz) (^{6,8})</td>
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<td>Excitation (20-40 Hz)(^{7,8}), (20-50 Hz)(^{9}) *</td>
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<td>Reduction in voiding dysfunction (^{13})</td>
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<td></td>
<td>Cranial Sensory (CSN), Proximal Urethra</td>
<td>N/A</td>
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<td>Excitation (2-5 Hz)(^{3}), (2, 10, 33 Hz)(^{8})</td>
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<td>No Excitation (2-5 Hz)(^{7,8}), (1-3, 20-50 Hz)(^{9}) *</td>
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<td>Bladder excitation (^{15,21})</td>
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<td>Bladder excitation (^{15,21})</td>
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Table 1.1: Summary of pudendal-to-bladder reflexes across species and injury models.

This table summarizes pre-clinical experiments performed across various animal models of bladder function and clinical studies of the effects of pudendal nerve stimulation on bladder activity.

1 (Thor et al. 1990)  
2 (Tai et al. 2007b; Tai et al. 2008)  
3 (Tai et al. 2006)  
4 (Boggs et al. 2006a; Boggs et al. 2006b)  
5 (Yoo and Grill 2007)  
6 (Woock et al. 2008)  
7 (Yoo et al. 2008a)  
8 (Woock et al. 2009b)  
9 (Bruns et al. 2009a)  
10 (Chen et al. 2010)  
11 (Goldman et al. 2008)  
12 (Kondo et al. 1982)  
13 (Peters et al. 2005)  
14 (Previnaire et al. 1996)  
15 (Yoo et al. 2011)  
16 (Sato et al. 1975)  
17 (Hotta et al. 2012)  
18 (Peng et al. 2008b)  
19 (Chen et al. 2012)  
20 (Morrison et al. 1995)  
21 (McGee et al. 2011)  
22 (Tai et al. 2011)  
23 (Sato et al. 1977)  
24 (Xiao et al. 2014b)  
N/A = Not Available

* The lack of distension-evoked bladder contractions after acute SCT typically prevents the investigation of bladder inhibition.
2. Selective Co-stimulation of Pudendal Afferents Enhances Reflex Bladder Activation and Improves Voiding Efficiency

This chapter was previously published in Neurourology and Urodynamics and is used with permission (McGee and Grill 2014b). The manuscript was accepted for publishing on July 8, 2013 and was available online on August 9, 2013 and in print in November 2014.

2.1 Introduction

Spinal cord injury (SCI) causes lower urinary tract (LUT) dysfunction, including incontinence, chronic retention, and detrusor-sphincter dyssynergia (DSD) (Abrams et al. 2002), which leads to decreased quality of life (Anderson 2004). Intermittent catheterization and anticholinergic medications are used to treat LUT dysfunction but are associated with frequent urinary tract infections, urinary tract damage and side-effects (Burns et al. 2001). Various techniques may be used to initiate voiding, including bladder tapping, Valsalva maneuvers, and reflex voiding, although the prevalence of DSD and associated poor-outcomes limit this in the SCI population (Benevento and Sipski 2002). Restoring LUT function in persons with neurological disease or injury, including SCI, remains a significant unmet need. Electrical stimulation is an alternative approach to restore LUT function, but current approaches are not widely employed (Gaunt and Prochazka 2006). Electrical stimulation of pudendal nerve (PN) afferents to
cause reflex activation or inhibition of the bladder is an alternative approach to restore control of bladder function.

Electrical stimulation of the PN is uniquely capable of producing both excitation of the bladder and voiding, and inhibition of the bladder and continence (Boggs et al. 2006b; Tai et al. 2007b). Stimulation frequency directly influences the bladder response to stimulation: low frequencies, 2-15 Hz, produce inhibition and continence while higher frequencies, 20-50 Hz, evoke excitation and sustained contractions (Boggs et al. 2006b; Woock et al. 2008). Importantly, comparable inhibitory and excitatory reflexes have been demonstrated in persons with SCI (Kirkham et al. 2001a; Gustafson et al. 2004; Yoo et al. 2007).

More recent work differentiated the effects of stimulation of individual distal PN branches on bladder function. Selective electrical stimulation of the cranial sensory nerve (CSN) and dorsal genital nerve (DGN) branches each evoked sustained bladder contractions and generated increases in voiding efficiency (VE) compared to distention-evoked voiding. Similarly, in humans with SCI, two excitatory pudendo-vesical reflexes were evoked by selective stimulation of the proximal and distal urethra (Yoo et al. 2011), presumably corresponding to the CSN and DGN.

However, VEs produced by PN stimulation were limited to ~60% (Yoo et al. 2008a), likely as a result of the bladder volume dependence of the evoked reflexes. Bladder activation by pudendal afferent stimulation requires volumes of 70-80% of the
threshold volume to elicit distension-evoked reflex contractions (Woock et al. 2008). This volume dependence does not result from length-tension properties of the detrusor (Boggs et al. 2005), but rather from a neural mechanism mediated by superposition of pelvic and pudendal afferent activity (Woock et al. 2011). Presumably, as the bladder empties during pudendal afferent stimulation-evoked contractions, pelvic afferent activity is reduced (Häbler et al. 1993), the superposed afferent activity declines, and the evoked contraction subsides, thereby limiting VE.

The purpose of this study was to test the hypothesis that stimulation of multiple PN afferents, either bilateral DGN stimulation or selective co-stimulation of both CSN and DGN, will evoke larger reflex bladder contractions and result in higher VEs than stimulation of any single afferent pathway alone. Producing efficient voiding with lower residual volumes is imperative for eventual clinical application, as large residuals are linked to risk of bacteriuria (Truzzi et al. 2008).

2.2 Materials and Methods

Animal care and experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee. Sexually intact, adult male cats (3.5-4.5 kg) were anesthetized with ketamine-HCl (35 mg·kg⁻¹ im) and anesthesia was maintained with α-chloralose (65 mg·kg⁻¹ iv, supplemented at 15 mg·kg⁻¹ iv). End-tidal CO₂ was maintained between 3-4% with artificial respiration. Blood pressure was monitored with a catheter placed in the carotid artery and a solid-state pressure
transducer. Body temperature was maintained at 38°C using a heating pad and intravenous fluids, 0.9% NaCl with 5% dextrose and 8.4 mg·L⁻¹ NaHCO₃, were administered (15 ml·kg⁻¹·h⁻¹).

A midline abdominal incision was made to expose the bladder, and a 3.5 Fr. suprapubic catheter was inserted into the dome and secured with a purse string suture. Bladder pressure was measured with a solid-state pressure transducer (Deltran, Utah Medical) in series with the catheter and recorded. The urethra was occluded with a 3.5-5 Fr catheter during isovolumetric experiments and filling of the bladder. The threshold volume at which distension-evoked reflex contractions (DECs) occurred was found by filling the bladder with saline at 1-2 ml/min. External anal sphincter (EAS) electromyographic (EMG) activity was recorded from two wire electrodes, amplified (1,000), filtered (10 Hz - 10 kHz), and recorded.

The PN was exposed through an incision between the base of the tail and the ischial tuberosity, transection of the gluteofemoralis, and dissection of the ischiorectal fossa. The sensory (SN) and rectal perineal (RP) branches of the PN were visible and further dissection of the distal portion of the SN branch revealed the CSN and dorsal nerve of the penis (DNP) branches (Yoo et al. 2008b; Yoo et al. 2008a).

In co-stimulation experiments, the CSN and DNP were stimulated by unilateral implantation of cuff electrodes around each branch, and in bilateral stimulation experiments, cuff electrodes were placed around both right and left DNP branches.
(Figure 2.1). Monopolar electrodes were composed of platinum contacts embedded within silicone cuffs, and two asynchronous pulse generators (Pulsar 6bp, FHC Inc.) were used to deliver stimulation (0.1 ms cathodic stimulus pulses, for 30 s) between each cuff and subcutaneous 20-gauge needles. The optimal frequencies identified in previous studies (12) were used for unilateral stimulation of CSN (2 Hz) and DNP (33 Hz) or bilateral stimulation of DNP (33 Hz). Stimulation amplitude was 3 times the threshold (T) to produce a reflex EMG response in the EAS with 1 Hz stimulation.

The effects of co-stimulation on isovolumetric bladder contractions, threshold volume, and VE were quantified (Figure 2.1A). Isovolumetric bladder contraction trials were conducted across 30 cats at approximately 80% of the threshold volume for DECs. Threshold volume experiments were conducted in 16 cats in which pudendal afferent stimulation generated reflex bladder contractions. The threshold volume to evoke bladder contractions with each stimulation type was determined by filling the bladder at 0.333 ml/min and delivering stimulation in randomized order every 30 seconds. Contractions were defined as increases in pressure greater than 25 cmH₂O or 10 cmH₂O above baseline. Voiding experiments were conducted in 16 cats in which pudendal afferent stimulation generated reflex bladder contractions. For each stimulation condition, the bladder was filled at 1-2 ml/min to 125% of the threshold volume for DECs. DNP stimulation at 10 Hz was used to inhibit bladder contractions and prevent voiding during the fill, if necessary (Woock et al. 2008). After reaching 125% of the
threshold volume, the urethral catheter was removed and continuous stimulation was delivered. Measurements of volume voided and the residual volume in the bladder after voiding were used to determine VE:

\[
\text{Voiding efficiency (VE) (\%) = } \left( \frac{V_{\text{voided}}}{V_{\text{voided}} + V_{\text{residual}}} \right) \times 100.
\]

Bladder pressures during isovolumetric trials were quantified by mean and maximum pressure during stimulation minus the average of the baseline pressure during the 3 seconds before stimulation, as well as the pressure-time integral (PTI) with respect to baseline (Figure 2.2A). The mean, maximum, and PTI were averaged across repeated trials within experiments and compared in an ANOVA with post hoc comparisons using Fisher’s Protected Least Significant Difference (PLSD) test. EAS EMG recordings were stimulus-triggered averaged, rectified and integrated over the 20 ms following the stimulus artifact, and compared in an ANOVA with post hoc PLSD. Volume thresholds to evoke contractions with stimulation were normalized to the threshold volumes for DECs in each cat and analyzed by repeated measures ANOVA and post hoc PLSD. Stimulation-evoked VEs were normalized to distention evoked VEs for each experiment and analyzed with repeated measures ANOVA and post hoc PLSD.

2.3 Results

The effects of bilateral DNP stimulation or selective unilateral co-stimulation of CSN and DNP on isovolumetric bladder contractions, threshold volumes, and VE were
measured in anesthetized cats and compared to single nerve branch stimulation and
distention-evoked reflexes.

2.3.1 Stimulation-Evoked Bladder Contractions

Robust bladder contractions were evoked in all cats by stimulation of the DNP
(T= 0.43±0.04 mA) or CSN (T= 0.44±0.05 mA) under isovolumetric conditions. Bilateral
DNP stimulation evoked larger mean bladder pressures (24.2±0.8 cmH\(_2\)O, mean ± SEM,
n=16) than either right (19.4±0.6 cmH\(_2\)O) or left (18.4±0.6 cmH\(_2\)O) unilateral stimulation
(Figure 2.2A-B). Maximum bladder pressures during stimulation were also larger for
bilateral stimulation (34.1±0.9 cmH\(_2\)O) than right (28.1±0.7 cmH\(_2\)O) or left (27.1±0.7
cmH\(_2\)O) unilateral stimulation. PTI values, representing the area under the contraction,
were larger for bilateral stimulation (652±26 cmH\(_2\)Os) than for unilateral stimulation (R
DNP: 474±14 cmH\(_2\)Os, L DNP: 494±23cmH\(_2\)Os). There was no consistent laterality across
animals, although laterality within animals was seen. In 12 cats where there were
substantial differences between bladder contractions evoked by right or left unilateral
stimulation, stimulation of the dominant side produced significantly larger mean
bladder contractions (20±1.5 cmH\(_2\)O) than the non-dominant side (14.9±1.4 cmH\(_2\)O)
(p=0.0229). Co-stimulation-evoked bladder contractions had larger mean and maximum
pressures (24.7±0.8 cmH\(_2\)O, 36.1±1.1 cmH\(_2\)O, mean ± SEM, n=14) than CSN (17.1±0.8
cmH\(_2\)O, 29.0±1.2 cmH\(_2\)O) or DNP (21.1±0.9 cmH\(_2\)O, 31.4±1.1 cmH\(_2\)O) stimulation alone
(Figure 2.2C-D). The PTI values for bladder contractions evoked by co-stimulation
(697±28 cmH₂Os) were also larger than those evoked by CSN (468±28 cmH₂Os) or DNP (580±24 cmH₂Os).

2.3.2 EAS Activation Varies with Stimulation Frequency and Spatial Pattern

Reflex activation of the EAS varied with stimulation frequency and pattern of co-stimulation (Figure 2.3). EAS responses (latency: 10-15 ms) were elicited in all cats with stimulation at 1 Hz of either DNP or CSN. Unilateral 33 Hz DNP stimulation evoked EAS responses that rapidly diminished after the first 1-2 pulses of the stimulation train (Boggs et al. 2006b; Woock et al. 2008), while unilateral 2 Hz CSN stimulation evoked a consistent reflex response following each pulse. Similar to unilateral 33 Hz DNP stimulation, bilateral DNP stimulation and CSN and DNP co-stimulation evoked a reflex that diminished after the first 1-2 pulses. Rectified and integrated EAS responses during 2 Hz CSN stimulation were significantly larger than either 33 Hz DNP stimulation alone (19.5 times larger) or CSN and DNP co-stimulation (15.7 times larger) (p < 0.0006, n=10 CSN and DNP co-stimulation experiments).

2.3.3 Effects of Stimulation on Threshold Volumes

Repeated randomized stimulation during slow bladder filling was used to determine volume thresholds to evoke sustained bladder contractions with each stimulation condition and for DECs (Figure 2.4A). Both unilateral and bilateral DNP stimulation evoked contractions at smaller bladder volumes than DECs. Robust, sustained bladder contractions were generated with unilateral or bilateral DNP
stimulation at 79±2.6% of DEC volume thresholds and bilateral DNP (75±2.6%) stimulation evoked contractions at smaller volumes than either right (80±2.8%) or left (83±2.5%) unilateral DNP stimulation (Figure 2.4B). Individual or selective co-stimulation of CSN and DNP (82±3.6%) evoked contractions at lower volumes than DECs and co-stimulation (76±3.9%) evoked contractions at smaller bladder volumes than stimulation of either CSN (88±3.8%) or DNP (81±3%) alone, although the latter difference was not significant (Figure 4C). In two of seven cats, the threshold volume for selective co-stimulation of CSN and DNP was the same as the threshold volume for DNP stimulation alone, while it was lower in the remaining five.

2.3.4 Effects of Stimulation on Voiding Efficiency

An example of bladder emptying with bilateral DNP stimulation is shown in Figure 2.5A. Voided volumes and VEs for both stimulation-evoked voiding and distention-evoked voiding (DEV) varied across cats (Figure 2.5B), and VEs with stimulation were normalized by distention-evoked VEs to combine data across experiments. VE was significantly higher than DEV with either unilateral or bilateral DNP stimulation (159±7%, mean ± SEM). Further, bilateral DNP stimulation (181±7%) increased VE over either right or left unilateral DNP stimulation (147±6%) alone in seven of eight cats, and produced significantly higher VEs (Figure 2.5C). Both individual and co-stimulation of CSN and DNP increased VE by 142±5% over distention-evoked
voiding. Selective co-stimulation (162±5%) produced significantly larger VEs than individual stimulation of CSN (122±5%) or DNP (143±5%) alone (Figure 2.5E).

2.4 Discussion

Electrical stimulation of PN afferents is a promising approach to restore control of bladder function. Although pudendal afferent stimulation generates robust reflex bladder contractions, residual bladder volumes following PN stimulation evoked voiding are too large for successful clinical translation. The results of this study indicate that selective co-stimulation of multiple PN afferents evokes larger bladder contractions, generates bladder contractions at smaller bladder volumes, and increases VE compared to stimulation of any single afferent pathway alone. Although statistically significant, the effect size of co-stimulation on voiding efficiency was limited from a functional perspective. The results may have been affected by the use of a spinal intact, anesthetized animal model, and additional experiments should be performed in persons with impaired voiding to determine the clinical promise of this approach.

2.4.1 Evoked Isovolumetric Bladder Contractions

Bilateral DNP stimulation and co-stimulation of CSN and DNP produced larger isovolumetric mean pressure, maximum pressure, and PTI than isolated stimulation of individual PN branches. Co-stimulation did not result in superposition of responses from individual stimulation, suggesting a ceiling effect on bladder activation where co-stimulation cannot increase bladder contraction size beyond some maximum.
Stimulation type during isovolumetric blocks was randomized, and the interaction between stimulation type and order was not significant for either bilateral or co-stimulation experiments. Thus, the larger bladder contractions evoked by co-stimulation were not dependent on the previous type of stimulation and did not result in carry-over effects to the next type of stimulation. All co-stimulation was asynchronous, or interleaved in time, preventing simultaneous electrical stimulation of different branches. Therefore, the larger bladder contractions produced by co-stimulation are the consequence of a neural process resulting from PN afferent signals, facilitated by converging spinal cord inputs (Thor et al. 1989), rather than an increase in effective stimulation amplitude.

2.4.2 Suppression of Dyssynergic Activity

Neither 33 Hz bilateral DNP stimulation nor co-stimulation of CSN at 2 Hz and DNP at 33 Hz produced reflex activation of the EAS. Pudendal afferent stimulation has been shown to suppress activity in the skeletal muscles of the pelvic floor, and was also seen with co-stimulation. Electroneurogram (ENG) activity from the deep perineal branch of the pudendal nerve (innervates the EUS) was suppressed (Shefchyk and Buss 1998; Boggs et al. 2006b) and intraurethral EMG recordings were similarly suppressed (Woock et al. 2008) during bladder contractions evoked by pudendal afferent stimulation in intact and spinal transected cats. Further, EMG from perineal muscles in persons with chronic SCI was suppressed during bladder contractions evoked by
pudendal afferent stimulation (Yoo et al. 2011). We used the EAS electromyogram as a proxy for PN efferent reflex activity produced by stimulation, as previous studies showed strong congruence between EAS and EUS electromyograms in response to pudendal afferent stimulation (Woock et al. 2008; Yoo et al. 2008a). However, differential activity in the EUS and EAS is well documented, and EAS activity may not reflect EUS activity or outlet pressure. The sphincters are capable of operating independently (Shafik 1998) and changes in activity seen in the EUS may not be seen in the EAS (Fedirchuk and Shefchyk 1993). While 2 Hz CSN produced dyssynergic activation of the EAS, stimulation-evoked EAS activation was suppressed with co-stimulation of CSN and DNP, and PN afferent stimulation can be used reflexively to activate the bladder without activating PN efferents. This is an improvement over previous methods, such as sacral anterior root stimulation, which produce concomitant activation of bladder and sphincter (Brindley 1977). It remains to be determined whether DSD during bladder activation following chronic SCI is also suppressed by DGN stimulation.

2.4.3 Lower Threshold Volumes

Electrical stimulation of PN afferents evokes bladder contractions at approximately 80% of the threshold volume for DECs. The threshold volume dependence of the reflex is mediated by the convergence of pudendal and pelvic afferents in the sacral spinal cord (Boggs et al. 2005; Woock et al. 2011). Voiding efficiency produced by PN afferent stimulation appears to be limited by the volume
threshold because as voiding occurs and the volume drops below the threshold volume, contractions cease. All types of pudendal afferent stimulation, including bilateral and co-stimulation, evoked bladder contractions at lower volumes than distension (Woock et al. 2009b). The reduced volume threshold for stimulation-evoked contractions provides a potential mechanism for the increased VE with stimulation.

The threshold volume with bilateral DNP stimulation was lower than with unilateral DNP stimulation, presumably as a result of reinforced pudendal afferent signaling to the sacral spinal cord. CSN and DNP co-stimulation reduced the threshold volume to evoke robust bladder contractions compared to CSN stimulation alone, apparently due to DNP stimulation, as DNP stimulation evoked contractions at a lower volume than CSN stimulation. Therefore, any improvement in VE produced by co-stimulation over DNP stimulation alone is not solely attributable to a change in threshold volume. Rather, stimulation of CSN and DNP activates two separate reflex pathways (Yoo et al. 2008a; Woock et al. 2009b), which may explain why co-stimulation did not reduce threshold volume compared to DNP stimulation alone, but did improve VE. In contrast, bilateral DNP stimulation likely reinforced the pudendal afferent input in one of the reflexes in the spinal cord, producing lower threshold volumes and increased VE.
2.4.4 Enhanced Voiding Efficiency

Stimulation of either DNP or CSN increased VEs over distension-evoked voiding (Yoo et al. 2008a), and selective co-stimulation of either bilateral DNP or CSN and DNP further increased VE. Voiding efficiencies may have been adversely affected by anesthesia with α-chloralose, which is known to affect spinal reflexes (Shimamura et al. 1968) and bladder activity (Rudy et al. 1991) and the use of another anesthetic may lead to different results. Pelvic and pudendal afferents converge in the dorsal horn (Thor et al. 1989) and modulate the sacral spinal network that controls the pelvic efferent output of the sacral parasympathetic nucleus (McMahon and Morrison 1982). Specifically, pudendal afferent fibers terminate in portions of the ipsilateral dorsal horn, intermediate zone, the dorsal gray commissure and contralateral dorsal horn (Thor et al. 1989). Bilateral stimulation may result in superposition of converging right and left pudendal afferent activity to produce reflex bladder contractions with lower levels of pelvic afferent signaling and thereby produce larger VEs than unilateral stimulation.

In contrast, the improved VE generated by co-stimulation of CSN and DNP over individual CSN or DNP stimulation is likely due to the activation of two separate reflex pathways (Yoo et al. 2008a; Woock et al. 2009b). The limited reduction in threshold volume with co-stimulation compared to DNP stimulation alone supports this mechanism.
Although not directly investigated, we can compare the VEs produced by bilateral stimulation and co-stimulation. Bilateral DNP stimulation improved VE more than co-stimulation of CSN and DNP. Additionally, in one cat where bilateral stimulation and co-stimulation evoked voiding was measured, bilateral DNP stimulation generated larger voided volumes than co-stimulation of DNP and CSN. This finding is supported by the significant decrease in threshold volume seen with bilateral stimulation, whereas co-stimulation did not reduce volume threshold compared to DNP stimulation.

2.4.5 Clinical Relevance

In a clinical setting, with a variety of causes of bladder dysfunction, ranging from neurological disease to traumatic injury, co-stimulation of multiple nerve branches may be more effective, especially in the case of inadequate benefit from individual stimulation. In cases of complete SCI, spinal circuitry and reflexes below the injury are likely still intact. Studies evaluating stimulation of pudendal sensory pathways suggest that PN afferent stimulation is effective after transection of the spinal cord (Tai et al. 2007b; Woock et al. 2011), although efficient voiding may be limited due to DSD, and the impact of co-stimulation in persons with SCI remains to be determined in clinical studies. In cases such as these, bilateral DNP stimulation is more likely to be successful for evoking on-demand bladder contractions and increasing VE. Both branches of the dorsal genital nerve (DGN) travel close together near the distal urethra.
(Bradley et al. 1973; Vaze et al. 2008), and bilateral DNP stimulation could be implemented with a single, midline electrode. Percutaneous stimulation of the DGN produced a 50% or greater reduction in overactive bladder symptoms with DGN stimulation in 81% of subjects (Goldman et al. 2008). Additionally, two independent pudendal-bladder reflex pathways are present in persons with SCI (Yoo et al. 2011) and co-stimulation of these pathways can improve bladder activation and voiding (McGee et al. 2011). However, this will require surgical access (Gustafson et al. 2005) and implantation of a cuff electrode (Kent and Grill 2013).

2.5 Conclusion

We quantified the effects of selective co-stimulation of multiple pudendal afferent pathways on bladder contractions and voiding in α-chloralose anesthetized cats. Both bilateral DNP stimulation and co-stimulation of CSN and DNP evoked larger bladder contractions and increased VE over single branch PN afferent stimulation and distention evoked voiding. Future development of neural prosthetics for restoration of bladder function should include these techniques of spatial patterning of pudendal afferent stimulation, particularly bilateral DNP stimulation, which produced large on-demand bladder contractions coupled with suppression of pelvic floor reflex activity, to improve bladder control in persons with bladder dysfunction from neurological disease or injury.
Figure 2.1: Experimental setup to measure the effects of selective co-stimulation of pudendal afferents on bladder function.
Figure 2.1: Experimental setup to measure the effects of selective co-stimulation of pudendal afferents on bladder function.

A. Summary of number of cat experiments performed, n. B. A suprapubic catheter placed in the bladder dome was used to measure bladder pressure ($P_{\text{bladder}}$). After dissection of the ischiorectal fossa, electrode cuffs were placed around each nerve branch for selective stimulation. During voiding experiments the urethral catheter used to occlude the urethra was removed and voided volume was measured with a scale. B. The pudendal nerve (PN) splits into a rectal perineal (RP) branch and sensory (SN) branch, which is further divided into the cranial sensory (CSN), innervating proximal urethra, and the dorsal genital nerve (dorsal nerve of penis in males – DNP), innervating the distal urethra (18). The placement of cuff electrodes for both bilateral DNP stimulation and CSN and DNP co-stimulation experiments are shown.
Figure 2.2: Selective co-stimulation of multiple afferent pathways evokes larger isovolumetric bladder contractions.
Figure 2.2: Selective co-stimulation of multiple afferent pathways evokes larger isovolumetric bladder contractions.

A. Representative bladder pressure traces from isovolumetric trials for right, left and bilateral DNP stimulation. Heavy black bars indicate when stimulation was applied. Inset indicates mean, maximum and pressure-time integral (PTI). B. Mean, maximum and PTI of bladder pressure were larger for bilateral stimulation than either right or left DNP stimulation (p < 0.0001, ANOVA, n=16 cats, post hoc Fisher’s PLSD, * p < .0001). Error bars indicate standard errors. C. Representative bladder pressure traces from isovolumetric trials for stimulation of DNP, CSN and CSN+DNP co-stimulation. D. Mean, maximum and PTI of bladder pressure were larger for CSN+DNP co-stimulation than either stimulation of CSN or DNP alone (p < 0.0001, ANOVA, n=14 cats, post hoc Fisher’s PLSD, * p < 0.05, ** p < 0.005, *** p < 0.0001).
Single trials (light lines) and averaged (dark lines) EMG responses time aligned to the stimulation pulse. Low frequency (1 Hz) stimulation of DNP and CSN evoked a strong bulbocavernosus reflex (BCR). Large spikes in EMG recordings are stimulus artifacts, indicating when stimulation was presented. The EAS reflex response is seen consistently after each pulse of 2 Hz CSN stimulation at 3T, while it disappears after the first 1-2 pulses with 33 Hz DNP at 3T (middle row). With 33 Hz bilateral DNP stimulation or co-stimulation of 2 Hz CSN and 33 Hz DNP at 3T, the response is either absent or disappears after the first 1-2 pulses (bottom row).
Figure 2.4: Selective co-stimulation can reduce threshold volume for stimulation-evoked contractions.

A. Threshold volume testing with bilateral DNP stimulation. Stimulation was presented for 30 sec every minute (light gray bars), in random block order, while the bladder was filled at a rate of 0.333 ml/min. The first robust contractions evoked by bilateral stimulation, R DNP stimulation, L DNP stimulation and distension are labeled by B, L, R and D, respectively. B. The threshold volumes to evoke contractions with right, left and bilateral DNP stimulation all were lower than the distention evoked volume threshold (p < 0.0001, 2-way repeated measures ANOVA, n= 9 cats, post hoc Fisher’s PLSD, * p < 0.001, ** p < 0.0001). The threshold volume to evoke contractions with bilateral DNP stimulation was lower those for R DNP or L DNP stimulation alone. C. The threshold volumes to evoke contractions with stimulation of CSN, DNP and co-
stimulation of CSN and DNP were lower than the distention evoked volume threshold (p = 0.0016, 2-way repeated measures ANOVA, n= 7 cats, post hoc Fisher’s PLSD, * p < 0.05, ** p < 0.005). The threshold volume to evoke contractions with DNP stimulation was lower than for CSN stimulation. The threshold volume to evoke contractions with co-stimulation of CSN and DNP was lower than for CSN stimulation.
Figure 2.5: Selective co-stimulation of multiple afferent pathways improves voiding efficiency.
Figure 2.5: Selective co-stimulation of multiple afferent pathways improves voiding efficiency.

A. Example of bilateral DNP stimulation-evoked voiding. Voided volume measured by weight with a scale converted into volume is shown over time during the void. Gray bar indicates when stimulation was on. B. Raw voiding efficiencies from bilateral stimulation experiments for each cat. C. Raw voiding efficiencies from co-stimulation experiments for each cat. D. Bilateral DNP stimulation increased voiding efficiency over stimulation of R or L DNP alone and distention (p = 0.0066, 2-way repeated-measures ANOVA, post hoc Fisher’s PLSD, * p < 0.001, ** p < 0.0001). E. Co-stimulation of CSN and DNP increased voiding efficiency over stimulation of CSN or DNP also and distention (p = 0.005, 2-way repeated-measures ANOVA, post hoc Fisher’s PLSD, * p < 0.0001).
3. Multiple Reflex Pathways Contribute to Bladder Activation by Intraurethral Stimulation in Persons with Spinal Cord Injury

3.1 Introduction

Lower urinary tract dysfunction, including neurogenic detrusor overactivity, urinary incontinence, chronic retention of urine, and detrusor-sphincter dyssynergia, can be caused by spinal cord injury (SCI) (Shingleton and Bodner 1993; de Groat et al. 1998; Benevento and Sipski 2002; McGee et al. 2015). Management of bladder dysfunction in persons with SCI typically includes anticholinergic medications and intermittent catheterization, although these often involve drug side effects and frequent urinary tract infections, further impacting quality of life (Shingleton and Bodner 1993; Ku 2006). Electrical stimulation is an alternative approach for restoration of bladder function (Gaunt and Prochazka 2006).

Several different stimulation locations have been investigated to restore bladder control in SCI with varying degrees of success. Sacral anterior root stimulation (SARS) is effective for bladder control following SCI, but has not been widely implanted due to the requirement for an irreversible dorsal rhizotomy (Brindley 1988; Rijkhoff 2004), which eliminates residual reflex erection or defecation. Sacral nerve stimulation (SNS) with Interstim® is less invasive but is generally ineffective in SCI (Chartier-Kastler et al. 2001; Kessler et al. 2010). Pudendal nerve stimulation (PNS) can produce inhibition of
bladder contractions and continence or bladder activation and voiding in persons with SCI (Vodušek et al. 1987; Wheeler et al. 1992; Previnaria et al. 1996; Kirkham et al. 2001b; Gustafson et al. 2003; Lee et al. 2003; Gustafson et al. 2004; Yoo et al. 2007).

Although promising, PNS in humans with SCI has not produced sufficiently efficient voiding. The purpose of the present study was to investigate the effects of co-stimulation of the proximal and distal urethra in persons with SCI to test the hypothesis that selective co-stimulation will enhance bladder activation, as compared to single site stimulation.

In the cat, selective co-stimulation of the distal branches of the PN, the cranial urethral sensory nerve (CSN) and the dorsal genital nerve (DGN), increases reflex bladder activation and produces more efficient voiding than single branch stimulation (McGee and Grill 2014b). These distal branches of the PN innervate the proximal and distal urethra (Yoo et al. 2008b) and intraurethral stimulation produces bladder responses comparable to those produced by direct nerve stimulation (Bruns et al. 2009a; Wooock et al. 2009b). Although the precise innervation of the human urethra is not fully known, studies in patients with SCI demonstrate bladder activation through selective stimulation in the proximal and distal urethra (Yoo et al. 2011). The present results demonstrate that bladder activation by co-stimulation of the proximal and distal urethra engages multiple reflex pathways. Bladder activation is dependent on stimulation
location, frequency, bladder volume, and intraurethral co-stimulation can be more effective than individual stimulation.

3.2 Methods

All study procedures were approved by the Institutional Review Board of Duke University Medical Center and written informed consent was provided by each subject. Seventeen subjects (13 male, 4 female) as least one year post suprasacral SCI were enrolled in the study (Table 3.1).

A 4 Fr catheter was inserted into the bladder to adjust bladder volume and measure bladder pressure, and a 9 Fr balloon catheter was inserted in the rectum to monitor abdominal pressure. A custom 12 Fr Foley stimulating catheter made of silicone with 12 (or 15) cylindrical, stainless steel electrode contacts (2.75 mm width) was inserted into the urethra such that the balloon lay in the bladder neck. Electrode locations in the proximal and distal urethra were based on typical anatomical landmarks and were shown to evoke bladder contractions in humans with SCI (Yoo et al. 2011). Given the significant inter-individual variability of these landmarks and the total length of the urethra, the electrodes were positioned with 5 (6) electrodes targeting the proximal urethra (0.9 or 0.5 cm spacing), and 7 (9) electrodes targeting the distal urethra (1.85 or 1.4 cm spacing). Pressure transducers were connected in series with the urethral and rectal catheters to record vesical pressure ($P_{ves}$) and abdominal pressure ($P_{abd}$).
Detrusor pressure ($P_{\text{det}}$) was calculated using $P_{\text{ves}}$ and $P_{\text{abd}}$ ($P_{\text{det}} = P_{\text{ves}} - P_{\text{abd}}$).

Electromyographic (EMG) activity was measured with percutaneous stainless steel wires inserted into the perineum and anal sphincter, amplified (1-5k), filtered (10 Hz – 10 kHz), and recorded.

Stimulating catheter electrode contacts were selected that minimized the threshold ($T$) to evoke reflex EMG activity in either the external anal sphincter (EAS) or perineal musculature (external urethral sphincter - EUS) with 2 Hz stimulation in the proximal or distal urethra when the bladder was empty. A control bladder fill was conducted at the beginning of each experiment to determine the volume threshold for distension-evoked contractions (DECs). Trials to measure the size of stimulation-evoked bladder contractions were conducted under isovolumetric conditions at approximately 80% of the volume necessary to produce DECs. Battery powered Empi 300PV electrical stimulators delivered trains of charge-balanced, asymmetric biphasic current pulses (pulse width = 0.2 ms, duration = 20 s). Blocks of sixteen randomized combinations of single site and co-stimulation at proximal and distal electrodes at different frequencies (2, 10, 20, and 40 Hz) were presented at stimulation amplitudes of 2 and 4 times the thresholds to evoke reflex EMG responses (2T and 4T, respectively).

Mean $P_{\text{det}}$ was calculated as the average pressure during stimulation minus the average pressure during the 5 seconds before stimulation. The maximum $P_{\text{det}}$ was the
maximum pressure evoked during stimulation, minus the baseline average. For all subjects who had stimulation-evoked contractions, the mean and maximum $P_{\text{det}}$ were compared in an ANOVA and post hoc paired comparisons with Bonferroni correction. EMG activity during stimulation-evoked bladder contractions was rectified and integrated, after stimulation artifact subtraction, and compared to pre-stimulation baseline values. Normalized, rectified and integrated (RI) EMG values for co-stimulation were compared in an ANOVA and post hoc paired comparisons with Bonferroni correction.

### 3.3 Results

We measured the effects of intraurethral stimulation on activity in pelvic floor muscles and bladder activation in persons with SCI. We tested multiple combinations of different frequencies at different locations under isovolumetric conditions.

#### 3.3.1 Reflex responses to intraurethral stimulation

The average electrode locations for proximal and distal stimulation were $3.9 \pm 0.4$ cm and $16.5 \pm 1.4$ cm from the bladder neck, respectively (Figure 3.1). Reflex EAS or EUS EMG activity in response to 2 Hz intraurethral stimulation was used to assess neural activation. Reflex responses were demonstrated in 10/17 subjects. In 7/10 subjects, reflex responses were evoked by stimulation in both the proximal and distal urethra, while in
the three remaining subjects, reflex responses were evoked by stimulation in only either the distal (1) or proximal (2) urethra.

Intraurethral stimulation-evoked reflex EMG responses fell into two distinct latency ranges: 30-50 ms and 60-90 ms (Figure 3.2), and these ranges corresponded to proximal and distal sites of intraurethral stimulation, respectively. The average latency of responses evoked by proximal stimulation was 76 ± 3.4 ms, while the average latency of responses evoked by distal stimulation was 48 ± 6.2 ms (p=0.0006, ANOVA, n=10). Table 32 shows reflex latencies and stimulation thresholds (T) for each subject. Four of 10 subjects had long both latency responses to proximal intraurethral stimulation and short latency responses to distal stimulation. In the two female subjects who had reflex responses to stimulation, only long latency responses were evoked with intraurethral stimulation of the proximal or distal portions of the urethra. There were no differences between reflex thresholds for proximal (13 ± 4.0 mA, n=11) or distal stimulation (12 ± 5.0 mA, n=6; p=0.934 ANOVA), or between thresholds to evoke short (20 ± 5.4 mA, n=11) or long latency (22 ± 3.9 mA, n=6; p=0.764 ANOVA) responses.

### 3.3.2 Effects of co-stimulation on bladder pressure and EMG activity

Increases in P\text{det} provoked by intraurethral stimulation were observed in 7 subjects. In some subjects, several factors limited our ability to conduct intraurethral stimulation: absence of distension-evoked bladder contractions (n=3), early termination due to
discomfort (n=2), stimulation amplitude limited by sensation (n=3), and inability to insert the stimulation catheter (n=1).

Figure 3.3A shows examples of bladder contractions evoked by intraurethral co-stimulation in comparison to a distension-evoked bladder contraction, and Figure 3.3B shows examples of bladder responses evoked by individual stimulation or co-stimulation. Mean stimulation-evoked $P_{\text{det}}$ was dependent on the stimulation frequency applied in the proximal and/or distal urethra (Figure 3.4A; $p<0.0001$, ANOVA, n=6). Post hoc comparisons with Bonferroni correction ($p<0.0001$) revealed that select combinations of co-stimulation produced larger increases in $P_{\text{det}}$ than individual stimulation, while other pairs of co-stimulation frequencies produced smaller contractions or reductions in $P_{\text{det}}$.

Proximal and distal intraurethral stimulation produced different effects on the mean and maximum $P_{\text{det}}$. For example, 10 Hz proximal stimulation produced excitation while 10 Hz distal stimulation produced inhibition. Additionally, there were significant differences in mean $P_{\text{det}}$ evoked with distal stimulation, and 2 Hz distal stimulation evoked significantly larger contractions than 10 or 20 Hz distal stimulation. Changing one of the frequencies in co-stimulation also had a significant effect on mean $P_{\text{det}}$. For example, 40P-20D (40 Hz proximal, 20 Hz distal) was significantly different than 20D alone, 10P-20D, 40P-40D, and 20P-20D.
On the other hand, there were no significant differences between the maximum $P_{det}$ produced by stimulation at different frequencies (Figure 3.4B). In subjects where stimulation produced increases in $P_{det}$, increasing the amplitude of stimulation produced an increase in mean $P_{det}$ ($1T=0.37\pm0.35$ cmH$_2$O, $2T=1.5\pm0.24$ cm H$_2$O, $4T=2.2\pm0.82$ cmH$_2$O; n=5), but this increase was not significant ($p=0.0736$).

To evaluate further the effectiveness of co-stimulation, we quantified the percent of subjects where stimulation produced maximum $P_{det}$ greater than 10 cmH$_2$O for each frequency pair (Figure 3.5). This revealed patterns of co-stimulation that were more effective ($2P-2D$, $40P-40D$) or less effective ($10D$, $10P-2D$).

There was a significant difference in the normalized RI EMG activity measured across subjects ($p<0.0001$, ANOVA, n=6). Stimulation produced a significant increase in EMG compared to stimulation-off periods (Figure 3.6; $p<0.0001$, ANOVA, n=6). However, there was a significant interaction effect in the ANOVA of normalized RI EMG between the frequency of stimulation and stimulation-on periods ($p=0.0057$), indicating that the significant increase in RI EMG activity when stimulation was applied was dependent on the stimulation frequencies.

### 3.3.3 Stimulation-evoked voiding efficiency

In one subject, two sets of proximal and distal stimulation frequencies that were effective in evoking bladder contractions were selected to evaluate the voiding efficiency
produced by stimulation. Voiding efficiency was improved with co-stimulation of 40 Hz proximal, 20 Hz distal (56%) and 20 Hz proximal, 20 Hz distal (36%) compared to control voiding (17%) (Figure 3.7).

3.4 Discussion

Following SCI, pudendal nerve stimulation can inhibit bladder contractions and promote continence or generate bladder contractions and emptying, but voiding efficiencies are smaller than required for clinical application. We conducted experiments to determine whether selective co-stimulation of the proximal and distal urethra could increase the effectiveness of bladder activation in persons with chronic SCI.

3.4.1 Reflex responses to stimulation

Intraurethral stimulation produced reflex activity in the EUS and / or EAS EMG. Neural recruitment was consistent at both locations in the urethra, as there was no significant difference in reflex thresholds, and reflex responses were evoked by proximal and distal urethral stimulation in a majority of subjects. However, the latency of reflex responses following stimulation in the proximal and distal urethra varied. The approximately 30 ms difference between the proximal and distal reflex latencies was not likely the result of nerve conduction delay due to urethral stimulation location. For example, if the conduction velocity of the pudendal nerve afferents was 29 m/s (Cueva-Rolon et al. 1994), a difference in 13 cm between electrode locations would result in a
latency difference of 4.5 ms, which does not account for the large differences in latencies between these two populations.

Instead, the different latencies of reflex responses evoked by proximal and distal stimulation are likely the result of activation of separate reflex pathways. In a prior study, proximal stimulation produced a reflex response in the bulbocavernosus muscle at 70 ms, approximately double the latency of distal stimulation of the DGN (30-40 ms) (Dick et al. 1974). Another study reported that short latency responses (36 ms) were found following stimulation of the glans penis in normal individuals and similar responses were evoked patients with neurogenic bladder from spinal cord disease (Vodusek et al. 1983). Proximal urethral stimulation in patients with multiple sclerosis evoked reflex responses at 78.5 ms latency (Andersen and Bradley 1976). Thus, the difference in reflex latencies is not due to differences in location along the same nerve or pathological changes from SCI, but rather differences in the complement of sensory fibers activated and the reflex that was engaged.

There was no significant difference in reflex thresholds, indicating that large diameter fibers were likely activated at both locations. Both pelvic and pudendal nerves have been identified in the prostatic portion of the urethra in the cat (Danuser et al. 1997) and pelvic afferents that innervate the urethra responded to low threshold mechanical stimuli (Bahns et al. 1987). Therefore, long latency reflex responses may be mediated by
pelvic rather than pudendal afferents. Distal stimulation, which evoked reflex responses at short latencies, consisted with previous studies, was likely mediated by stimulation of pudendal afferents, as expected. In two subjects, proximal stimulation, at the most proximal electrode locations, produced both a short latency reflex response and long latency response. This suggests that in some humans the proximal urethra may include both pudendal and pelvic afferent innervation.

3.4.2 Co-stimulation of the proximal and distal urethra

Intraurethral co-stimulation produced enhanced bladder activation compared to stimulation of individual sites alone, similar to results in the cat where co-stimulation of CSN and DGN produced synergistic bladder activation and improvements in voiding efficiency compared to individual stimulation (McGee and Grill 2014b).

There was a significant effect of stimulation frequency on mean $P_{det}$ evoked by co-stimulation, consistent with other work in the cat where the size of bladder contractions evoked with stimulation was dependent on stimulation frequency and location (Boggs et al. 2006b; Yoo et al. 2008a; Bruns et al. 2009a; Woock et al. 2009b). However, interpreting these results is complicated because of the interactions of stimulation frequency and stimulation location and/or reflex activated. In each subject, various combinations of frequencies evoked robust bladder contractions, i.e., there was no optimal combination of frequencies that was effective in every subject. There were,
however, patterns that were more or less likely to evoke increases in bladder pressure across the population (Figure 3.5).

Similar to results from the cat, some frequencies produced excitation while others were typically ineffective or produced inhibition (Woock et al. 2008; Yoo et al. 2008a) and the location of stimulation affected bladder activation with stimulation (Bruns et al. 2009a; Woock et al. 2009b). Differential effects of stimulation frequency were also seen in chronic SCI cat models (Tai et al. 2006; Tai et al. 2007b); low frequencies (3-10 Hz) of stimulation produced inhibition while high frequencies (> 20 Hz) evoked bladder contractions. In our work, distal urethral stimulation at 10 Hz failed to produce robust contractions in all subjects, caused bladder inhibition in some, and was comparable to low frequency stimulation in the cat. The mean detrusor pressure evoked with 10 Hz distal stimulation was also significantly different from 2 Hz distal stimulation or 10 Hz proximal stimulation. Further relating to the results from cats, co-stimulation with high frequencies (e.g., 40P-20D) produced larger bladder contractions than co-stimulation with pairs of lower frequencies (10P-10D).

The results from intraurethral co-stimulation may be further complicated by the fact that some subjects exhibited long latency reflex responses to distal co-stimulation or short latency responses to proximal stimulation, indicating that distal stimulation may have activated pelvic urethral afferents or proximal stimulation may have activated
pudendal afferents. These differences in neural innervation could explain the large variability between patients in response to stimulation and frequency combinations.

Changes in detrusor pressure were observed in 7 subjects with SCI in response to individual stimulation or co-stimulation of the proximal and distal urethra. In the remaining subjects, no stimulation-evoked changes in detrusor pressure were observed. The absence of distension-evoked bladder contractions or stimulation-evoked reflexes in those subjects limited our ability to set the proper bladder volume (80% of volume for DECs) and stimulation amplitude (2T or 4T). This led to ineffective stimulation because the bladder volume may have been subthreshold for stimulation-evoked contractions, noted to be approximately 70% DEC volume in other studies in persons with SCI (Kennelly et al. 2010), or the stimulation amplitude may have been subthreshold for sufficient neural recruitment. Additionally, in some patients with incomplete SCI, sensation of the electrical stimulation was uncomfortable and limited the stimulation amplitude or the number of trials performed.

The increase in RI EMG activity with stimulation varied with frequency combinations; some patterns of co-stimulation produced larger increases in RI EMG activity over baseline. A correlation between the increase in RI EMG with co-stimulation and the reflex that was engaged (response latency) could not be obtained because of the heterogeneity of reflex latencies, and two of the subjects exhibited co-stimulation-evoked
contractions without stimulation-evoked reflex EMG responses at either location in the urethra.

The prevalence of co-stimulation patterns that increased EMG activity may be due to the activation of pelvic afferents by stimulation in the proximal urethra, indicated by long latency reflex responses. In the cat, high frequency pudendal afferent stimulation did not produce an increase in EMG activity (Woock et al. 2008, McGee and Grill 2014), whereas pelvic nerve stimulation produced concomitant sphincter and bladder activation in dogs (Holmquist and Olin 1968) and a previous study of intraurethral stimulation showed that proximal stimulation produced more EMG activity than distal stimulation (Yoo et al. 2011). Thus, stimulation that produced long latency responses across locations and subjects suggests that pelvic afferents may have been activated and could explain the increases in RI EMG activity seen with stimulation. However, excitatory pudendal-pudendal reflexes exist (McKenna and Nadelhaft 1989), and pudendal afferents may thus have contributed to the increase in RI EMG with proximal and distal co-stimulation.

In one subject, voiding was demonstrated with patterns of co-stimulation that were found to be effective in isovolumetric conditions. Although there were 7 patients who demonstrated stimulation-evoked changes in detrusor pressure, the limited effectiveness of stimulation in some subjects and time constraints limited our testing of
voiding efficiency in all 7. Voiding trials were performed in one other subject, who failed to void under control or co-stimulation conditions, due to increased EMG activity from dyssynergia and/or the presence of the stimulation catheter. In the subject where voiding was demonstrated, voiding efficiency was increased with co-stimulation of both of the selected frequency combinations. Individual distal or proximal stimulation was not used in this patient for voiding trials and could not be compared to voiding efficiency produced by co-stimulation.

3.4.3 Significance

This work demonstrates the importance of stimulation frequency and stimulation location, or reflex pathway activated, on the effects of electrical stimulation on the bladder and muscles of the pelvic floor. Stimulation frequency affected the size of bladder contractions evoked by stimulation, similar to the results from the cat (Yoo et al. 2008a).

Electrical stimulation of urethral afferents produced bladder activation through two separate pathways and selective co-stimulation of these pathways can produce enhanced bladder activation, as was demonstrated previously in the cat (McGee and Grill 2014b). In the human, these two pathways were differentiated by different latency responses to intraurethral stimulation and likely distinct reflex pathways.
Further, the results reveal that the reflex circuitry mediating these effects of afferent stimulation is present in the lumbosacral spinal cord and preserved after suprasacral SCI. Furthermore, for other applications of stimulation of afferent pathways in the pelvic region, reflex latency may be used as an indicator of which neural pathways was activated. Further development of afferent stimulation for neural prosthetic restoration of bladder function should include these techniques of co-stimulation to improve bladder control when individual site stimulation is ineffective.
Table 3.1: Summary of spinal cord injured participant population and experimental outcomes.

<table>
<thead>
<tr>
<th>n</th>
<th>Sex</th>
<th>Injury Level</th>
<th>Complete or Incomplete SCI</th>
<th>Years Since Injury</th>
<th>Proximal or Distal Stim-Evoked Reflex</th>
<th>$\Delta P_{det}$ with Co-stimulation</th>
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<tbody>
<tr>
<td>1</td>
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<td>N</td>
</tr>
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<td>T12</td>
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<td>Y</td>
</tr>
<tr>
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<td>N</td>
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<td>T7</td>
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</tr>
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<td>-</td>
</tr>
<tr>
<td>9</td>
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</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>11</td>
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</table>

Seventeen individuals with either complete or incomplete SCI above the sacral level participated in the study. The mean number of years since injury was 12.8 years. Stimulation-evoked reflex responses from either proximal or distal urethra stimulation were demonstrated in 10/17 subjects. Changes in bladder pressure ($P_{det}$) with intraurethral stimulation were observed in 7 subjects.
Table 3.2: Summary of reflex responses to proximal and distal intraurethral stimulation for each participant.

<table>
<thead>
<tr>
<th>n</th>
<th>Proximal Stimulation</th>
<th>Distal Stimulation</th>
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<td></td>
<td>Electrode (#)</td>
<td>Latency (ms)</td>
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<tr>
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<tr>
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<td>3-4*</td>
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<tr>
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<td>1-2*</td>
<td>-</td>
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<tr>
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<td>1-2*</td>
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<tr>
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</table>

Reflex response to low frequency stimulation at either location, proximal or distal, was found in 10/14 patients. Electrode location, reflex latency, and stimulation amplitude necessary to evoke reflex response are listed for each location. Asterisk (*) indicates experiments that were conducted with a 15 electrode catheter and reflect different anatomical locations than the same electrode on a 12 electrode catheter. R indicates that an electrode patch on the leg was used as the stimulation return electrode. If the patient terminated study procedures prior to stimulation-evoked reflex testing, n/a is indicated.
Figure 3.1: Anatomy of the lower urinary tract and intraurethral stimulation locations.

Generalized human anatomy of the male lower urinary tract is shown, including expected urethral innervation by the pudendal and pelvic nerves. The pudendal and pelvic nerves originate from the sacral level (S2-S4 in the human) and innervate the urethra and external genitalia, and bladder and bladder neck, respectively. The urethral meatus and distal portion of the urethra is innervated by a distal branch of the pudendal nerve, the dorsal genital nerve (DGN). The pudendal nerve also carries motor fibers for the control of the external urethral sphincter (EUS) and external anal sphincter (EAS). The proximal urethra may be innervated by both the pelvic and pudendal nerves. The
custom stimulating electrode catheter with 12 electrode contacts is shown; electrodes were positioned to target both the proximal and distal urethra.
Figure 3.2: Reflex EMG responses to proximal and distal intraurethral stimulation.

A) Representative examples of reflex electromyographic (EMG) activity evoked in the perineum (external urethral sphincter – EUS) by 2 Hz stimulation of the proximal or distal urethra. Light gray traces represent individual responses to stimulation, black trace is the average. B) The latency of reflex response evoked by proximal stimulation was significantly longer than distal stimulation (*p=0.0006, ANOVA, n=10). C) Reflex latency with respect to stimulating electrode location, measured as the distance from bladder neck. Electrodes positioned closer to the bladder neck evoked reflex responses with longer latencies than stimulation at electrodes farther from the bladder neck.
Figure 3.3: Bladder contractions evoked by intraurethral stimulation.
Figure 3.3: Bladder contractions evoked by intraurethral stimulation.

A) Co-stimulation of proximal and distal urethra produced increases in bladder pressure which were comparable to control, distension-evoked contractions (DECs). Traces show changes in detrusor pressure for distention (top) and stimulation with 2 Hz proximal, 10 Hz distal (middle) and 2 Hz proximal, 2Hz distal (bottom). Bar indicates when stimulation was applied. B) Changes in detrusor pressure with stimulation were dependent on stimulation frequency. Traces represent detrusor pressure in response to individual stimulation of either proximal or distal urethra or co-stimulation in another subject. The first column of plots represent responses to only proximal stimulation at 2, 10, 20 and 40 Hz, while the first row of plots represents stimulation with distal stimulation. Each of the plots of co-stimulation was produced by stimulation with frequency of stimulation shown by the row of proximal stimulation and column of distal stimulation. Bars indicate when stimulation was applied.
Figure 3.4: Mean and maximum detrusor pressures evoked by intraurethral stimulation.

A) Bars represent mean change in detrusor pressure ($P_{\text{det}}$) evoked by stimulation compared to baseline pressure. There was a significant effect of stimulation frequency presented on mean detrusor pressure ($p<0.001$, ANOVA, $n=6$). Post hoc comparisons with Bonferroni correction revealed stimulation frequency combinations which were significantly different from each other ($^* p < 0.001$).

B) Plot shows the maximum detrusor pressure evoked by stimulation over baseline pressure for each frequency combination. There was no significant main effect of stimulation frequency on maximum bladder pressure.
Figure 3.5: Percent of subjects with robust bladder contraction at each frequency combination.

Figure represents % of subjects who demonstrated at least a 10 cmH₂O increase in maximum $P_{\text{det}}$ for each stimulation combination. Some frequency combinations were less effective than others. 10 Hz distal stimulation failed to evoke a robust contraction in any subject, while 2P-2D and 40P-40D evoked robust contractions in 4/7 subjects (57%).
Figure 3.6: Normalized reflex responses to intraurethral co-stimulation to evoke bladder contractions.

The rectified and integrated (RI) EMG responses were normalized to pre-stimulation EMG activity after artifact subtraction of both. Plot shows normalized RI EMG for each stimulation frequency combination. There was a significant difference in normalized RI EMG with stimulation (p<0.0001 ANOVA, n=6), but a significant interaction effect on normalized RI EMG between stimulation frequency and stimulation-on periods (p<0.0057), indicating that some frequency combinations of co-stimulation evoked smaller increases in RI EMG with stimulation to baseline activity.
Figure 3.7: Voiding with intraurethral co-stimulation in one subject.

A) Trace shows increase in detrusor pressure evoked by stimulation with 20 Hz proximal, 20 Hz distal which produced voiding. B) Equation used to calculate voiding efficiency is shown. The volume voided was divided by the sum of the residual and voided volumes. C) Voiding efficiency (VE) for co-stimulation and control, distension-evoked contractions is shown. Stimulation pairs of 40 Hz proximal, 20 Hz distal and 20 Hz proximal, 20 Hz distal produced robust contractions under isovolumetric conditions. Co-stimulation with these stimulation frequencies led to successful voiding of volume from the bladder. Control voiding was inefficient (16%), while co-stimulation produced an increase in VE.
4. Temporal Pattern of Stimulation Modulates Reflex Bladder Activation by Pudendal Nerve Stimulation

4.1 Introduction

Neurological disease or injury can result in lower urinary tract (LUT) dysfunction, including urinary incontinence, chronic retention of urine, and detrusor-sphincter dyssynergia (Abrams et al. 2002). Bladder dysfunction leads to substantial decreases in quality of life (Costa et al. 2001; Anderson 2004; Oh et al. 2005), and medical complications including urinary tract infections, skin breakdown, bladder and kidney damage, and rehospitalization (Van Kerrebroeck et al. 1993b). Restoring LUT function remains a significant unmet need in those with neurological disease or injury. For example, in a study of paraplegic persons with spinal cord injury (SCI), restoration of bladder control was the second highest priority after recovery of sexual function, and ranked ahead of restoration of sensation or elimination of chronic pain (Anderson 2004).

The dominant clinical approach to treat bladder dysfunction is intermittent catheterization and anticholinergic medications, although this approach is often associated with frequent urinary tract infections and undesirable drug side-effects (Burns et al. 2001). Electrical stimulation is an alternative treatment for urinary incontinence and dysfunctional voiding. Stimulation of the sacral anterior roots produces effective voiding following SCI, but requires an irreversible dorsal rhizotomy for restoration of continence and to prevent detrusor sphincter dyssynergia (Brindley...
Sacral nerve stimulation using Interstim ® (Medtronic) is effective for the treatment of some etiologies of urge urinary incontinence and urinary retention (Siegel et al. 2000), but is less effective in persons with SCI (Chartier-Kastler et al. 2001).

Electrical stimulation of afferents in the pudendal nerve (PN) is a promising approach to restore LUT function through activation of reflexes that either inhibit the bladder to maintain continence or activate the bladder to cause micturition (Tai et al. 2007b). In a blinded study comparing the effectiveness of sacral nerve stimulation to pudendal nerve stimulation for voiding dysfunction, the pudendal lead was chosen as the superior lead and produced greater overall reduction in symptoms in the majority of participants (Peters et al. 2005), indicating the potential for success with this approach.

Higher frequency (20-50 Hz) stimulation of the dorsal genital nerve (DGN) branch of the PN evokes bladder contraction in both cats (Boggs et al. 2006b; Tai et al. 2008; Woock et al. 2008) and humans with SCI (Yoo et al. 2011). However, voiding efficiencies produced by DGN stimulation are limited (Yoo et al. 2008a) and may be insufficient for clinical translation. Innovative approaches are needed to increase voiding efficiency by pudendal afferent stimulation. For example, selective co-stimulation of multiple pudendal afferent branches improved reflex bladder activation and voiding (McGee and Grill 2014b). Modifying the temporal pattern of stimulation is another approach that may improve the efficacy of stimulation. For example, bursting patterns of pudendal afferent stimulation improved reflex bladder activation in cats (Bruns et al. 1988).
2008; Bruns et al. 2009a; Bruns et al. 2009b), producing more robust bladder contractions than corresponding regular frequencies.

The objective of this work was to determine whether, in addition to the strong dependence on stimulation frequency (Boggs et al. 2006), the bladder response to pudendal afferent stimulation was also dependent on the temporal pattern of stimulation, and to determine whether specific patterns of stimulation produced larger bladder contractions than constant frequency stimulation. The results show that although none of the tested patterns evoked larger bladder contractions, several temporal patterns were equally as effective as regular stimulation. These results contribute to understanding the mechanisms of lumbosacral spinal processing of pudendal afferent activity for bladder control, and may be helpful for revealing novel approaches to treat LUT dysfunction.

4.2 Methods

All animal care and experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee. Twelve sexually intact, adult male cats (3.0-4.25 kg) were anesthetized with ketamine-HCl (35 mg·kg\(^{-1}\) i.m.) and anesthesia was maintained with α-chloralose (65 mg·kg\(^{-1}\) i.v., supplemented at 15 mg·kg\(^{-1}\) i.v.). Animals were intubated and end-tidal CO\(_2\) was maintained between 3-4% with artificial respiration. Blood pressure was monitored with a catheter placed in the carotid artery and a solid-state pressure transducer. Body temperature was maintained at 38°C.
using a heating pad and intravenous fluids, 0.9% NaCl with 5% dextrose and 8.4 mg·L⁻¹ NaHCO₃, were administered (15 ml·kg⁻¹ h⁻¹).

A midline abdominal incision was made to expose the bladder and a suprapubic catheter (3.5 Fr) was inserted into the bladder dome and secured with a purse string suture. A solid-state pressure transducer (Deltran, Utah Medical) in series with the catheter was used to measure bladder pressure. The ureters were tied, cut and drained externally and the urethra was occluded with a 3.5-5 Fr catheter for the duration of the isovolumetric experiments. The threshold volume for distension-evoked contractions (DECs) was determined by filling the bladder with room temperature saline at 1-2 ml·min⁻¹. Electromyographic (EMG) activity from the external anal sphincter (EAS), and in some animals the external urethral sphincter (EUS), was recorded differentially between two stainless steel wires inserted into the EAS (or EUS) with respect to a subcutaneous needle reference electrode, amplified (gain=10,000), and filtered (10 Hz - 10 kHz).

In a prone position, the PN was accessed through an incision between the base of the tail and the ischial tuberosity, transection of the gluteofemoral muscle, and dissection of the ischiorectal fossa. A monopolar electrode, composed of a platinum contact (1.5x3 mm) embedded within a silicone cuff was placed around the dorsal genital nerve (dorsal nerve of the penis - DNP) branch of the PN (Martin et al. 1974; Yoo et al. 2008a). Electrical stimulation was delivered between the cuff and a subcutaneous
20-gauge needle in the leg using a current pulse generator (Pulsar 6bp, FHC Inc.). The patterns were generated using a customized computer program (LabChart Stimulator, AD Instruments) and output to the pulse generator to trigger current pulse delivery. Stimulation trains varied in frequency and pattern of pulse delivery, but were fixed in length (30 sec) and pulse width (0.1 ms). The optimal frequency of DNP stimulation identified in previous studies (Yoo et al. 2008a), 33 Hz, was selected for regular frequency stimulation (Pattern #1). Six other patterns had mean frequencies of 33 Hz but different temporal patterns; two additional patterns contained 33 Hz components but had different overall mean rates (Figure 4.1). Stimulation amplitude was fixed for all patterns at 3 times the threshold to produce a bulbocavernosus reflex EMG response in the EAS or EUS with 1 Hz DNP stimulation.

In addition to regular 33 Hz stimulation (Pattern #1), frequency ramp, random, alternating frequency, pauses, and doublet patterns of stimulation were evaluated (Figure 4.1). Frequency ramps were trains in which the length of the inter-pulse-interval (IPI) gradually changed and could be described as decreasing (high to low frequency, Pattern #2) or increasing (low to high frequency, Pattern #3) ramps. Random trains (Pattern #4) had IPIs selected from a uniform random distribution (IPIs were limited between 2 and 100 ms), with mean rate of 33 Hz. Stimulation trains with on and off periods were also presented: Pattern #5 was composed of 100 ms of 66 Hz stimulation followed by 100 ms pauses (33 Hz mean frequency), while Pattern #8 was composed of
1000 ms of 33 Hz stimulation followed by 200 ms pauses for 30 s (27.5 Hz mean frequency). Alternating frequency patterns, where the IPI alternated between two values, were also delivered: 10 and 50 ms IPIs (Pattern #6) and 20 and 40 ms IPIs (Pattern #7). A doublet or bursting pattern, 33 Hz inter-burst-interval with 10 ms IPI (66 Hz mean frequency), was also delivered (Pattern #9).

Further, we included three additional patterns that were generated to resemble the burst-like patterns suggested by Bruns et al. (2008) to be most effective in evoking robust bladder contractions with PN stimulation: B1 (2 pulses with 5 ms IPIs repeated at 25 Hz), B2 (2 pulses with 5 ms IPIs repeated at 0.5 Hz), and B3 (5 pulses with 5 ms IPIs repeated at 1 Hz).

Patterns of stimulation were presented in a randomized order under isovolumetric bladder conditions, at approximately 80% of the threshold volume to evoke DECs, with approximately one minute between each trial. Bladder contractions were quantified by the mean pressure and maximum pressure during stimulation with respect to baseline bladder pressure before stimulation. EAS EMG recordings were stimulus-triggered averaged, rectified and integrated over the 20 ms following the stimulus artifact. The mean and maximum bladder pressures were averaged across repeated trials within experiments and analyzed by ANOVA with post-hoc paired comparisons made with Bonferroni corrected p-values to maintain \( \alpha = 0.05 \).

4.2.1 Model of Spinal Processing of Temporal Patterns
We used a computational model of the lumbosacral neural network mediating the pudendo-vesical reflex (Figure 4.7A) to investigate the basis for the responses to different temporal patterns of pudendal afferent stimulation. The model takes as input activity in pelvic and pudendal afferents that project to dorsal and medial interneurons in the lateral dorsal horn or dorsal gray commissure, and generates as outputs estimates of sacral parasympathetic nucleus (SPN) neuron firing and bladder pressure (which, via a closed loop mechanism, drives pelvic afferent activity). Supraspinal control by the periaqueductal gray (PAG) and pontine micturition center (PMC) was included in the model to mediate distension-evoked contractions.

The model was composed of a network of linear integrate and fire (LIAF) neurons that were synaptically coupled. When an action potential reached a synapse it produced an excitatory or inhibitory post-synaptic potential in the post-synaptic cell. An action potential or spike was triggered in the post-synaptic cell when the transmembrane voltage crossed a specified threshold. The interactions of excitatory and inhibitory interneurons in the model reproduce the stimulation frequency dependent bladder responses to pudendal afferent stimulation observed in animal experiments (12, 14). The same temporal patterns of stimulation tested experimentally were applied to the model to determine the effects of stimulation pattern on interneuron and SPN neuron firing. For further detail and a quantitative description of the model, see Chapter 6.
4.3 Results

The objective was to determine whether bladder contractions evoked by pudendal afferent stimulation were dependent on the temporal pattern of stimulation. Robust bladder contractions were evoked in all cats with regular 33 Hz stimulation. The average mean bladder contraction size evoked by regular 33 Hz stimulation was 15.8±1.3 cmH$_2$O; 86% of all individual trials with regular 33 Hz stimulation evoked a robust bladder contraction (greater than 10 cmH$_2$O increase over baseline). Different temporal patterns of stimulation generated bladder contractions of different magnitudes and pressure-time profiles, or shapes. Examples of typical bladder contractions evoked by each pattern of stimulation from one cat are shown in Figure 4.2.

4.3.1 Bladder Contraction Magnitude across Temporal Patterns

There was a significant main effect of varying the temporal pattern of pudendal afferent stimulation on the mean bladder pressure evoked by stimulation (p < 0.0001, ANOVA, n=12) (Figure 4.3A). Several temporal patterns evoked bladder contractions with magnitudes equal to those evoked by constant frequency 33 Hz stimulation, there was no pattern that evoked significantly larger mean bladder pressures than regular 33 Hz stimulation (Pattern #1), and Pattern #4, #5, and #9 evoked bladder contractions with significantly lower mean bladder pressures than constant frequency 33 Hz stimulation (p<0.05, post hoc with Bonferroni correction).

The maximum bladder pressure evoked by stimulation was similarly affected by
the temporal pattern of electrical stimulation (p < 0.0001, ANOVA, n=12) (Figure 4.3B). Pattern #1 and #7 evoked bladder contractions with the largest maximum bladder pressures, and no stimulation pattern evoked significantly larger maximum bladder pressure recordings than Pattern #1. Pattern #5 and #9 evoked bladder contractions with significantly smaller maximum bladder pressures (p<0.05, post hoc with Bonferroni correction).

Pattern #5 and #9 evoked both smaller mean and maximum bladder contractions than regular 33 Hz stimulation. Pattern #5 (with 100 ms pauses) had a mean rate of 33 Hz and still resulted in reduced bladder contraction size. Patterns with alternating IPI patterns (#6, #7) or increasing and decreasing ramp trains (#2, #3) produced robust bladder contractions that were not significantly different from those evoked by regular 33 Hz stimulation.

Stimulation with a random temporal pattern (Pattern #4) produced bladder contractions with significantly lower mean bladder pressures than regular 33 Hz stimulation (p<0.05, post hoc with Bonferroni correction). The responses to 33 Hz regular stimulation were consistent across animals and trials, but the bladder response to random trains, where each train was created from the same distribution of IPIs but was not fixed, varied greatly across and within animals (Figure 4.4A). The distribution of mean bladder pressures for Pattern #4 was much larger than Pattern #1. The coefficient of variation (CV; the standard deviation of the population divided by the population
mean) of responses to Pattern #1 was 0.29 while the responses to Pattern #4 were more variable and had a CV of 0.73 (Figure 4.4B).

Three additional temporal patterns of stimulation, inspired by the bursting patterns from Bruns et al. (2008) that produced the largest evoked bladder pressures (Figure 4.5), were evaluated in 5 animals. Pattern B1 consisted of a burst of 2 pulses with 5 ms IPI repeated at 25 Hz (mean rate of 50 Hz), Pattern B2 was a burst of 2 pulses with 5 ms IPI repeated at 0.5 Hz (mean rate 1 Hz), and Pattern B3 was a burst of 5 pulses with 5 ms IPIs repeated at 1 Hz (mean rate 5 Hz). Patterns B2 and B3 had lower mean frequencies because the previous study reported that some animals may respond preferentially to either low or high frequency stimulation. The evoked pressures were highly variable across the bursting patterns (Figure 4.5B, C). There was a significant effect of stimulation pattern on the size of the evoked bladder contractions (n=5, ANOVA, p<0.001), and Pattern #1 evoked significantly larger bladder contractions than B1, B2, and B3 (p<0.05, post hoc with Bonferroni correction). There was no significant difference between the contractions evoked by Pattern #9 (bursts of 2 pulses with 10 ms IPI repeated at 33 Hz) and patterns B1, B2 or B3.

4.3.2 EMG Responses across Temporal Pattern

EMG activity from the EAS was recorded to evaluate sphincter activation in response to pudendal afferent stimulation, as reflex EAS and EUS activation is strongly dependent on stimulation frequency (Woock et al. 2008), and different temporal patterns
of stimulation may differentially impact sphincter response. Stimulation with regular 33 Hz stimulation (Pattern #1) evoked transient EAS responses that rapidly diminished after the first 1-2 pulses of the stimulation train. This transient response was seen in single responses to the first couple of pulses in the train, but the average EMG response during the 30 s revealed no overall EAS EMG activity (Figure 4.6). Similarly, the decreasing and increasing ramp train patterns (#2, #3), alternating IPI patterns (#6,#7), pause patterns (#5,#8), and doublet pattern (#9) all evoked transient sphincter responses following the first few pulses of stimulation. The 33 Hz random stimulation train (Pattern #4) however, in addition to evoking EAS responses which diminished at the beginning of the train, also occasionally evoked reflex responses throughout the stimulation train. These additional reflex responses often followed paired pulses of stimulation with short, approximately 2 ms, IPIs.

### 4.3.3 Model of Spinal Processing of Temporal Patterns

Figure 4.8 shows the activity of individual model neurons for 1 s during application of regular 33 Hz stimulation (Pattern #1) and Pattern #4 and #5. The bladder contractions evoked by stimulation with Pattern #1 and #5 in the model (Figure 4.7B) mimic those in the experiments, shown in Figure 4.2A. The magnitude of bladder contractions produced by different temporal patterns of stimulation applied to the model (Figure 4.7C) paralleled those seen in animal experiments (Figure 4.3A). Common temporal features, including pauses and high frequency activity, reduced the firing rate
of the SPN neuron in the model and produced lower evoked bladder pressures, as seen in the animal experiments. Pauses in stimulation, as in Pattern #5, silenced the activity of the interneurons, interrupting the excitatory activation of the SPN. Periods of high frequency stimulation (Pattern #4, #5 or #9) failed to generate an increase in interneuron firing rate to match the input, and dominance by the inhibitory interneuron (INM- further decreased the SPN firing rate.

4.4 Discussion

Electrical stimulation of PN afferents can generate both inhibition and excitation of the bladder (Boggs et al. 2006b; Tai et al. 2007b; Tai et al. 2008; Woock et al. 2008) and is a promising approach to restore control of bladder function in persons with neurological disorders or injury (Yoo et al. 2007; Yoo et al. 2011; McGee et al. 2015). However, residual bladder volumes following PN stimulation-evoked voiding may be too large for successful widespread clinical translation. The temporal pattern of stimulation is an important stimulation parameter dimension that could improve voiding with PN stimulation. We measured the size of isovolumetric bladder contractions produced by a wide range of temporal patterns of stimulation. The bladder response to pudendal afferent stimulation was strongly dependent on the pattern of stimulation, similar to the dependence on stimulation frequency (Boggs et al. 2006b; Yoo et al. 2008a), but none of the patterns evoked larger bladder contractions than regular 33 Hz stimulation.
4.4.1 Temporal Pattern and Reflex Bladder Activation

Stimulation of dorsal genital afferents of the PN with 33 Hz regular stimulation evoked robust bladder contractions in all cats, and equally robust contractions were evoked with select temporal patterns (#7, #6, #3, and #2). These patterns featured only small changes in IPI compared to regular 33 Hz stimulation that did not produce substantial differences in interneuron or SPN neuron firing rates in the model, as compared to 33 Hz regular stimulation. This is similar to previous results from studies of pudendal afferent stimulation in cats following acute SCI where a range of frequencies were found to be evoke bladder contractions (Boggs et al. 2005; Boggs et al. 2006b; Woock et al. 2008).

Interestingly, two temporal patterns with mean frequencies of 33 Hz (Pattern #4 and #5) produced significantly lower mean bladder pressures than regular 33 Hz stimulation. However, the maximum bladder pressure evoked by Pattern #4 was not significantly different from Pattern #1. This indicates that although peaks in bladder pressure produced by random trains occasionally matched those produced by regular 33 Hz stimulation, the random train did not consistently produce enough bladder activation to increase the mean bladder pressure to the level of regular stimulation. In the computational model, Pattern #4 also produced a reduction in SPN neuron firing and lower bladder pressures because interneuron firing was interrupted by the random pattern of stimulation. Therefore, the specific temporal pattern of pulse delivery, not
simply the mean frequency, is an important determinant of the magnitude of the evoked bladder contraction.

The bladder response to random trains was variable both within and across animals. The coefficient of variation of the mean bladder contraction responses to random trains with Pattern #4 was much higher than the CV of responses to regular 33 Hz stimulation. This may be due to the fact that the random temporal patterns of pulses were not fixed and each train was composed of a different arrangement of IPIs drawn from the same distribution. The differences seen within a single animal, e.g., Figure 4.4A, support the conclusion that the specific arrangement of stimulation pulses in time is critical to the resulting bladder pressure response. However, the changes in bladder pressure in the cat occurred too slowly to correlate directly with the changes in IPIs in the random trains. The high sensitivity of the reflex circuit to specific combinations of IPIs within the random trains suggests that a combination of patterns or IPIs could exist to increase the strength of evoked bladder contractions.

The results from stimulation with Patterns #4 and #5 suggest that bursts, pauses, or random IPIs preclude generation of robust bladder contractions. Pattern #5, with 100 ms pauses and a mean frequency of 33 Hz, produced contractions with lower mean and maximum bladder pressures than regular 33 Hz. The results from stimulation with Pattern #5 in the model indicate that to produce robust bladder contractions requires more than simply a certain number of pulses delivered over a period of time. The 100 ms
pauses interrupted the interneuron firing activity and decreased SPN firing, and the increased frequency during the “on phases” of Pattern #5 did not to evoke an increase in SPN firing, making Pattern #5 an ineffective stimulus train, despite the mean rate of 33 Hz.

Bladder contractions evoked by Pattern #8, composed of repeating 1000 ms periods of 33 Hz stimulation periods and 200 ms silent periods, were smaller than regular 33 Hz stimulation, but not significantly different following post hoc paired comparisons with Bonferroni correction. This result indicates that although accommodation or pauses are detected in recordings of PN afferent activity (Snellings et al. 2012), when stimulating at 33 Hz, the introduction of pauses produces a reduction in the effectiveness of stimulation. Pauses in stimulation with Patterns #5 and #8 silenced the firing of the model interneurons, producing a sharp decrease in SPN neuron firing and bladder pressure.

Pattern #9, 100 Hz doublets at 33 Hz, was the least effective pattern of all tested, rarely producing a robust bladder contraction. Pattern #9 had a higher mean frequency (66 Hz) than Pattern #1, and the doublet of two pulses at 100 Hz may have effectively pushed stimulation beyond the range of effective frequencies for bladder activation (Yoo et al. 2008a). This was consistent with the model results, as Pattern #9 failed to evoke an increase in SPN firing, or bladder pressure, because the interneuron firing rates could not follow the high rate of stimulation. This result was surprising however, since burst
stimulus patterns evoked greater bladder pressures than continuous stimulation by either direct PN stimulation or intraurethral stimulation of pudendal afferents (Bruns et al. 2008; Bruns et al. 2009a).

Following our findings of the lack of effectiveness of bursting patterns (#9) we broadened the test set to include patterns previously reported to be effective (Bruns et al. 2008). The mean and maximum bladder contraction amplitudes evoked by all three patterns (B1, B2, B3) were significantly lower than those evoked by regular 33 Hz stimulation. The addition of a burst of pulses to regular frequency patterns may increase bladder activation in some cases when compared to the original stimulation frequency; however, it does not appear that this can improve reflex bladder activation beyond regular 33 Hz stimulation. These results suggest that bursting patterns may not be better suited for application in bladder control than regular patterned stimulation, although burst stimulation of the compound PN or intraurethral stimulation may produce different results than burst stimulation of the DNP. Further, the previous studies suggested the existence of low and high frequency responders across animals, where large bladder contractions were evoked in some animals with lower frequency burst stimulation. We cannot exclude the possibility of such a phenomenon, particularly with the heterogeneity seen in our results in response to random patterned stimulation trains (Pattern #4). However, regular high frequency (33 Hz) stimulation was consistently effective across all 12 animals.
4.4.2 EMG Activity

The EAS EMG was used as a proxy for PN efferent activity produced by stimulation, as previous studies showed strong congruence between EAS EMG and EMG from periurethral striated muscles in response to pudendal afferent stimulation (Woock et al. 2008). Both regular and patterned stimulation of PN afferents produced robust reflex activation of the bladder without sustained activation of PN efferents. None of the 9 patterns produced a change that could be detected in the averaged EMG signal. The random 33 Hz train (Pattern #4), occasionally evoked more reflex activity in the EAS than constant 33 Hz stimulation, depending upon the specific IPIs, and highlights the importance of temporal pattern in determining the reflex response to stimulation. This characteristic of PN afferent stimulation, which is preserved across different temporal patterns, is a potential advantage over previous methods to produce bladder emptying, such as sacral anterior root stimulation, which produce concomitant activation of the bladder and external urethral sphincter (Brindley 1977).

4.4.3 Mechanisms of Spinal Processing of Temporal Patterns of Stimulation

The results from the computational model reproduced the effect on bladder activation of different temporal patterns of pudendal afferent stimulation. The differential effects of patterns arose from the temporal filtering of afferent signals by interactions between excitatory and inhibitory interneurons in the model neural
network. Reflex selection by temporal features of input signals in lumbosacral spinal circuits has been modeled for locomotion (Jilge et al. 2004) and suggested by Boggs et al. (Boggs et al. 2006b) for processing of pudendal afferent stimulation. The results from our relatively simple neural network model demonstrate how such reflex selection can be accomplished by a network of neurons in the spinal cord, and indicate that this behavior does not require processing in higher order circuits, consistent with the preservation of stimulation frequency dependent effects following acute (Boggs et al. 2005; Boggs et al. 2006b; Woock et al. 2009b) and chronic (Tai et al. 2006; Tai et al. 2007b) spinal transection. Additionally, the network does not control bladder activation by acting as a simple integrator of pulses over a time period. Instead, interactions between interneurons control the SPN neuron firing rate (bladder pressure) in response to the temporal pattern of afferent activity.

4.5 Conclusion

The temporal pattern of pudendal afferent stimulation significantly influenced the magnitude of isovolumetric bladder contractions evoked by stimulation. The effects of temporal patterns of stimulation were mediated by interactions between local interneurons that controlled the firing rate of the output to the bladder, the SPN neuron. This work demonstrates the importance of studying temporal patterns of pudendal afferent stimulation for bladder control and indicates that temporal patterns may be used to modulate the neural network response to stimulation. Temporal patterns of
pudendal nerve stimulation for bladder control have not been adequately explored and should be considered in the development and optimization of novel neural prosthetic approaches for efficient bladder control.
Figure 4.1: Temporal patterns of pudendal afferent stimulation.

Mean frequencies, calculated over the 30 s stimulation train, are shown. Pattern #2 and #3 are decreasing and increasing ramp trains, respectively, where the inter-pulse-interval (IPI) changes gradually at each pulse. Pattern #4 is a random train with mean 33 Hz. Pattern #5 consists of repeating 100 ms periods of either 66 Hz stimulation or no stimulation, for an overall mean rate of 33 Hz. Pattern #6 and #7 contained alternating 10 and 50 ms IPIs and 20 and 40 ms IPIs, respectively, for an overall mean rate of 33 Hz. Pattern #8 was 1000 ms of 33 Hz stimulation followed by a 200 ms pause, for an effective stimulation rate of 27.5 Hz. Pattern #9 was inspired by a previous study (17), and contained two pulses of 100 Hz stimulation repeated at 33 Hz, for a mean rate of 66 Hz.
Figure 4.2: Bladder contractions evoked by temporal patterns of pudendal afferent stimulation.

Representative bladder contractions evoked by the 9 temporal patterns of stimulation from one cat are shown. Stimulation was presented for 30 s (amplitude = 3T) and is represented by the thick bar beneath the pressure trace.
Figure 4.3: Effect of temporal patterns of pudendal afferent stimulation on the magnitude of evoked bladder contractions.

A) Mean evoked bladder contraction pressure varied across stimulation pattern (p <0.0001, ANOVA, n=12). Patterns with different letters above each bar are significantly different from each other (p<0.05, post hoc paired tests with Bonferroni correction). B) Maximum pressure evoked by stimulation was dependent on the pattern of stimulation (p <0.0001, ANOVA, n=12). Post hoc paired tests with Bonferroni correction indicated that some patterns evoked contractions with smaller peak pressures than regular 33 Hz stimulation (Pattern #1).
Figure 4.4: Bladder contractions evoked by a random pattern of pudendal afferent stimulation.

Bladder contractions evoked by random patterns of 33 Hz stimulation (Pattern #4) varied within and across animals, while 33 Hz regular stimulation consistently evoked robust bladder contractions. **A** ) Contractions evoked by stimulation with either Pattern #1 or #4 in one animal. Bladder contractions evoked with regular 33 Hz stimulation (Pattern #1) were more consistent in amplitude than random trains with Pattern #4. **B** ) Mean bladder contraction pressure evoked by Pattern #4 was significantly less than Pattern #1 (n=12, p<0.05 post hoc with Bonferroni correction). Dots indicate average response within each animal; responses for each animal across the two patterns are connected with lines. The coefficient of variation (CV) of the responses across animals to stimulation was greater for Pattern #4 than Pattern #1.
Figure 4.5: Bladder contractions evoked by stimulation of pudendal afferent with temporal patterns containing bursts.

A) Patterns #1, #9, B1, B2, and B3. B1 was a burst of 2 pulses at 200 Hz every 25 Hz, B2 was 2 pulses at 200 Hz every 0.5 Hz, and B3 was 5 pulses at 200 Hz every 1 Hz.

B) Representative bladder contractions evoked by stimulation with each pattern from one animal. Stimulation was applied as noted below each trace. B1, B2, and B3 failed to evoke larger bladder contractions than Pattern #1.

C) Mean bladder pressure evoked by each pattern. There was a significant main effect of temporal pattern on mean bladder contraction pressure (n=5, ANOVA, p<0.0001) and Pattern #1 evoked larger bladder contractions than Pattern #9, B1, B2, and B3 (* p<0.05, post hoc with Bonferroni correction).
Figure 4.6: EMG response to temporal patterns of pudendal afferent stimulation.

Representative EMG recordings of reflex responses from the external anal sphincter (EAS) during each temporal patterns of pudendal afferent stimulation in a single cat. Single trials (light lines) and averaged (dark lines) EMG responses were time aligned to the stimulation pulse. Sharp spikes in EMG recordings are stimulus artifacts, indicating when stimulation was presented (stimulation times indicated by * in first example).
Figure 4.7: Effects of temporal patterns of pudendal nerve stimulation in a neural
network model of the pudendo-vesical reflex.

A) Topology of the neural network model of the pudendo-vesical reflex. The
network included neurons representing pelvic (Pel) and pudendal (Pud) afferents,
interneurons (IN_D, IN_M+, IN_M-, FB), and preganglionic motor neurons in the sacral
parasympathetic nucleus (SPN). Supraspinal control by the periaqueductal gray (PAG)
and pontine micturition center (PMC) was included in the model to evoke distension-
evoked contractions. See Chapter 6 for more details. B) Simulated bladder contractions
produced as output of the neural network model. Bladder pressure is shown in response
to stimulation with Patterns #1 and #5. Stimulation is marked by the bar under
contraction. C) Mean bladder pressures produced in the computational model across the
temporal patterns of stimulation replicated the results from animal experiments.
Figure 4.8: Model neuron firing in response to temporal patterns of pudendal afferent stimulation.

Firing activity of the model neurons is shown for 1 s of stimulation with Patterns #1, #4, and #5. Each row represents the firing activity of each model neuron over time. Stimulation with pauses and high frequency bursts, present in Pattern #4 or #5, produced SPN firing rates that were lower than regular stimulation (Pattern #1). During pauses (★), the interneurons’ firing rates decreased, causing the SPN to stop firing. During bursts of high frequency stimulation (★), the interneurons failed to increase their firing rate to match the input, and dominance by the inhibitory interneuron (IN$_M$-) prevented the SPN from firing more than once.
5. A Spinal GABAergic Mechanism is Necessary for Bladder Inhibition by Pudendal Afferent Stimulation

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5.1 Introduction

Improved bladder control is an unmet need in persons with urinary incontinence (Abrams et al. 2002) and in persons with neurological disease or injury, such as spinal cord injury (Costa et al. 2001). Electrical stimulation is a promising approach to treat overactive bladder (OAB), which can produce urgency and incontinence and impair quality of life (Patrick et al. 1999; Chiaffarino et al. 2003). In particular, pudendal afferent stimulation inhibits distension-evoked bladder contractions and improves continence (Vodusek et al. 1986). We sought to identify the mechanism(s) of bladder inhibition by pudendal afferent stimulation.

Pudendal nerve (PN) stimulation may be an alternative to sacral nerve stimulation to improve continence (Peters 2010), and in patients who do not respond to sacral neuromodulation it may be a successful treatment option (Sherman and Amundsen 2007; Mashni and Peters 2010; Peters et al. 2010). Low frequency (~ 10 Hz) pudendal afferent stimulation inhibits bladder contractions, thereby promoting continence in preclinical animal studies (Boggs et al. 2006b; Tai et al. 2006; Wenzel et al. 2006).
Inhibition of bladder contractions and increased bladder capacities elicited by low frequency pudendal afferent stimulation can also be achieved after chronic spinal cord injury (Tai et al. 2007b; Tai et al. 2011). However, the mechanisms of action of PN stimulation mediated bladder inhibition are unclear, and such knowledge may improve patient selection and enable improvements in the therapy.

Initial studies suggested that pudendal afferent stimulation produced bladder inhibition via activation of sympathetic efferents in the hypogastric nerve causing α-adrenergic receptor mediated inhibition at the vesical ganglia and / or β-adrenergic receptor mediated direct inhibition of the detrusor muscle (De Groat and Saum 1972; de Groat and Theobald 1976; Lindstrom et al. 1983; Craggs and McFarlane 1999), while recent studies suggest that other central mechanisms may play a role in pudendal afferent evoked inhibition (Mally et al. 2013). Stimulation of afferents in the dorsal genital branch of the PN continued to produce robust bladder inhibition following bilateral transection of the hypogastric nerve and pharmacological blockade of α- and β-adrenergic receptors (Woock et al. 2011), challenging the notion that bladder inhibition with PN stimulation requires activation of sympathetic outflow and suggesting the existence of an alternate mechanism. The purpose of the present experiments was to identify other potential mechanisms of PN afferent stimulation-evoked inhibition of isovolumetric reflex bladder contractions in anesthetized cats. We found that spinal GABAergic mechanisms play a critical role in mediating bladder inhibition evoked by
electrical stimulation of pudendal afferents, while adrenergic, glycinergic, and opioidergic mechanisms did not appear to be necessary for inhibition.

5.2 Methods

Acute, non-survival experiments were conducted in 33 intact, adult male cats (2.8-4.5 kg). All animal care and experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee. Initial anesthesia was with ketamine-HCl (35 mg·kg\(^{-1}\) i.m.) and anesthesia was maintained with α-chloralose (65 mg·kg\(^{-1}\) i.v., supplemented at 15 mg·kg\(^{-1}\) i.v.). End-tidal CO\(_2\) was maintained between 3-4% with artificial respiration. Blood pressure was monitored via a catheter placed in the carotid artery and a solid-state pressure transducer. Body temperature was maintained at 38°C using a heating pad, and 0.9% NaCl with 5% dextrose and 8.4 mg·L\(^{-1}\) NaHCO\(_3\) was administered intravenously (15 ml·kg\(^{-1}\)·h\(^{-1}\)).

The bladder was accessed through a midline abdominal incision, and a 3.5 Fr suprapubic catheter was inserted into the bladder dome and secured with a purse string suture. Bladder pressure was measured with a solid-state pressure transducer in series with the catheter and recorded. A 3.5-5 Fr catheter was used to occlude the urethra during bladder filling and isovolumetric conditions. The threshold volume for distension-evoked reflex contractions (DECs) was found by filling the bladder at 1-2 ml/min with either room temperature saline or 0.25% acetic acid (AA), used to irritate the bladder and mimic overactive bladder symptoms (Tai et al. 2012).
Electromyographic (EMG) activity was detected from the external anal sphincter (EAS) with paired wire electrodes, amplified (1,000), filtered (10 Hz - 10 kHz), and recorded. The pudendal nerve (PN) was exposed through an incision between the base of the tail and the ischial tuberosity and transection of the gluteofemoralis. After dissection of the ischiorectal fossa, the sensory (SN) and rectal perineal (RP) branches of the PN were visible. Further dissection of the distal portion of the SN branch revealed the dorsal nerve of the penis (DNP) (Yoo et al. 2008b; Yoo et al. 2008a). For animals receiving intrathecal administration of picrotoxin, a partial laminectomy was performed from L6-S3. A 20G needle was used to puncture the dura and insert a PE 10 catheter for intrathecal infusion. The location of the intrathecal catheter and spread of injected volume was confirmed in a separate experiment using dye and post-mortem dissection.

The PN and DNP were stimulated unilaterally with cuff electrodes around each branch (Figure 5.1A). Electrodes were composed of single platinum contacts embedded within silicone cuffs, and pulse generators (Pulsar 6bp, FHC Inc.) were used to deliver stimulation (0.1 ms cathodic stimulus pulses, for 20-30 s) between the cuffs and subcutaneous 20G needles. Both the PN and DNP were stimulated at 10 Hz, a frequency previously shown to produce strong bladder inhibition in cats (Woock et al. 2008). The stimulation amplitude was 1, 2 or 3 times the threshold (T) that produced a bulbocavernosus reflex (BCR) EMG response in the EAS with 1 Hz stimulation.

Trials to evaluate bladder response to 10 Hz PN or DNP stimulation were
conducted under isovolumetric conditions after saline infusion to 100% of the threshold volume for DECs under control conditions and following drug administration. In some animals, individual stimulation of both the PN and DNP was performed to compare the drug effects on bladder inhibition at each stimulation location. For all drugs, a control trial was conducted before drug administration to confirm bladder inhibition with 10 Hz PN or DNP stimulation (Figure 5.1B). In some animals where drug administration affected bladder inhibition, an additional trial was performed after a drug washout period of at least two hours. To prevent interaction effects following the administration of multiple drugs, each animal received only one pharmacological treatment.

Picrotoxin, an antagonist for GABA (γ-aminobutyric acid) receptor chloride channels, was administered intravenously (n=11, 1.5 mg·kg⁻¹ i.v.) with saline (n=8) or AA (n=3) bladder infusion or intrathecally (n=3, 5 mM, 0.2 ml, based on doses and volumes found to be effective in similar experiments (Reddy and Yaksh 1980; Garcia-Rill et al. 1985)) with saline bladder infusion. In three additional animals, picrotoxin (0.5 mg·kg⁻¹ i.v.) was administered to evaluate the effectiveness of picrotoxin at a lower dose. Phentolamine (2 mg·kg⁻¹ i.v.) and propranolol (1 mg·kg⁻¹ i.v.) (Edvardsen 1968; Koley et al. 1984; Danuser et al. 1995; Woock et al. 2011) were co-administered to block α- and β-adrenergic receptors (n=7). Unilateral and bilateral hypogastric nerve transection was performed to eliminate sympathetic innervation of the bladder (n=2). To block glycinergic receptors, increasing cumulative doses of strychnine (n=4, 0.01-0.1 mg·kg⁻¹
i.v.) were administered, including two animals who received cumulative doses up to 0.25 mg·kg\(^{-1}\) i.v (Shefchyk et al. 1998). Increasing cumulative doses of naloxone (0.1-4.0 mg·kg\(^{-1}\) i.v.), a competitive opioid antagonist, were administered to identify any opioidergic contribution to bladder inhibition (n=3), as this was ambiguous from prior studies (Chen et al. 2010; Mally et al. 2013).

Gallamine triethiodide (10 mg·kg\(^{-1}\) i.v. initial dose with 5 mg·kg\(^{-1}\) i.v. supplemented every 45 min), a paralytic and muscle relaxant, was administered throughout experiments with picrotoxin and strychnine to prevent convulsions caused by the pharmacological antagonists. Additional control trials were conducted after administration of gallamine to detect any change in stimulation-evoked inhibition of bladder contractions caused by administration of the paralytic.

Bladder inhibition produced by stimulation was quantified as normalized bladder pressure (NBP): the ratio of the mean bladder pressure during the entire time of stimulation to the mean bladder pressure during control DECs. Statistical significance was determined either by ANOVA or repeated measures ANOVA with \textit{post hoc} paired comparisons with Bonferroni correction (p<0.05). Trials of stimulation of PN or DNP were pooled for analysis, as noted in the results. Data are shown as mean ± standard error, unless otherwise stated.

### 5.3 Results

Ten Hz stimulation of the pudendal nerve (PN, n=21 cats) or the dorsal nerve of
the penis (DNP, n=12) inhibited distension-evoked bladder contractions in all animals in which each target nerve was stimulated. The DECs evoked by bladder filling were consistent over time, as reported in a previous study (Boggs et al. 2005), and did not change following repeated stimulation of PN or DNP (Figure 5.1B). The reduction in bladder pressure during PN and DNP stimulation was dependent on the amplitude of stimulation (p=0.011, ANOVA, Figure 5.2), but there was no significant difference in the normalized bladder pressure during stimulation of PN or DNP (p=0.337, ANOVA). In the following results, we combined cases of PN and DNP stimulation for analysis because there was no significant interaction effect in the two-way ANOVA for bladder inhibition between stimulation site and drug delivered (p=0.914), indicating that there was no difference in drug effect on bladder inhibition by PN or DNP stimulation. Although the magnitude of bladder inhibition varied across animals, stimulation producing any decrease in normalized bladder pressure occurred in 100 %, 87.5 % and 41.2 % of trials at 3T, 2T and 1T stimulation of the PN and in 100 %, 83.3 % and 50 % of trials at 3T, 2T and 1T stimulation of the DNP. Bladder inhibition evoked by PN or DNP stimulation was disrupted by the administration of picrotoxin, either intravenously or intrathecally, but was not blocked by phentolamine, propranolol, HGN transection, strychnine or naloxone.

5.3.1 Antagonism of GABA\textsubscript{A} reversibly blocked bladder inhibition

Intravenous picrotoxin (1.5 mg·kg\textsuperscript{-1}) blocked bladder inhibition by PN and DNP
stimulation at 1T, 2T and 3T amplitudes. The normalized bladder pressure during pudendal afferent stimulation was significantly higher following administration of picrotoxin \( (p<0.001, \text{ANOVA, } n=11: 9 \text{ PN, } 2 \text{ DNP}; \text{Figures 5.2 and 5.3}) \). Administration of 1.5 mg·kg\(^{-1}\) picrotoxin blocked bladder inhibition and produced significantly higher normalized bladder pressures than controls and paralyzed control trials \( (p<0.001, \text{post hoc with Bonferroni correction}) \). Although the average normalized bladder pressure produced by stimulation was dependent on stimulation amplitude, picrotoxin blocked the decrease in normalized bladder pressure for all amplitudes (Figure 5.2). The loss of inhibition following intravenous picrotoxin was reversible and stimulation-evoked inhibition returned following a washout period of approximately two hours \( (n=4) \), indicating that the loss of bladder inhibition was not due to a loss of stimulation effectiveness (Figures 5.3 and 5.4). In three animals, a lower dose of picrotoxin \( (0.5 \text{ mg·kg}\(^{-1}\) i.v.) \) was administered and did not block inhibition of bladder contractions by pudendal afferent stimulation (Figure 5.4A). There was no significant difference between normalized bladder pressures during control and lower dose picrotoxin trials \( (p=0.908, \text{post hoc with Bonferroni correction}) \). Normalized bladder pressure during stimulation-evoked inhibition following administration of the paralytic but before administration of picrotoxin was not significantly different from that of control trials \( (p=0.569, \text{post hoc with Bonferroni correction}) \), demonstrating that the loss of inhibition was not a result of co-administration of the paralytic (Figures 5.3 and 5.4). In one cat, 33
Hz stimulation, previously demonstrated to evoke reflex contraction of the bladder (Woock et al. 2008), continued to evoke robust bladder contractions after the administration of picrotoxin, even though 10 Hz stimulation-evoked inhibition was blocked.

Pudendal afferent stimulation-evoked bladder inhibition was also blocked following intrathecal application of picrotoxin (Figure 5.5), resulting in significantly larger bladder pressures during stimulation (p=0.005, ANOVA, n=3: 2 PN, 1 DNP; p<0.05 post hoc paired comparisons with Bonferroni correction). A separate experiment using dye and post-mortem dissection demonstrated successful placement of the intrathecal catheter at the lumbosacral spine. Inhibition and the consequent stimulation-evoked reduction in bladder pressure returned after two hours of washout following intrathecal picrotoxin administration (n=2).

Intravenous picrotoxin also blocked stimulation-evoked inhibition of bladder contractions generated by acetic acid (AA). Infusion of acetic acid into the bladder produced large distension-evoked contractions at lower volumes than saline infusion (69 ± 4.5 % of saline threshold volume), but there was no change in the effectiveness of stimulation-evoked inhibition from saline to AA trials. Normalized bladder pressure during pudendal afferent stimulation with AA was higher following intravenous picrotoxin than during pudendal afferent stimulation prior to picrotoxin (p<0.001, ANOVA, n=3: 2 PN, 1 DNP; p<0.001 post hoc paired comparisons with
Bonferroni correction; Figure 5.6). Similar to intravenous picrotoxin administration with saline bladder infusion, bladder inhibition by pudendal afferent stimulation with AA bladder infusion returned after more than two hours of washout.

5.3.2 Administration of other pharmacological antagonists

We tested a number of other interventions to investigate other mechanisms that might contribute to pudendal afferent stimulation evoked bladder inhibition. Table 1 summarizes the experiments performed where we did not observe effects of the interventions on pudendal afferent stimulation-evoked inhibition of DECs.

Ten Hz stimulation of either PN or DNP at 3T continued to evoke consistent bladder inhibition, and there was no significant difference between the reduction in normalized bladder pressure during stimulation after any of the following interventions (Table 1): 1) naloxone administered in cumulative doses (0.1-4.0 mg·kg\(^{-1}\) i.v., \(p=0.568\), ANOVA, \(n=3\): 2 DNP, 1 PN), 2) strychnine, administered in cumulative doses (0.025-0.25 mg·kg\(^{-1}\) i.v., \(p=0.541\), ANOVA, \(n=4\): 3 DNP, 1 PN), and 3) neither unilateral nor bilateral HGN transection suppressed or eliminated bladder inhibition caused by PN stimulation ranging from 2T to 10T (\(p=0.909\), ANOVA, \(n=2\)). Although 3T PN and DNP stimulation continued to evoke consistent bladder inhibition, there was a main effect of co-administration of α- and β-adrenergic antagonists (phentolamine, 2 mg·kg\(^{-1}\) i.v.) and (propranolol, 1 mg·kg\(^{-1}\) i.v.) with repeated measures ANOVA (\(p=0.039\), \(n=7\): 4 PN, 3 DNP). Post hoc paired comparisons with Bonferroni correction revealed that normalized
bladder pressure was significantly higher following phentolamine (p=0.015), but not propranolol (p=0.065). However, normalized bladder pressure during stimulation remained low following phentolamine, indicating that robust bladder inhibition was still intact (Table 1).

Although a smaller number of animals was used in some of the non-GABAergic antagonist experiments, the risks of a type II error for strychnine (β=0.04), naloxone (β=0.17), and HGN transection (β=0.02), where a statistically significant difference in normalized bladder pressure may not have been detected, were still quite low.

5.4 Discussion

This study of the mechanisms of bladder inhibition by pudendal afferent stimulation was prompted by studies suggesting that stimulation-evoked bladder inhibition is not mediated by the activation of sympathetic outflow (Woock et al. 2011; Mally et al. 2013), as previously asserted, and by previous reports of differing contributions of opioidergic mechanisms to stimulation-evoked bladder inhibition (Chen et al. 2010; Mally et al. 2013). The present results reveal that a spinal GABAergic mechanism mediates the inhibitory pudendo-vesical pathway. Picrotoxin, a GABA\textsubscript{A} receptor antagonist, blocked pudendal afferent stimulation-evoked bladder inhibition, and was effective when administered in a sufficient dose either systemically or locally to the lumbosacral spinal cord. Consistent with our results, other studies have reported that GABA and muscimol (GABA\textsubscript{A} receptor agonist) when administered intravenously
or intrathecally inhibit the micturition reflex in rats (Maggi et al. 1987). Further, a recent report also found that lower doses of picrotoxin did not disrupt pudendal afferent stimulation mediated inhibition of bladder contractions during cystometry with saline (Xiao et al. 2014a). These results identify a clear and compelling mechanism for pudendal afferent stimulation evoked inhibition of the bladder, and may inspire the development of therapies for restoration of bladder control.

5.4.1 GABAergic mechanisms are necessary for bladder inhibition by pudendal afferent stimulation

Picrotoxin, administered intravenously (1.5 mg·kg⁻¹) to block GABAₐ receptors, reversibly blocked inhibition of reflex bladder contractions by pudendal afferent stimulation at all stimulation amplitudes. PN and DNP stimulation-evoked bladder inhibition was blocked by picrotoxin similarly across multiple amplitudes of stimulation, suggesting that they both employ the same neurotransmitter mechanisms. The loss of pudendal afferent stimulation-evoked inhibition was not due to a loss of the effect of stimulation at the electrode because inhibition returned after a washout period of at least 2 hours. Because the pharmacokinetics of picrotoxin are not well documented, we chose a washout period of 2 hours that corresponded to the time when the effects on blood pressure diminished. Low dose picrotoxin (0.5 mg·kg⁻¹) failed to block inhibition by pudendal afferent stimulation, indicating that the effect on inhibition was dose dependent and required sufficient blockade of GABAₐ receptor activity.
Picrotoxin was administered intrathecally in some animals to identify the location of picrotoxin-mediated blockade of bladder inhibition. Intrathecal picrotoxin produced results similar to those following administration of intravenous picrotoxin, and blocked bladder inhibition by pudendal afferent stimulation. Inhibition and the consequent stimulation-evoked reduction in bladder pressure returned after two hours of washout. Therefore, although the pharmacodynamics of intrathecal administration and the concentration of drug delivered were different from intravenous administration, picrotoxin’s effects were still reversible.

The key finding that spinal GABAergic mechanisms mediate bladder inhibition by pudendal afferent stimulation does not rule out contributions of other mechanisms, although our data suggest that adrenergic, glycinergic and opioidergic mechanisms may not be necessary for robust inhibition. Parallel pathways at the spinal and supraspinal levels may both be involved in bladder control and activated by pudendal afferent stimulation, as bladder excitation with pudendal afferent stimulation involves both spinobulbospinal and spinal reflexes (Woock et al. 2009b).

Neural circuits in the periaqueductal gray (PAG) and pontine micturition center (PMC) are known to employ GABA (de Groat and Wickens 2013), but are unlikely to be primarily responsible for pudendal stimulation-evoked reflex bladder inhibition, as bladder inhibition by pudendal afferent stimulation remains intact following chronic spinal cord injury (Tai et al. 2007b; Tai et al. 2008). Any central GABAergic pathways
that are involved would have also been antagonized after administration of intravenous picrotoxin. Intrathecal blockade of GABA was important because central GABAergic mechanisms are known to exist in the PAG (Stone et al. 2011), and these results demonstrated that the GABAergic neurons participating in pudendal afferent stimulation-evoked inhibition are located in the lumbosacral spinal cord.

The GABA\textsubscript{A} pathway identified here may control sacral parasympathetic output via inhibition of preganglionic cells in the sacral parasympathetic nucleus (SPN) by sacral interneurons or recurrent inhibition from the SPN (Shefchyk 2001). GABA\textsubscript{A} receptors are located throughout the dorsal horn and in the dorsal gray commissure (DGC) (Alvarez et al. 1996). GABA\textsubscript{A} receptors also occur on neurons in the SPN (Araki 1994), whose dendrites extend to the DGC (de Groat et al. 1981; Morgan et al. 1981), a site of pudendal afferent innervation (Thor et al. 1989). There may be multiple inhibitory pathways to the SPN (DeGroat 1971), including both presynaptic and postsynaptic GABAergic inhibition from other areas of the sacral spinal cord (Ramírez-León et al. 1994; Alvarez et al. 1996). Inhibitory feedback neurons from the SPN are known to produce recurrent inhibition of the bladder circuit (de Groat 1976) and may be GABAergic. Presynaptic inhibition of pelvic afferents by primary afferent depolarization may also be GABAergic (Buss and Shefchyk 1999; Rudomin and Schmidt 1999).

5.4.2 GABAergic mechanisms participate in inhibition of both nociceptive and non-nociceptive bladder reflexes
Intravenous picrotoxin also reversibly blocked pudendal afferent stimulation-evoked inhibition of reflex bladder contractions induced by AA installation. Although previous reports suggested that separate pathways may govern inhibition of nociceptive and non-nociceptive bladder contractions (Tai et al. 2012; Xiao et al. 2014a), GABAergic mechanisms appear critical for inhibition of both types of contractions. A recent study of low dose picrotoxin found that picrotoxin blocked inhibition of nociceptive, but not non-nociceptive, bladder contractions (Xiao et al. 2014a). This finding, when combined with our results, suggests that the nociceptive bladder state produced by infusion of AA may shift the response to picrotoxin to lower doses since blockade of pudendal afferent inhibition of nociceptive bladder contractions occurred at a lower dose than non-nociceptive bladder contractions.

Because AA infusion is frequently used as a model of overactive bladder in animal models (Mally et al. 2013), this finding has important implications for research for the treatment of overactive bladder. Following spinal cord injury, an alternate reflex pathway for bladder control emerges, driven by nociceptive C-fiber afferent activity from the bladder (Fowler 2002). Therefore, pudendal afferent inhibition of nociceptive bladder contractions that result from either overactive bladder or spinal cord injury is likely to be mediated by GABA_A.

5.4.3 Other mechanisms may not be necessary for bladder inhibition by pudendal afferent stimulation
We investigated a number of potential contributors to bladder inhibition, and found little to no effect on bladder inhibition by pudendal afferent stimulation of the blockade of adrenergic, sympathetic, glycinergic, or opioidergic mechanisms. Although it is not clear that 3T stimulation can be considered supramaximal (Figure 5.2), subtle effects of non-GABAergic antagonists may have been obscured by the use of 3T as opposed to lower amplitudes of stimulation.

Bladder inhibition by low frequency PN or DNP stimulation was not blocked by co-administration of α- and β-adrenergic antagonists, indicating that adrenergic mechanisms, which were assumed to be a primary mechanism of bladder inhibition evoked by afferent stimulation (de Groat and Theobald 1976), may not be necessary for this type of inhibition. Administration of phentolamine resulted in a statistically significant increase in normalized bladder pressure during pudendal afferent stimulation; however, the small effect size indicates robust bladder inhibition persisted under this treatment. In contrast to the large effect of picrotoxin (change in NBP = 0.473), the change in normalized bladder pressure following phentolamine was quite small (change in NBP = 0.071), indicating that α-adrenergic signaling may play a modulatory role.

Inhibition was preserved following unilateral and bilateral HGN transection, replicating prior results (Woock et al. 2011; Zhang et al. 2013), and indicating that activity in the hypogastric nerve is not required for pudendal afferent mediated bladder
inhibition of DECs. Previous studies have suggested that sympathetic mechanisms mediate bladder inhibition evaluated stimulation of pelvic afferents (de Groat and Theobald 1976) or intravaginal stimulation (Lindstrom et al. 1983), which might employ different mechanisms than stimulation of somatic afferents in the PN. Further, mechanisms of inhibition may be different at low bladder pressures (< 5 cmH₂O), where hypogastric transmission is thought to be required, and high bladder pressures (> 15 cmH₂O), where inhibition is preserved following hypogastric transection (Fall et al. 1977).

Administration of strychnine to block glycine receptors also failed to block bladder inhibition consistent with a previous study where strychnine failed to block bladder inhibition produced by rectal distension or perianal stimulation (DeGroat 1971). In two animals, stimulation continued to produce strong inhibition of reflex bladder contractions after the dose of strychnine was increased to 0.25 mg·kg⁻¹ i.v., confirming that the absence of effect was not caused by too low of a dose of strychnine. This dose approaches the lethal dose (0.33 mg·kg⁻¹ i.v.) (National Institute for Occupational and Health 1975) and was shown to adequately block glycine receptors in other reflex pathways (Shefchyk et al. 1998).

Bladder inhibition by pudendal afferent stimulation remained intact following intravenous administration of naloxone, a competitive antagonist of µ-opioid receptors, that also blocks, albeit with lower affinity, κ- and δ-opioid receptors (Helm et al. 2008).
Prior studies reported variable effects of blocking opioid receptors on pudendal afferent stimulation-evoked bladder inhibition. Naloxone blocked bladder inhibition by low amplitude pudendal afferent stimulation in cats (Chen et al. 2010) and mechanical skin stimulation in rats (Hotta et al. 2012). Inhibition of bladder contractions evoked by the infusion of acetic acid, but not saline, with tibial nerve stimulation was blocked by naloxone (Tai et al. 2012). Later experiments demonstrated that naloxone had no effect on pudendal stimulation-evoked inhibition of bladder overactivity in cats (Mally et al. 2013). We found that inhibition by either PN or DNP stimulation remained intact following administration of naloxone across a range of doses and stimulation amplitudes. The maximum dose (4.0 mg·kg\(^{-1}\) i.v.) was much higher than in previous studies and pudendal afferent stimulation continued to produce robust inhibition of DECs. Although opioids did not provide a major contribution to reflex bladder inhibition by evoked by stimulation of pudendal afferents, it is possible that they modulate neurotransmitter release or modify parasympathetic outflow (Roppolo et al. 1983).

### 5.4.4 Effectiveness of stimulation–evoked inhibition

Our results demonstrate that 3T PN or DNP stimulation at 10 Hz robustly produced bladder inhibition in control trials. Stimulation at 1T and 2T produced significant bladder inhibition, but was less robust, consistent with results of other studies (Woock et al. 2008). The nomenclature used here for threshold and stimulation
amplitude (1T = BCR reflex EMG threshold) should be distinguished from other studies where 1T is determined as the threshold to evoke inhibition and would, in general, represent a higher amplitude.

There were no significant differences in bladder inhibition or the effect of pharmacological antagonists on stimulation of the compound PN or DNP. Different mechanisms of inhibition could be employed by stimulation of different nerve pathways (van der Pal et al. 2006), however bladder inhibition was consistent across DNP and PN stimulation. Additionally, because stimulation amplitude was normalized to the BCR threshold for each nerve, differences in neural activation between the nerves likely did not affect the results. Stimulation of the sacral roots, which activates both pelvic and pudendal afferents, could engage neural network mechanisms other than those activated with PN or DNP stimulation (van der Pal et al. 2006).

The administration of the paralytic, gallamine, served two purposes in these experiments: first, preventing convulsions due to systemic intravenous administration of the pharmacological antagonists, and second, eliminating any contribution of re-afference to bladder activity. Re-afference, the activation of sensory (afferent) mechanisms by muscle contraction evoked by electrical stimulation of efferent axons, can produce reflex bladder contractions (Thon et al. 1991; Yoo et al. 2008a), and it was important to eliminate the secondary effects of stimulation of PN efferents to study clearly the effect low frequency pudendal afferent stimulation-evoked bladder
In these experiments, we found that picrotoxin increased excitability of the bladder, and distention-evoked bladder contractions occurred at lower bladder volumes than control trials. This is consistent with previous studies where administration of GABA\(A\) antagonists excited the bladder (Maggi et al. 1987; Igawa et al. 1993) although a recent study reported that picrotoxin increased bladder capacity in cats (Xiao et al. 2014a).

5.4.5 Perspectives and Significance

Pudendal afferent stimulation-evoked inhibition was previously thought to be mediated through bladder relaxation mechanisms used in normal control of the lower urinary tract via the hypogastric nerve and adrenergic receptors. The results presented here indicate that bladder inhibition evoked by pudendal afferent stimulation relies on inhibition of the excitatory control of the bladder, rather than activation of a bladder relaxation pathway. Bladder inhibition by stimulation of pudendal afferents may be via synaptic inhibition of the SPN via GABA\(A\) from local interneurons or presynaptic inhibition of parasympathetic afferents. Although additional inhibitory mechanisms exist at the pelvic ganglia and bladder muscle (De Groat and Saum 1972; Lindstrom et al. 1983), these are not necessary for pudendal afferent evoked bladder inhibition as systemic adrenergic blockade failed to block inhibition by pudendal afferent stimulation.

These experiments demonstrated that GABA\(A\) in the lumbosacral spinal cord is
required for bladder inhibition by pudendal afferent stimulation, and that glycinergic, adrenergic, and opioidergic mechanisms may not be necessary. These results have important implications for neuromodulation via pudendal nerve stimulation and may impact the understanding of the mechanisms of sacral neuromodulation, which may activate afferents from both the pelvic and pudendal nerves. Bladder inhibition by sacral neuromodulation, particularly in cases after spinal cord injury, likely utilizes a sacral spinal network that is mediated by GABA. These results also indicate that pharmacological interventions, such as GABA agonists for OAB, should be investigated to improve the efficacy of existing neuromodulation techniques.
Figure 5.1: *In vivo* experiments to determine the mechanisms of bladder inhibition by pudendal afferent stimulation.

A) A suprapubic catheter placed in bladder dome was used to record bladder pressure. A catheter in the urethra was used to occlude the urethra and fill the bladder with saline or 0.25% acetic acid. Wire electrodes in the EAS (external anal sphincter) measured electromyographic (EMG) activity. After surgical dissection custom silicone cuff electrodes with platinum contacts were placed around the PN (pudendal nerve) and DNP (dorsal nerve of the penis). Intrathecal (IT) injections were made through a subdural catheter placed via a sacral laminectomy. B) Control distension-evoked contractions (DECs) following saline infusion (top trace) and robust bladder inhibition by 1T or 3T PN stimulation at 10 Hz (second and third traces). Bottom trace shows consistent reproducibility of DECs within one animal. Bar indicates when stimulation was on.
Figure 5.2: Effect of stimulation amplitude on bladder inhibition generated by PN and DNP stimulation.

Average normalized bladder pressure under control conditions and after administration of picrotoxin for 1T, 2T, and 3T stimulation of PN (n=8) and DNP (n=5). Picrotoxin significantly blocked the reduction in normalized bladder pressure for all amplitudes of both PN and DNP stimulation (p<0.001, ANOVA). There was a significant effect of stimulation amplitude on normalized bladder pressure (p=0.011, ANOVA) normalized bladder pressure was significantly higher with 1T stimulation than 3T stimulation (p=0.004, post hoc paired comparisons with Bonferroni correction).
Figure 5.3: Intravenous picrotoxin (1.5 mg·kg⁻¹) reversibly blocked inhibition of reflex bladder contractions by pudendal afferent stimulation.
Figure 5.3: Intravenous picrotoxin (1.5 mg·kg⁻¹) reversibly blocked inhibition of reflex bladder contractions by pudendal afferent stimulation.

Representative bladder pressure traces showing the effect of 3T stimulation of the PN (pudendal nerve) or DNP (dorsal nerve of the penis) on bladder contractions before and after administration of high dose picrotoxin. Control and paralytic trials were performed before administration of picrotoxin. Washout trials occurred at least 2 hours after picrotoxin administration. Bar indicates when stimulation was on.
Figure 5.4: Intravenous picrotoxin blocked inhibition of reflex bladder contractions by pudendal afferent stimulation.
Figure 5.4: Intravenous picrotoxin blocked inhibition of reflex bladder contractions by pudendal afferent stimulation.

A) Bladder pressure during PN stimulation, normalized to pressure during control distension-evoked contractions (DECs) across conditions. Each animal received either 0.5 mg·kg⁻¹ picrotoxin (n=3) or 1.5 mg·kg⁻¹ picrotoxin (n=8). There was a significant effect of drug administered on inhibition of DECs with pudendal stimulation (p<0.001, ANOVA, n=11: 9 PN, 2 DNP). High dose picrotoxin blocked inhibition of DECs resulting in significantly larger normalized bladder pressures during stimulation (* p<0.001, post hoc Bonferroni correction). There was no significant difference in bladder inhibition between control, paralytic and low dose picrotoxin trials. In a subset of animals receiving 1.5 mg·kg⁻¹ picrotoxin, inhibition and consequent reduction in normalized bladder pressure returned after a washout period of 2+ hours post picrotoxin administration and was not significantly different from control trials. B) Normalized bladder pressure during stimulation for individual animals for high dose picrotoxin (1.5 mg·kg⁻¹ picrotoxin). There was a significant effect of drug administration on normalized bladder pressure (p=0.002, ANOVA, n=8). Post hoc paired comparisons with Bonferroni correction showed that while normalized bladder pressure was significantly higher with picrotoxin (* p<0.001), there was no significant difference between control, paralytic, and washout trials.
Figure 5.5: Intrathecal picrotoxin reversibly blocked inhibition of reflex bladder contractions by pudendal afferent stimulation.

A) Representative bladder pressure traces from control, paralytic, and intrathecal (i.t.) picrotoxin trials of 3T PN (pudendal nerve) stimulation. Bar indicates when stimulation was on. The low bladder pressure seen in the picrotoxin trial example is a result of periodic DECs. After the onset of the next DEC inhibition of the contractions was blocked by i.t. picrotoxin. B) Bladder pressure during PN or DNP (dorsal nerve of the penis) stimulation, normalized to pressure during control distension-evoked contractions (DECs), following i.t. picrotoxin and wash-out (p=0.005, ANOVA, n=3: 2 PN, 1 DNP; * p <0.05 post hoc paired comparisons with Bonferroni correction).
Figure 5.6: Intravenous picrotoxin reversibly blocked inhibition of acetic acid induced, bladder contractions by pudendal afferent stimulation.

A) Representative bladder pressure traces from one animal with 3T PN (pudendal nerve) stimulation for control fill with acetic acid (AA), following intravenous picrotoxin, and following picrotoxin washout. Bar indicates when stimulation was on. B) Bladder pressure during 3T stimulation, normalized to pressure during control distension-evoked contractions (DECs), was significantly different following administration of picrotoxin (p<0.001, ANOVA, n=3: 2 PN, 1 DNP; * p<0.001 post hoc paired comparisons with Bonferroni correction).
Table 5.1: Summary of experiments performed to identify the mechanisms of pudendal afferent inhibition of distension-evoked contractions under saline bladder infusion.

<table>
<thead>
<tr>
<th>Mechanism Investigated</th>
<th>Treatment or Pharmacological Antagonist</th>
<th>Dose and Route</th>
<th>n</th>
<th>Average NBP Before Treatment</th>
<th>Average NBP After Treatment</th>
<th>Average Within-Animal Δ NBP</th>
<th>post hoc Tests</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic</td>
<td>Phentolamine (α-adrenergic antagonist)</td>
<td>2 mg kg⁻¹ i.v.</td>
<td>n=7</td>
<td>0.383 ± 0.03</td>
<td>0.454 ± 0.03 *</td>
<td>0.071 ± 0.01</td>
<td>NSP compared to control was significantly higher for phentolamine, but not propranolol.</td>
<td>Bladder inhibition remained for both</td>
</tr>
<tr>
<td></td>
<td>Propranolol (β-adrenergic antagonist)</td>
<td>1 mg kg⁻¹ i.v.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sympathetic</td>
<td>Hypogastric Nerve Transection</td>
<td>Unilateral, Bilateral</td>
<td>n=2</td>
<td>0.332 ± 0.09</td>
<td>0.351 ± 0.04</td>
<td>0.019 ± 0.13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Glycinergic</td>
<td>Strychnine (glycine antagonist)</td>
<td>0.1 mg kg⁻¹ i.v.</td>
<td>n=4</td>
<td>0.525 ± 0.11</td>
<td>0.544 ± 0.13</td>
<td>0.019 ± 0.06</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Opioidergic</td>
<td>Naloxone (opioid antagonist)</td>
<td>0.1 - 4.0 mg kg⁻¹ i.v.</td>
<td>n=3</td>
<td>0.452 ± 0.09</td>
<td>0.388 ± 0.09</td>
<td>-0.064 ± 0.16</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>GABAergic</td>
<td>PicROTOXIN (GABA₅ antagonist)</td>
<td>0.5 mg kg⁻¹ i.v.</td>
<td>n=3</td>
<td>0.599 ± 0.11</td>
<td>0.646 ± 0.06</td>
<td>0.046 ± 0.08</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>PicROTOXIN (GABA₅ antagonist)</td>
<td>1.5 mg kg⁻¹ i.v.</td>
<td>n=8</td>
<td>0.625 ± 0.06</td>
<td>1.098 ± 0.02 **</td>
<td>0.473 ± 0.06</td>
<td>Stimulation-evoked inhibition was significantly blocked by PicROTOXIN. Control, paralytic and washout trials were not significantly different.</td>
<td>Inhibition returned following washout period (n=4)</td>
</tr>
<tr>
<td></td>
<td>PicROTOXIN (GABA₅ antagonist)</td>
<td>5 mM bol i.t.</td>
<td>n=3</td>
<td>0.595 ± 0.07</td>
<td>1.115 ± 0.04 **</td>
<td>0.519 ± 0.11</td>
<td>Stimulation-evoked inhibition was significantly blocked by PicROTOXIN. Control, paralytic and washout trials were not significantly different.</td>
<td>Inhibition returned following washout period (n=2)</td>
</tr>
</tbody>
</table>
Table 5.1: Summary of experiments performed to identify the mechanisms of pudendal afferent inhibition of distension-evoked contractions under saline bladder infusion.

Experiments evaluating bladder inhibition following the administration of phentolamine and propranolol, hypogastric nerve transection, strychnine, naloxone and picrotoxin were performed independently in each animal. The dose and route of treatment or drug administration and number of experiments performed (n) are each listed. The average normalized bladder pressure (NBP) is listed before and after treatment and represents the normalized fraction of bladder inhibition with stimulation across animals at the highest dose point (mean ± standard error). Average NBP values >1 indicate that pudendal afferent stimulation did not inhibit bladder contractions. The average within-animal change in NBP is also shown to illustrate that trends in NBP before and after treatment were consistent across animals. There was a significant main effect of co-administration of adrenergic antagonists on normalized bladder pressure (p=0.039, ANOVA), although robust bladder inhibition was preserved. Post hoc paired comparisons with Bonferroni correction showed that normalized bladder pressure was significantly higher than control following phentolamine (* p=0.015), but not propranolol (p=0.065). There was a significant effect of treatment on stimulation-evoked inhibition with high dose intravenous or intrathecal picrotoxin administration (** p< 0.001, ANOVA). Post hoc paired comparisons with Bonferroni correction (p<0.05) were performed only when there was a general drug effect on stimulation effectiveness.
Washout trials were performed in a subset of animals that showed loss of bladder inhibition with picrotoxin. In 2 of 4 animals receiving strychnine, the maximum dose was 0.1 mg·kg⁻¹, in the remaining 2 animals, additional doses were given to 0.25 mg·kg⁻¹.
6. Modeling the Spinal Pudendo-Vesical Reflex for Bladder Control by Pudendal Afferent Stimulation

6.1 Introduction

Bladder dysfunction resulting from neurological disease or injury, such as spinal cord injury (SCI), produces symptoms of urinary incontinence, chronic retention of urine, and detrusor sphincter dyssynergia (Abrams et al. 2002), which greatly reduce quality of life (Anderson 2004; Ku 2006). Electrical stimulation of pudendal afferents is a promising method to restore continence and micturition through reflex inhibition or excitation of the bladder by the activation of spinal circuits (Boggs et al. 2006b; Woock et al. 2008). Although the input-output properties of the pudendo-vesical reflex have been characterized empirically, there is limited understanding of the underlying neural network mechanisms that mediate the reflexes governing the effects of PN stimulation on bladder function. The objective of this work was to develop and validate a biophysically-motivated model of the neural network underlying the pudendo-vesical reflex.

Several key features of the bladder response to pudendal afferent stimulation indicate that the pudendo-vesical reflex is a spinal network-mediated phenomenon, rather than a product of higher order processing in the brainstem. Electrical stimulation of pudendal afferents generates robust bladder contractions in animals (Tai et al. 2006; Yoo and Grill 2007; Yoo et al. 2008a) and humans (Yoo et al. 2007; Yoo et al. 2011), but
the excitatory pudendo-vesical reflex is strongly dependent on bladder volume (Woock et al. 2011), suggesting a convergence of pelvic and pudendal afferents in the spinal cord network (Woock et al. 2011). The effects of PN afferent stimulation on the bladder are strongly dependent on the frequency of stimulation, with high frequencies evoking reflex bladder contractions and low frequencies producing bladder inhibition (Boggs et al. 2006b; Woock et al. 2008; Yoo et al. 2008a). These frequency-dependent effects are mediated in spinal neural networks, as they are preserved after spinal cord transection (Woock et al. 2008). Finally, eliminating pudendal sensory feedback by nerve transection in animal experiments (Peng et al. 2008b) and intraurethral anesthesia in humans (Shafik et al. 2003) reduces voiding efficiency, indicating that these feedback signals critically interact with the network that produces normal voiding behavior. Collectively, these studies indicate that interactions between neurons in a spinal network are responsible for the pudendo-vesical reflex.

A limited number of quantitative models have been developed that explore the spinal neural network control of bladder function. Of the models that describe mechanisms of control of the lower urinary tract, most do not model neural activity with sufficient detail or include contributions by pudendal afferents. Hosein and Griffiths (1990) developed a quantitative computer simulation of a proposed lower urinary tract control system, simulating changes in bladder volume, pressure, and flow rate from inhibitory and excitatory control signals that represented neural activity in the brainstem
and peripheral nerves. Bastiaanssen et al. (1996) developed a biomechanical neuromuscular model of the bladder that incorporated neuroanatomy, physiology, and muscular mechanisms, and responded to input signals from a simulated neural network. A computer model of the neural control and mechanical properties of the bladder and urethra by van Duin et al. (van Duin et al. 1999) demonstrated the importance of contributions by urethral afferents for simulation of normal lower urinary tract behavior. A more recent model of the periaqueductal gray (PAG) and pontine micturition center (PMC) in the brainstem reproduced the micturition cycle, but did not include pudendal afferents or the associated spinal reflex pathways (de Groat and Wickens 2013).

The goal of this work was to develop a computational model of the lumbosacral spinal neural network responsible for the pudendo-vesical reflex. The model structure was based upon established neuroanatomical connections and inspired by a prior model of frequency-dependent selection of spinal locomotor reflexes (Jilge et al. 2004), as the lower urinary tract reflexes evoked by pudendal afferent stimulation similarly depend on the frequency of afferent activation (Boggs et al. 2006b). Our model of the neural network that mediates the spinal pudendo-vesical reflex replicated the effects of pudendal afferent stimulation frequency and pattern on bladder pressure measured experimentally and enabled dissection of the underlying mechanisms.

6.2 Methods
We developed, implemented, and validated a computational model of the spinal neural network mediating the pudendo-vesical reflex.

6.2.1 Model Topology

The network model incorporated sensory inputs from pelvic and pudendal nerve afferent fibers, excitatory and inhibitory interneurons, and preganglionic neurons in the sacral parasympathetic nucleus (SPN) that innervated the bladder via the pelvic nerve (Figure 1). A simplified descending excitatory pathway from the PAG and PMC was included to generate distension-evoked contractions (DECs). Table 1 includes a description of each neuron and the references that informed the structure of the model.

Pudendal afferents have cell bodies in the dorsal root ganglia and enter the dorsal horn at the sacral level (L6-S1 in rats, S1-S3 in cats, and S2-S4 in humans), diverging into both medial and lateral projections (Roppolo et al. 1985; Thor et al. 1989). The lateral projections synapse on interneurons along the lateral edge of the dorsal horn, while the medial projections synapse on interneurons in the dorsal gray commissure (DGC) or send rostral projections via the dorsal columns. In this model, we excluded ascending projections by pudendal afferents and focused on the local spinal reflex-mediated mechanisms of bladder control with electrical stimulation.

Pelvic afferents, also with cell bodies in the dorsal root ganglia, enter the dorsal horn at the sacral level (L6-S1 in rats, S1-S3 in cats, and S2-S4 in humans). Pelvic
afferents make predominately lateral projections (Nadelhaft et al. 1983; Roppolo et al. 1985) to the dorsal interneurons, in addition to rostral projections to the brainstem via the dorsal columns. Pelvic mechanoreceptors in the bladder are sensitive to changes in bladder pressure, increases in tension of the bladder wall, or bladder distension. The pelvic afferent neuron in this network was modeled to match the response of low threshold mechanoreceptors sensitive to bladder distension (Sengupta and Gebhart 1994). Bladder activation is via the output of the pelvic preganglionic efferent neurons with cell bodies in the SPN (de Groat et al. 1982).

Both excitatory and inhibitory medial interneurons modulate pelvic efferents through synaptic connections onto neurons in the SPN (de Groat et al. 1998). Additionally SPN neurons have dendrites that extend to the DGC (Nadelhaft et al. 1983; Nadelhaft and Booth 1984), where medial interneurons are likely to originate. Stimulation of the DGC evokes coordinated increases in bladder pressure and decreases in urethral sphincter pressure, further suggesting it is a critical part of the network (Blok et al. 1998; Grill et al. 1999; Pikov et al. 2007). The dorsal interneuron receives inputs from pelvic and pudendal afferents and acts as an excitatory input to the SPN (Araki and de Groat 1997). The inhibitory feedback interneuron (FB) is excited by the SPN neuron, and provides negative feedback to modulate the transmission of incoming afferent activity (de Groat and Ryall 1968; de Groat 1976; Shefchyk 2001).

A supraspinal node, which simulated sensory integration performed by the PAG
and PMC, provided descending activation of the SPN via excitation the dorsal interneuron. The PAG/PMC “switched on” and fired at a rate of 15 Hz when pelvic afferent firing was high (> 10 Hz) and the bladder volume exceeded the volume threshold for DECs. We included this descending excitatory pathway because it is necessary for the generation of DECs and interacts with the lumbosacral spinal circuits (Fowler et al. 2008; Sasaki and Sato 2013).

### 6.2.2 Linear Integrate and Fire (LIF) Neurons

The model was implemented in Matlab (Mathworks, Natick, MA, USA) and was composed of 8 linear integrate and fire (LIF) neurons. The baseline firing rate of the pelvic afferents was ~ 1 Hz when bladder volume was low, as described in electrophysiological experiments (Häbler et al. 1993). The other neurons were silent until activated and their firing rates were dependent on the integration of their respective synaptic inputs. Table 2 shows the equations used to model the network of LIF neurons and the parameters that were used in the model.

Although the selection and optimization of the model neuron parameters was based on studies of the electrophysiological properties of neurons and behavior of the network, there was no explicit biophysical representation in this model. When an action potential occurred in the presynaptic neuron, a change in membrane conductance was generated in the postsynaptic neuron, causing a change in transmembrane voltage. The transient change in conductance was specified for each neuron to match the properties
of the specified neurotransmitter receptor type likely to be found in each cell group. Excitatory postsynaptic potentials were parameterized to model glutamatergic synapses with NMDA receptor kinetics. Glutamate is a primary neurotransmitter employed by the spinal cord, and the excitatory connections between interneurons and SPN neurons are mediated by NMDA receptors (de Groat et al. 1998). Conversely, inhibition of the SPN by PN stimulation is mediated by GABA\(\text{A}\) receptors (McGee et al. 2014), and inhibitory postsynaptic potentials were parameterized to model GABA\(\text{A}\) receptor kinetics.

An action potential was initiated in the postsynaptic neuron when the transmembrane voltage surpassed the specified threshold. Spike-triggered adaptation of neuron firing rates, described in Table 2, was implemented to increase the threshold voltage following repeated firing of the neuron. The parameters describing each neuron and synapse were selected to generate realistic SPN neuron firing rates and reproduce behavior from pelvic nerve recordings (Satchell and Vaughan 1989).

### 6.2.3 Closed-Loop Calculation of Bladder Pressure and Pelvic Afferent Firing Rate

Bladder activity is dependent on both pelvic efferent firing and the volume in the bladder (de Groat and Ryall 1969; Sasaki 1998; Mendez et al. 2013), and we incorporated both into the calculation of the equivalent bladder pressure. Bladder pressure was recurrently simulated using functions of bladder volume and a polynomial fit of bladder
pressure and SPN neuron firing rate in anesthetized cats (Sasaki 1998). The pelvic afferent firing rate was continually updated based on the bladder pressure from the previous time step using a polynomial fit of low threshold pelvic afferent activity as a function of bladder pressure (Sengupta and Gebhart 1994). The functions used to calculate bladder pressure from the firing rate of the model SPN neuron and bladder volume, and to calculate pelvic afferent firing from bladder pressure are shown in Table 2.

6.2.4 Model Evaluation of Pudendal Afferent Stimulation

We applied a variety of pudendal afferent stimulation inputs and tested multiple bladder volumes to evaluate the function of the neural network model. We assessed the effects of simulated slow infusion of fluid into the bladder to determine whether the model reproduced realistic responses to bladder filling. We also studied the effects of stimulation of pudendal afferents at different frequencies on the firing rate of the model SPN neuron and bladder pressure. The change in mean bladder pressure evoked by stimulation was calculated for each stimulation period as the mean bladder pressure during stimulation minus the pre-stimulation mean pressure. Further, we applied temporal patterns of pudendal afferent stimulation to compare the effects of stimulation pattern on the size of bladder contractions evoked by the model to previous experimental data in cats (McGee and Grill 2013) and explore the behavior of each model neuron.
6.3 Results

We developed a computational model of the sacral spinal neural circuit mediating the pudendo-vesical reflex and used the model to investigate mechanisms underlying the frequency dependence of bladder responses evoked by pudendal afferent stimulation.

6.3.1 SPN Neuron Firing Rate and Bladder Pressure during Bladder Filling

The firing rate of the model SPN neuron and the simulated bladder volume were used to calculate bladder pressure, which was compared directly to results from experimental studies. The model SPN neuron firing rate was modulated by interneuron activity, which was dependent upon the firing rates of the pelvic and pudendal afferents. The model SPN neuron exhibited both tonic and burst-like firing activity (Satchell and Vaughan 1989), and the firing rate and behavior (Figure 3) were within physiological limits reported in previous studies (de Groat et al. 1982; Sasaki 1998).

Figure 2B shows the response of the neural network to a constant rate bladder infusion, which resulted in a DEC similar to what is seen with constant rate bladder infusion in the cat (Figure 2A). The SPN neuron firing rate was sparse but burst-like at smaller bladder volumes and pressures, and increased slowly with volume during bladder filling. At larger bladder volumes, increased bladder pressure and pelvic afferent activity produced additional increases in SPN firing rate. Prior to the onset of a
coordinated DEC, transient increases and decreases in bladder pressure were observed, corresponding to bursts and pauses in the SPN neuron firing rate. A DEC was triggered by the PAG/PMC when bladder volume and pelvic afferent activity exceeded threshold. Above the DEC threshold volume, the pressure-time profile of bladder contractions mimicked those seen \textit{in vivo} (Figure 2), and the SPN neuron fired at ~15 Hz (Figure 3D).

6.3.2 Model Reproduces in Vivo Responses to Pudendal Afferent Stimulation

The response of the neural network to different frequencies of pudendal afferent stimulation was simulated to investigate reflex activation or inhibition of the bladder. The synaptic weights of model neurons in the network model were adjusted to reproduce the tuning curve of bladder excitation across frequencies of pudendal afferent stimulation (Figure 4). Similar to what is observed experimentally (Boggs et al. 2006b; Yoo et al. 2008a), high frequencies of stimulation evoked bladder contractions, but frequencies above 33 Hz were less effective than 33 Hz. Stimulation at frequencies of 2-20 Hz failed to evoke robust bladder contractions and 10 Hz stimulation produced a reduction in bladder pressure.

Further, the size of bladder contractions evoked by stimulation of pudendal afferents was dependent on bladder volume. Pudendal afferent stimulation at 33 Hz failed to evoke robust bladder contractions (mean bladder pressure $>10$ cmH$_2$O) below ~70% of the volume necessary to evoke DECs, a hallmark of the pudendo-vesical reflex.
The firing rate of the SPN neuron and bladder pressure evoked by 33 Hz stimulation increased with increases in bladder volume. Figure 2C shows an example where 33 Hz pudendal afferent stimulation produced an increase in SPN neuron firing rate and bladder pressure during filling (prior to the initiation of DECs), while 10 Hz pudendal afferent stimulation produced a decrease in SPN neuron firing rate and a reduction in bladder pressure, consistent with experimental results (Woock et al. 2008; Woock et al. 2011). Isovolumetric bladder contractions evoked by 33 Hz pudendal afferent stimulation and inhibition of isovolumetric DECs by 10 Hz pudendal afferent stimulation also mimicked results from pudendal afferent stimulation under isovolumetric bladder conditions in the cat (McGee et al. 2014; McGee and Grill 2014b) (Figure 3A-B).

Changes in bladder pressure during stimulation of the pudendal afferents in the model were a product of changes in SPN neuron firing rate. For example, during a robust bladder contraction evoked by 33 Hz pudendal afferent stimulation, the SPN neuron firing rate increased from ~3 Hz to ~22 Hz. The changes in firing rate of all model neurons in response to 10 or 33 Hz pudendal afferent stimulation are shown in Figure 3C-D. During 33 Hz pudendal afferent stimulation, the SPN neuron firing rate was increased and regularized by an increase in the firing rate of the excitatory interneurons. During 10 Hz pudendal afferent stimulation, domination of firing by the medial inhibitory interneuron silenced the SPN neuron, which produced a decrease in
bladder pressure (Figure 3B).

6.3.3 Network Structure Mediates Frequency and Bladder Volume Dependent Effects of Stimulation

To validate the structure of the network model and evaluate potential mechanisms underlying the frequency-dependent effects of PN stimulation on the bladder, we removed the contribution of the medial interneurons and evaluated bladder responses to different frequencies of pudendal afferent stimulation. Removing the medial interneurons, IN\(_{M+}\) and IN\(_{M-}\), produced an increase in neural activity throughout the network and monotonically increasing bladder pressure with increasing stimulation frequency (Figure 5). Further exploration of the model parameter space could not resolve the inability of this topology to reproduce the frequency-dependent effects of stimulation. Thus, neither inhibition with 10 Hz stimulation, nor a decline in the evoked bladder pressure at stimulation frequencies > 40 Hz was observed when the medial interneurons were removed, revealing that feedback inhibition alone was not sufficient to produce the frequency-dependent effects of stimulation.

We also evaluated the effects of removing the feedback (FB) interneuron, or dorsal interneuron, IN\(_{D}\), from the network to determine their contributions to the frequency-dependent effects of pudendal afferent stimulation on bladder pressure and SPN neuron firing rate. The firing rate of the SPN neuron and bladder pressure during stimulation were not substantially different following elimination of the FB interneuron.
from the network. Without feedback inhibition on IN_D, the SPN neuron exhibited intermittent bursting in response to high frequency inputs, but the overall firing rates and bladder pressures evoked by stimulation were unaffected.

To determine the role of the IN_D, we removed or reduced the synaptic weight (0.8 → 0.2) of the IN_D. The frequency-dependent excitation of the SPN neuron and consequent increases in bladder pressure with pudendal afferent stimulation remained intact, but the volume-dependent effects of stimulation and pelvic afferent contribution to SPN firing rate were lost. Pudendal afferent stimulation with 33-50 Hz caused the SPN neuron to fire, but the rate was too low to evoke robust contractions. Stimulation frequencies between 2-20 Hz and above 66 Hz failed to evoke SPN neuron firing. This revealed that the IN_D was not necessary to produce the stimulation frequency-dependent changes in SPN neuron firing rate or bladder pressure, but was critical to reproduce the volume-dependence of stimulation-evoked bladder contractions.

6.3.4 Blockade of GABAergic Activity Abolished Bladder Inhibition by Pudendal Afferent Stimulation

Bladder inhibition by low frequency pudendal afferent stimulation is mediated by GABAergic mechanisms in the spinal cord (McGee et al. 2014), and we simulated GABAergic blockade by reducing the synaptic weights of the inhibitory medial interneuron (0.65 → 0.2) and feedback interneuron (0.6 → 0.2). Similar to what was seen experimentally following blockade of GABA_A receptors with picrotoxin (McGee et al.
bladder inhibition evoked by 10 Hz pudendal afferent stimulation was abolished following blockade of the inhibitory interneurons (Figure 6). The inhibitory medial interneuron (IN$_{M^-}$) was most important for the bladder inhibition evoked by 10 Hz stimulation, as inhibition persisted when only the FB interneuron was removed from the network. Bladder excitation by 33 Hz pudendal afferent stimulation remained intact following blockade of the inhibitory interneurons, as observed experimentally (Figure 6).

### 6.3.5 Response to Temporal Patterns of Pudendal Afferent Stimulation

We evaluated the effects of temporal patterns of pudendal afferent stimulation, which produced different bladder responses in experiments in cats (McGee and Grill 2013) (Chapter 4), on the firing rates of neurons in our validated model. As in the experiments, bladder activation differed across patterns of pudendal afferent stimulation (Figure 7). Patterns that featured small changes in inter-pulse-interval (IPI) (Pattern #2, #3, #6 or #7) produced bladder contractions and SPN firing rates comparable to Pattern #1. Temporal patterns of stimulation that contained pauses, bursts, or random trains of stimulation produced bladder contractions with smaller mean bladder pressures than 33 Hz stimulation, as also observed experimentally. These smaller bladder contractions were mediated by changes in interneuron firing rate during pauses or bursts that produced a reduction in SPN neuron firing rate (Figure 8). Pauses in
stimulation in Pattern #5 or Pattern #8 silenced the interneurons and interrupted the excitatory activation of the SPN, causing a decrease in SPN firing rate and bladder pressure. During bursts of high frequency stimulation (Pattern #4, #5 or #9), the interneurons failed to increase their firing rate to match the input, and dominance by the inhibitory interneuron (IN\textsubscript{M-}) prevented the SPN from firing at a high rate.

6.4 Discussion

We implemented and validated a computational model of the spinal neural network that mediates the effects of pudendal afferent stimulation on the bladder. The model reproduced the effects of pudendal afferent stimulation frequency and pattern measured experimentally, and allowed exploration of the mechanisms underlying the strong stimulation frequency-dependent effects. Although the model included a simple representation of the descending pathway from the PAG/PMC to the SPN, the frequency and pattern-dependent effects of pudendal afferent stimulation were determined by changes in firing rate of spinal interneurons, suggesting that neural network interactions at the sacral level can mediate the bladder response to different frequencies or temporal patterns of pudendal afferent stimulation.

6.4.1 Model Bladder Pressure and Firing Rate

Simulated bladder filling produced realistic changes in SPN firing rate and bladder pressure, eventually triggering a DEC (Figure 2B). At small volumes, SPN firing rate and bladder pressure were low. Transient increases and decreases in bladder
pressure were observed at volumes and bladder pressures below the threshold for DECs, mimicking non-voiding contractions seen in cystometric studies (Hashim and Abrams 2006). These non-voiding contractions resulted from irregular, burst-like activity from the network processing of afferents that failed to produce a sustained increase in the firing rates of the interneurons or SPN neuron. At larger volumes and higher bladder pressures, continuous descending excitation from PAG/PMC, combined with increased pelvic afferent activity, produced DECs in the model. This matched a previous experimental study where DECs were evoked only by electrical stimulation of the PMC at high bladder pressures via polysynaptic excitation of SPN neurons (Sasaki and Sato 2013). The pressure-time profile of distension-evoked and stimulation-evoked bladder contractions in the model exhibited transient increases and decreases in pressure (Figure 2), which corresponded to brief changes in the firing rate of the SPN neuron. Bursting by preganglionic SPN neurons corresponded to rhythmic bladder activity in vivo (de Groat et al. 1982). Our model revealed that these bursts and pauses in the SPN firing rate were the product of competing excitation and inhibition of the SPN neuron by the network interneurons.

6.4.2 Model Reproduced Responses to Pudendal Afferent Stimulation

Our model reproduced the hallmarks of the pudendo-vesical reflex: volume-dependence of stimulation-evoked bladder contractions and stimulation frequency-dependent bladder activation. Pudendal afferent stimulation at 33 Hz during low
bladder volumes (< ~ 70 % DEC volume threshold) failed to evoke robust bladder contractions, and the size of stimulation-evoked bladder contractions increased with bladder volume. Previous studies suggested that the volume-dependence of pudendal afferent stimulation-evoked bladder contractions was the result of a convergence of pudendal and pelvic afferent inputs (Woock et al. 2011). Manipulation of the model corroborates this suggestion, as the IND, which receives both pelvic and pudendal afferent inputs, mediated the volume-dependence.

The bladder response evoked by pudendal afferent stimulation in the model exhibited a strong dependence on the frequency of stimulation. In the cat, 33 Hz stimulation evokes robust bladder contractions and augments ongoing bladder contractions, while 10 Hz stimulation inhibits bladder contractions (Woock et al. 2008); the synaptic weights of neurons in the model were manually adjusted to reproduce these frequency-dependent responses and fixed for the remainder of the analysis. High frequency stimulation (33 Hz) evoked large bladder contractions that were sustained during the period of stimulation, caused by an increase in SPN neuron firing rate that was driven by increased excitatory medial and dorsal interneuron activity. Low frequency (10 Hz) pudendal afferent stimulation did not evoke bladder contractions and inhibited ongoing bladder contractions, due to firing of the inhibitory medial interneuron and silencing of SPN neuron activity.

Stimulation from 2 to 20 Hz was not as effective at evoking bladder contractions
as 33 Hz (Boggs et al. 2006b; Yoo et al. 2008a). In previous experiments, responses to 20 Hz stimulation were variable, producing bladder contractions approximately 54% of the time. In the model, 20 Hz stimulation did not evoke bladder contractions; however a slightly higher frequency (25 Hz) did evoke bladder contractions, consistent with a frequency threshold between 20-25 Hz. Frequencies higher than 33 Hz evoked robust bladder contractions in the model, but the amplitude of the bladder contractions did not exceed that evoked by 33 Hz stimulation, producing a peak in the effect of pudendal afferent stimulation at approximately 33 Hz that matched experimental results (Yoo et al. 2008a). These results indicate that the structure of the lumbosacral spinal network is sufficient to account for the frequency-dependent effects of pudendal afferent stimulation on bladder activity, as suggested by animal studies where pudendal afferent stimulation evoked robust bladder contractions and inhibition following acute or chronic SCI (Tai et al. 2006; Woock et al. 2008; Xiao et al. 2014b).

Further, the model reproduced the bladder response to specific and widely differing temporal patterns of pudendal afferent stimulation (McGee and Grill 2013). Temporal patterns that featured small changes in IPI did not produce substantial differences in interneuron or SPN firing rates compared to regular 33 Hz stimulation (Figure 8). Patterns that included pauses and high frequency bursts were ineffective at increasing SPN firing rate in the model and did not evoke bladder contractions in the experiments. The pauses interrupted the interneuron firing and silenced the SPN
neuron, while the bursts of high frequency (66 Hz) stimulation periods failed to increase the SPN firing rate to follow the high input rate, producing an ineffective stimulus train despite the mean train rate of 33 Hz. Randomly patterned stimulation with a mean rate of 33 Hz, was less effective than regular 33 Hz stimulation in the model, matching experimental results. This decreased effectiveness was caused by the random bursts and pauses of stimulation that interrupted the regular firing of model interneurons and decreased the SPN firing rate (Figure 8), resulting in smaller and less consistent bladder contractions.

6.4.3 Mechanisms of Sacral Processing of Pudendal Afferent Stimulation

The model enabled manipulation of the neural network to study the underlying mechanisms of the frequency-dependent effects of pudendal afferent stimulation. Removing the contribution of the medial interneurons resulted in a simple network with a single inhibitory feedback neuron that produced a response to different frequencies of pudendal afferent stimulation inconsistent with experimental results. This suggests that the excitatory and inhibitory medial interneuron contributions were critical to the frequency dependent effects of stimulation on bladder activity. Thus, the structure of this network and interactions between excitatory and inhibitory interneurons mediate the effects of pudendal afferent stimulation on SPN firing rate and bladder pressure.

The model also reproduced the bladder response to pudendal afferent
stimulation following selective pharmacological blockade of GABA\textsubscript{A}, simulated by reducing the strength of model GABAergic synapses. Picrotoxin reversibly blocked 10 Hz pudendal afferent stimulation-evoked inhibition in cats (McGee et al. 2014) and in the model, bladder inhibition was abolished after blockade of the inhibitory synapses. Instead, slight bladder excitation was seen in response to 10 Hz stimulation, which matched the \textit{in vivo} results. Bladder excitation by 33 Hz stimulation was preserved following picrotoxin in the cat (McGee et al. 2014), and in the model, bladder excitation by 33 Hz pudendal afferent stimulation persisted following simulated blockade of GABAergic synapses.

The anatomical structure of the model of excitatory and inhibitory interneurons in the network was critical to both the volume-dependence of stimulation-evoked bladder contractions and frequency-dependence of stimulation on bladder activity. The frequency and pattern-dependent effects of pudendal afferent stimulation were determined by changes in firing rate of spinal interneurons, suggesting that neural network interactions at the lumbosacral level can mediate the bladder response to different frequencies or temporal patterns of pudendal afferent stimulation.
<table>
<thead>
<tr>
<th>Neuron</th>
<th>Description</th>
<th>Neuroanatomy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pud</td>
<td>Pudendal afferents</td>
<td>Pudendal afferents enter the dorsal horn of the sacral spinal cord. Medial projections innervate the dorsal gray commissure (DGC) and lateral projections innervate the lateral edge of the dorsal horn.</td>
<td>(Roppolo et al. 1985; Thor et al. 1989)</td>
</tr>
<tr>
<td>Pel</td>
<td>Pelvic afferents</td>
<td>Pelvic afferents enter the dorsal horn of the sacral spinal cord. Pelvic afferents primarily make lateral projections along the lateral border of the dorsal horn.</td>
<td>(Nadelhaft et al. 1983; Roppolo et al. 1985; Sengupta and Gebhart 1994)</td>
</tr>
<tr>
<td>IN&lt;sub&gt;M+&lt;/sub&gt;</td>
<td>Excitatory interneuron</td>
<td>Interneuron that excites neurons in the sacral parasympathetic nucleus (SPN), located medial to the SPN.</td>
<td>(de Groat et al. 1998)</td>
</tr>
<tr>
<td>IN&lt;sub&gt;M&lt;/sub&gt;-</td>
<td>Inhibitory interneuron</td>
<td>Interneuron that inhibits neurons in the SPN, located medial to SPN. GABAergic neurons project from DGC and dendrites from SPN have GABAergic receptors.</td>
<td>(Nadelhaft et al. 1983; Nadelhaft and Booth 1984; de Groat et al. 1998)</td>
</tr>
<tr>
<td>IN&lt;sub&gt;D&lt;/sub&gt;</td>
<td>Excitatory interneuron</td>
<td>Interneuron along lateral edge of dorsal horn that receives input from peripheral nerve afferents and excites the SPN.</td>
<td>(Araki and de Groat 1997; de Groat et al. 1998)</td>
</tr>
<tr>
<td>FB</td>
<td>Inhibitory feedback interneuron</td>
<td>Feedback interneuron that is controlled by output of SPN. This interneuron inhibits the dorsal interneuron and incoming peripheral afferents.</td>
<td>(de Groat and Ryall 1968; de Groat 1976; Shefchyk 2001)</td>
</tr>
<tr>
<td>PMC</td>
<td>Pontine Micturition Center</td>
<td>Descending pathway from the PMC produces polysynaptic excitation of the SPN.</td>
<td>(Sasaki and Sato 2013)</td>
</tr>
<tr>
<td>SPN</td>
<td>SPN neuron (Pelvic efferent)</td>
<td>Neurons with cell bodies in the SPN make up the efferent pelvic nerve.</td>
<td>(de Groat et al. 1982; Sasaki 1998)</td>
</tr>
</tbody>
</table>
Table 6.2: Model Equations and Parameterization

<table>
<thead>
<tr>
<th>Equations</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>The linear integrate and fire neuron follows the form</td>
<td>( V_{\text{rest}} = -65 \text{mV} )</td>
</tr>
<tr>
<td>( C_m \frac{dv}{dt} = I_{\text{leak}} + I_{\text{syn}} + I_{\text{inj}} = (V_{\text{rest}} - V)/R_m + I_{\text{syn}} + I_{\text{inj}} )</td>
<td>( V_{\text{ap}} = 60 \text{mV} )</td>
</tr>
<tr>
<td>The dynamics of the neuron's membrane potential include contributions</td>
<td>( V_{\text{th}} = -50 \text{mV} )</td>
</tr>
<tr>
<td>from membrane leak current, synaptic current and injected current. Using</td>
<td>( E_{\text{rev, ex}} = 0 \text{mV} )</td>
</tr>
<tr>
<td>( \tau_m = C_m R_m ), the equation can be rewritten as</td>
<td>( E_{\text{rev, in}} = -80 \text{mV} )</td>
</tr>
<tr>
<td>( \tau_m \frac{dv}{dt} = (V_{\text{rest}} - V) + R_m g(t)(V_{\text{rest}} - V) )</td>
<td>( \text{dt} = 0.1 \text{ms} )</td>
</tr>
<tr>
<td>Terms for a adaptation and synaptic conductance were included to yield</td>
<td>( \tau_m = 10 \text{ms} )</td>
</tr>
<tr>
<td>( \tau_m \frac{dv}{dt} = (V_{\text{rest}} - V)(1 + R_m \cdot \omega_{\text{ad}}) + R_m g_{\text{syn}}(E_{\text{rev}} - V) )</td>
<td>( R_m = 10 \text{M}\Omega )</td>
</tr>
<tr>
<td>The properties of the excitatory and inhibitory synaptic currents were</td>
<td>( \text{dur}_{\text{sp}} = 1 \text{ms} )</td>
</tr>
<tr>
<td>defined by the parameters selected for ( g_{\text{peak}}, E_{\text{rev}}, \tau_{\text{rise}} ) and ( \tau_{\text{decay}} ). A scaling factor, ( g ),</td>
<td>( \tau_{\text{ex}} = 0.9 \text{ms} )</td>
</tr>
<tr>
<td>controlled the relative weight of each synapse.</td>
<td>( \tau_{\text{td}} = 12.15 \text{ms} )</td>
</tr>
<tr>
<td>The change in synaptic conductance following a spike was modelled by</td>
<td>( \tau_{\text{td}} = 1.1 \text{ms} )</td>
</tr>
<tr>
<td>( g_{\text{syn}}(t) = g_{\text{syn}} f(e^{-(t-t_0)/\tau_{\text{decay}}} - e^{-(t-t_0)/\tau_{\text{rise}}}) ), which was represented by coupled, linear</td>
<td>( \tau_{\text{td}} = 10 \text{ms} )</td>
</tr>
<tr>
<td>ordinary differential equations, ( \frac{dg}{dt} = \frac{-\omega_{\text{ad}}}{\tau_{\text{ad}}} + z(t) \text{ and} ) ( \frac{dz}{dt} = \frac{-s}{\tau_{\text{rise}}} + \tilde{g}_{\text{syn}} u(t) ).</td>
<td>( g_{\text{peak, ex}} = 0.28 \text{mS/cm}^2 )</td>
</tr>
<tr>
<td>Neuronal adaptation was modeled by increasing the voltage threshold of the</td>
<td>( g_{\text{peak, in}} = 1.5 \text{mS/cm}^2 )</td>
</tr>
<tr>
<td>neuron following each spike. The adaptation variable decayed with time</td>
<td>( \omega_{\text{ad}, u} = 0.1 )</td>
</tr>
<tr>
<td>constant ( \tau_{\text{ad}} ).</td>
<td>( \omega_{\text{ad}, \text{inc}} = 0.5 )</td>
</tr>
<tr>
<td>( \frac{d\omega_{\text{ad}}}{dt} = \omega_{\text{ad}} - \omega_{\text{ad}, u} \text{ and} \frac{d\omega_{\text{ad}, u}}{dt} = \frac{-s}{\tau_{\text{ad}}} + \tilde{g}_{\text{syn}} u(t) ).</td>
<td>( \tau_{\text{ad}} = 35 \text{ms} )</td>
</tr>
<tr>
<td>When a spike fired in the presynaptic neuron, ( \omega_{\text{ad}} ) was</td>
<td>( \tilde{g}_{\text{p}, \text{IN}} = 0.45 )</td>
</tr>
<tr>
<td>incremented by ( \omega_{\text{ad}, \text{inc}} ) and the membrane potential was reset</td>
<td>( \tilde{g}_{\text{p}, \text{IN+}} = 0.6 )</td>
</tr>
<tr>
<td>( V_m(t) \geq V_{\text{th}} { \omega_{\text{ad}} \rightarrow \omega_{\text{ad}} + \omega_{\text{ad}, \text{inc}} } ).</td>
<td>( \tilde{g}_{\text{p}, \text{IN}} = 0.44 )</td>
</tr>
<tr>
<td>Simulated bladder pressure was recurrently calculated using functions of</td>
<td>( \tilde{g}_{\text{p}, \text{IN+}} = 0.7 )</td>
</tr>
<tr>
<td>bladder volume and pelvic efferent firing rate. The pelvic firing rate</td>
<td>( \tilde{g}_{\text{IN}-, \text{OUT}} = 0.65 )</td>
</tr>
<tr>
<td>calculation was generated from a polynomial fit of prior experimental</td>
<td>( \tilde{g}_{\text{IN+}-, \text{OUT}} = 0.6 )</td>
</tr>
<tr>
<td>results (30).</td>
<td>( \tilde{g}_{\text{IN-}} = 0.8 )</td>
</tr>
<tr>
<td>( P_{\text{B}}(t) = f(FR_{\text{pel}}(t - w_l)) + f(V_{\text{ol}}(t - 1)) )</td>
<td>( \tilde{g}_{\text{PAC}, \text{IN}} = 0.33 )</td>
</tr>
<tr>
<td>where,</td>
<td>( \tilde{g}_{\text{BB}, \text{IN}} = 1.0 )</td>
</tr>
<tr>
<td>( f(FR_{\text{pel}}) = 2 \times 10^{-2}FR_{\text{pel}}^2 - 3.3 \times 10^{-2}FR_{\text{pel}}^2 + 1.8FR_{\text{pel}} - 0.5 ) and</td>
<td>( \tilde{g}_{\text{BB}, \text{IN}} = 0.6 )</td>
</tr>
<tr>
<td>( f(V_{\text{ol}}) = (1.5V_{\text{ol}} - 10) ).</td>
<td>( \text{FR}_{\text{pel, in}} = 1 \text{Hz} )</td>
</tr>
<tr>
<td>Pelvic afferent firing rate was calculated from a polynomial fit of ( P_B )</td>
<td>( \text{FR}_{\text{pud}} = 0 \text{Hz} )</td>
</tr>
<tr>
<td>from recordings of low threshold pelvic afferents (33) and fed back into</td>
<td>( \text{FR}_{\text{PMC}} = 15 \text{Hz} )</td>
</tr>
<tr>
<td>the model.</td>
<td>( \text{Vol}_{\text{SEC}} = 10 \text{mL} )</td>
</tr>
<tr>
<td>( FR_{\text{pel}}(t) = f(P_{\text{B}}(t - 1)) )</td>
<td>( w_l = 1000 \text{ms} )</td>
</tr>
<tr>
<td>( = -3 \times 10^{-8}P_{\text{B}}^5 + 1 \times 10^{-8}P_{\text{B}}^4 - 1.5 \times 10^{-2}P_{\text{B}}^2 + 7.9 \times 10^{-2}P_{\text{B}} - 0.6P_{\text{B}} )</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.1: Structure of Neural Network Model of the Pudendo-vesical Reflex.

The network was composed of linear integrate and fire (LIF) neurons. Pudendal afferents innervate both medial and lateral portions of the dorsal horn (DH) and were represented by a single neuron with projections onto dorsal and medial interneurons (Pud). Pelvic afferents make primarily lateral projections in the dorsal horn and were represented by a single neuron (Pel). The dorsal interneuron (IN\textsubscript{D}) received input from both pelvic and pudendal nerves, while two interneurons (IN\textsubscript{M+}, IN\textsubscript{M-}) in the dorsal gray commissure (DGC) provided excitatory and inhibitory control of the sacral parasympathetic nucleus (SPN) neuron, the neural output to the bladder. The feedback
interneuron (FB) provided negative feedback from the SPN on the dorsal interneuron. Pelvic afferents also projected via the dorsal columns (DC) to a supraspinal processing node that simulated the periaqueductal gray (PAG) and pontine micturition center (PMC) and provided descending excitation of the IN_{D} via the lateral funiculus (LF).
Figure 6.2: Behavior in Response to Bladder Filling and Pudendal Afferent Stimulation.
Figure 6.2: Behavior in Response to Bladder Filling and Pudendal Afferent Stimulation.

A) Bladder pressure during slow, constant rate bladder filling in the adult cat anesthetized with α-chloralose (McGee et al. 2014). Bladder pressure slowly increases until a distension-evoked contraction (DEC) is evoked. Transient non-voiding contractions were present before the DEC. B) Neuronal spiking activity is represented with rasters of the model pudendal afferents (Pud), pelvic afferents (Pel), and sacral parasympathetic nucleus (SPN) neuron. Increasing the model bladder volume (used in calculation of pelvic afferent firing rate) produced a gradual increase in bladder pressure until a DEC was evoked, via the PMC, wherein bladder volume and pelvic afferent activity are high. Transient changes in bladder pressure that were produced by burst-like activity of the SPN neuron preceded the coordinated bladder contraction. C) 33 Hz stimulation of pudendal afferents evoked a stimulation-evoked bladder contraction (SEC) coincident with an increase in SPN neuron firing rate. 10 Hz stimulation of pudendal afferents produced stimulation-evoked inhibition (SEI) of DECs coincident with silencing of SPN neuron firing.
Figure 6.3: Reflex Bladder Excitation and Inhibition by Pudendal Afferent Stimulation.
Figure 6.3: Reflex Bladder Excitation and Inhibition by Pudendal Afferent Stimulation.

A) Bladder pressure responses to pudendal afferent stimulation at 33 or 10 Hz in the cat from previous experiments (McGee et al. 2014; McGee and Grill 2014b). Heavy bar indicates when stimulation was applied. B) Bladder pressure in response to stimulation of pudendal afferents in the model. C) Model neuron activity at specific time points in B is shown. During times of low pressure (Low $P_B$), SPN neuron firing remained low and irregular (1). During pudendal nerve stimulation at 33 Hz (2), the SPN neuron firing rate was regularized and increased by the excitatory interneurons, leading to an increase in bladder pressure. During distension-evoked contractions (DEC), SPN firing was increased, due to descending signals from the pontine micturition center (PMC) and the INd, producing high bladder pressures (3). 10 Hz PN stimulation (4) silenced the SPN firing via domination by of INm and produced a decrease in bladder pressure. D) Mean firing rate of each neuron under the four different conditions.
Figure 6.4: Stimulation Frequency Dependent Activation of the Bladder in the Model Matched Experimental Results

A) Change in bladder pressure during stimulation, relative to baseline, varied with the frequency of pudendal afferent stimulation. B) Percentage of trials during which a sustained bladder contraction (SBC) was evoked across different frequencies of pudendal (dorsal genital nerve, DGN) afferent stimulation (Boggs et al. 2006b; Yoo et al. 2008a).
Figure 6.5: Stimulation Frequency Dependent Responses in Model without Medial Interneurons.

Removal of the medial interneurons (INM+ and INM-) produced a thresholded monotonic increase in normalized bladder pressure with stimulation frequency that is not seen in experimental studies.
Figure 6.6: Effects of GABAergic Blockade on Response to Pudendal Afferent Stimulation.

A) Bladder pressure traces from experiment in α-chloralose anesthetized adult cat (McGee et al. 2014; McGee and Grill 2014b). Thick bar indicates when stimulation was applied. Inhibition of bladder contractions by 10 Hz pudendal DGN afferent stimulation was eliminated following administration of the GABA\textsubscript{A} receptor antagonist picrotoxin. Stimulation-evoked bladder contractions were produced with 33 Hz stimulation before and after the administration of picrotoxin. B) Simulated blockade of GABAergic inhibitory synapses in the model mimicked the effects of administration of picrotoxin. Pudendal afferent stimulation-evoked inhibition with 10 Hz was lost following blockade of model GABAergic synapses. Bladder contractions evoked by 33
Hz pudendal afferent stimulation were unaffected by the reduction in synaptic weight of inhibitory synapses in the neural network, consistent with experimental results (McGee et al. 2014).
Figure 6.7: Bladder Responses Evoked by Different Temporal Patterns of Pudendal Afferent Stimulation.
A) Temporal patterns of stimulation applied to the model and in experiments (Chapter 4). Patterns #2 and #3 were decreasing and increasing ramp trains, respectively, where the inter-pulse-interval (IPI) changed gradually at each pulse. Pattern #4 was a random train with mean 33 Hz. Pattern #5 consisted of repeating 100 ms periods of either 66 Hz stimulation or no stimulation, for an overall mean rate of 33 Hz. Patterns #6 and #7 were patterns of stimulation with alternating IPI presentation for an overall mean rate of 33 Hz, 10 and 50 ms IPIs and 20 and 40 ms IPIs, respectively. Pattern #8 was 1000 s of 33 Hz stimulation followed by a 200 ms pause for an effective stimulation rate of 27.5 Hz. Pattern #9 was inspired by a previous study (Bruns et al. 2008), and contained two pulses of 100 Hz stimulation repeated at 33 Hz, for a mean rate of 66 Hz. B) Bladder pressure evoked by stimulation of pudendal afferents in the model varied according to temporal pattern applied. C) Bladder pressure evoked by different temporal patterns of pudendal afferent (DGN) in α-chloralose anesthetized adult cats (n=12) (Chapter 4).
Figure 6.8: Model Neuron Firing Varies with the Temporal Pattern of Stimulation.
Figure 6.8: Model Neuron Firing Varies with the Temporal Pattern of Stimulation.

Firing activity of the model neurons is shown for 1 s of stimulation with each pattern of stimulation (Patterns #1 to #9). Each row represents the firing activity of each model neuron over time. Stimulation with pauses and high frequency bursts, Pattern #4, #5, #8, #9, produced SPN firing rates that were lower than regular stimulation (Pattern #1). Patterns with small changes in stimulation pattern (#2, #3, #6, #7) produced SPN firing rates similar to Pattern #1. During pauses (●), the interneurons’ firing rates decreased, causing the SPN to stop firing. During bursts of high frequency stimulation (●), the interneurons failed to increase their firing rate to match the input, and dominance by the inhibitory interneuron (INM–) prevented the SPN from firing at a high rate.
7. Discussion

Spinal cord injury (SCI) and neurological diseases cause lower urinary tract dysfunction (LUTD), significantly disrupting the ability to store urine (continence) and efficiently empty the bladder (micturition). LUTD has a negative impact on quality of life, and restoration of bladder and bowel function is a top priority for persons with SCI (Anderson 2004). When compared to traditional pharmacological or surgical treatments of LUTD, electrical stimulation provides a unique opportunity to interface with existing neural circuits that control bladder function. Sacral anterior root stimulation (SARS) is effective for bladder control following SCI, but has not been widely implanted due to the requirement for an irreversible dorsal rhizotomy (Brindley 1988; Rijkhoff 2004), which eliminates residual reflex erection or defecation. Sacral nerve stimulation (SNS) with Interstim® is less invasive but is generally ineffective in SCI (Chartier-Kastler et al. 2001; Kessler et al. 2010).

Recent clinical studies demonstrate that pudendal nerve stimulation (PNS) can produce both inhibition of bladder contractions, or at different stimulation parameters, bladder activation in persons with SCI (Vodušek et al. 1987; Wheeler et al. 1992; Previnaire et al. 1996; Kirkham et al. 2001b; Gustafson et al. 2003; Lee et al. 2003; Gustafson et al. 2004; Yoo et al. 2007). The objective of this dissertation was to advance our understanding of the effects of patterns of pudendal afferent stimulation on bladder activation and voiding, and determine the neural mechanisms of action by: 1)
investigating the size of reflex bladder contractions and voiding efficiencies evoked by
selective co-stimulation of pudendal afferents in anesthetized cats and urethral afferents
in persons with SCI, 2) exploring the importance of temporal pattern of stimulation on
the size of bladder contractions evoked in anesthetized cats, 3) identifying the
neurotransmitters responsible for low frequency stimulation-evoked bladder inhibition,
and 4) constructing a computational model of the neural network underlying the
pudendo-vesical reflex to explore mechanisms of action of pudendal afferent
stimulation.

The results revealed that selective co-stimulation of pudendal afferents generated
significantly larger isovolumetric bladder contractions and increased voiding efficiencies
as compared to individual stimulation and distention-evoked voiding (Chapter 2). The
increase in voiding efficiency with co-stimulation was facilitated by a decrease in
threshold volumes compared to individual stimulation and a suppression of
dyssynergic activity in the external anal sphincter (Chapter 2). In persons with SCI, the
size of reflex bladder contractions evoked with stimulation was dependent on
stimulation location, frequency, and amplitude and increased by co-stimulation of two
intraurethral sites (Chapter 3). Reflex electromyographic (EMG) activity suggested that
multiple reflex pathways contributed to bladder activation (Chapter 3). Moreover, the
temporal pattern of pudendal afferent stimulation in anesthetized cats significantly
affected the magnitude of evoked bladder contractions (Chapter 4). The results also
revealed the mechanisms of pudendal afferent stimulation-evoked bladder control; reflex bladder inhibition by low frequency pudendal afferent stimulation required a lumbosacral spinal GABAergic mechanism, as picrotoxin, a noncompetitive GABA$_A$ antagonist, significantly and reversibly blocked stimulation-evoked inhibition of bladder contractions (Chapter 5). Further, the frequency and pattern-dependent effects of pudendal afferent stimulation resulted from changes in firing rate of spinal interneurons in a computational model of the pudendo-vesical reflex, suggesting that neural network interactions at the lumbosacral level can mediate the bladder response to pudendal afferent stimulation (Chapter 6). The outcome of this dissertation is a better understanding of how spatial and temporal patterns of pudendal afferent stimulation can modulate reflex bladder activation via a network of neurons in the lumbosacral spinal cord.

7.1 Summary of Results

7.1.1 Spatial patterns improve reflex bladder activation and voiding

The first aim was to explore the effects of co-stimulation of multiple pudendal afferent pathways on reflex bladder activation. We quantified bladder contractions and voiding efficiencies produced by selective co-stimulation of distal sensory branches of the PN, the cranial sensory nerve (CSN) and dorsal nerve of the penis (DNP), or bilateral DNP stimulation in α-chloralose anesthetized cats. Previous studies demonstrated unique frequency tuning properties with stimulation of each afferent branch; robust
bladder contractions were evoked with high frequency stimulation of DNP (~ 33 Hz) or low frequency of stimulation of the CSN (~ 2 Hz) (Yoo et al. 2008a). However, the voiding efficiencies produced by stimulation of either of these branches individually were too low for successful clinical application, and this aim investigated the use of co-stimulation to improve voiding efficiency.

Selective co-stimulation of CSN and DNP, or bilateral DNP stimulation, evoked significantly larger bladder contractions and increased voiding efficiency over single branch stimulation and control, distention-evoked voiding. The effects of co-stimulation were volume dependent, and co-stimulation evoked bladder contractions at lower bladder volumes than individual site stimulation or distension-evoked contractions.

The results presented in Chapter 2 provided the first evidence of the use of stimulation of multiple afferent pathways in the pudendal nerve to improve voiding efficiency. Previous studies characterized the effects of stimulation at many different locations on the pudendal nerve or distal sensory branches (Boggs et al. 2006b; Tai et al. 2007a; Yoo et al. 2008a; Woock et al. 2011), but did not evaluate co-stimulation. Co-stimulation of the sacral nerve and high-frequency blockade of pudendal efferent signals to the urethral sphincter improved voiding efficiency (Boger et al. 2008), but our work was the first to demonstrate improved voiding efficiencies with co-stimulation of multiple afferent pathways.

The increased bladder activation and reduced volume thresholds with co-
stimulation suggest a bilateral convergence of afferents in each sensory branch and pelvic afferents at the spinal and/or supraspinal level in the central nervous system. Stimulation of the DNP and CSN engages supraspinal and spinal pathways (Yoo et al. 2008a), respectively, and pelvic afferents make both spinal and supraspinal projections (Roppolo et al. 1985), so it is unclear where such interactions take place. The results reported in Chapter 6 suggests that a neural network in the lumbosacral spinal cord controls the volume-dependence of the bladder response to pudendal afferent (DNP) stimulation and this model could be extended to explore bilateral stimulation; however the current model cannotexplain the interaction effects seen with co-stimulation of CSN and DNP without inclusion of additional supraspinal pathways. Further investigation into the mechanisms that mediate the volume-dependence of co-stimulation could lead to the design of more effective stimulation paradigms that can overcome the strong volume-dependence of the reflex that appears to limit voiding efficiency.

The results from Chapter 2 suggest that selective co-stimulation should be considered in the development of new neural prosthetic applications of electrical stimulation for restoration of bladder function. For example, asymmetric pudendal reflexes exist in humans (Hamdy et al. 1999; Enck et al. 2004), and bilateral PNS improve bladder activation and voiding when individual site stimulation is insufficient.

7.1.2 Multiple reflex pathways contribute to bladder activation by intraurethral stimulation in persons with SCI
The objective of this aim was to evaluate the effects of intraurethral co-stimulation, targeting the proximal and distal urethra, in persons with chronic, suprasacral SCI. We measured the effects of different frequencies of intraurethral stimulation at different locations on bladder activation and the electromyographic (EMG) activity of pelvic floor muscles during urodynamics. This study was prompted by results from our pre-clinical experiments of pudendal afferent co-stimulation in cats, where co-stimulation of pudendal sensory pathways produced larger bladder contractions and more efficient voiding (McGee and Grill 2014b), coupled with results from previous experiments where similar excitatory pathways were identified in persons with SCI (Yoo et al. 2011).

The size of reflex bladder contractions evoked with stimulation was dependent on stimulation location and frequency. Reflex EMG activity suggested that multiple reflex pathways contributed to bladder activation. These findings are consistent with previous clinical studies showing that stimulation frequency affected the size of bladder contractions evoked by stimulation (Gustafson et al. 2004; Yoo et al. 2011), and similar studies in the cat, where unique frequency tuning responses were seen for stimulation of different pathways (Boggs et al. 2006b; Yoo et al. 2008a) and selective co-stimulation of these pathways can produce enhanced bladder activation (McGee and Grill 2014b).

In the human, the two urethral pathways were differentiated by the latencies of reflex responses to intraurethral stimulation. The ~30 ms latency difference, as noted in
Chapter 3, may occur due to differences in the complement of sensory fibers activated and the reflexes engaged by stimulation. Although a previous study reported bladder activation via multiple reflex pathways in the proximal and distal urethral stimulation in persons with SCI (Yoo et al. 2011), this was the first study to quantify the difference in reflex latencies evoked by stimulation of these pathways.

Additional exploration of these multiple pathways is needed to determine if pelvic urethral afferents are responsible for long latency reflex responses to proximal urethral stimulation. This is important for future interventions because pelvic and pudendal afferent stimulation are likely to yield different effects on bladder and sphincter activation. Pudendal afferent stimulation does not increase sphincter EMG activity during stimulation (Woock et al. 2008; McGee and Grill 2014b), but pelvic stimulation produced concomitant sphincter and bladder activation in dogs (Holmquist and Olin 1968) and proximal urethral stimulation produced more EMG activity than distal stimulation in persons with SCI (Yoo et al. 2011). Concurrent activation of the bladder and sphincter with proximal stimulation may explain why co-stimulation of proximal and distal urethra in our results produced a significant increase in EMG activity (Chapter 3).

Although the size of bladder contractions evoked by intraurethral co-stimulation varied greatly with frequency across stimulation locations and subjects, these results indicate that intraurethral co-stimulation can evoke larger bladder contractions than
individual site stimulation in persons with SCI. Further identification of stimulation parameters (e.g., location, frequency, amplitude) that reliably evoke bladder contractions via the targeted reflex pathway(s) is necessary prior to implementation of co-stimulation methods for the treatment of bladder dysfunction. The heterogeneity in reflex pathway activated and the size of bladder contractions evoked by stimulation in our study indicated that effective stimulation parameters may differ for each patient.

This work demonstrates the importance of stimulation frequency and location, or reflex pathway activated, on the size of bladder contractions evoked by electrical stimulation. The results reveal that the reflex circuitry mediating the effects of afferent stimulation is present in the lumbosacral spinal cord and preserved after suprasacral SCI. Co-stimulation of multiple afferent reflex pathways can enhance activation of spinal circuits and may enable improved bladder emptying in SCI when stimulation of a single pathway is insufficient. Furthermore, for other applications of stimulation of afferent pathways in the pelvic region, reflex latency may be used as an indicator of which neural pathway was activated.

### 7.1.3 Temporal patterns of stimulation modulate reflex bladder activation

The objective of the second aim was to study the effects of temporal patterns of pudendal afferent stimulation on reflex bladder activation, and identify patterns which were more effective than regular 33 Hz. We quantified the effects of novel temporal
patterns of stimulation on the size of evoked bladder contractions in anesthetized cats. Previous studies demonstrated that bladder excitation and inhibition are evoked by different frequencies of pudendal afferent stimulation, indicating that the temporal pattern of may be an important determinant of bladder activation by pudendal afferent stimulation (Peng et al. 2008b; Yoo et al. 2008a; Yoo et al. 2011). High frequencies of pudendal afferent stimulation evoked robust bladder contractions while low frequencies failed to evoke bladder contractions or inhibited the bladder (Woock et al. 2008). In other studies, select patterns of burst stimulation improved reflex bladder activation over regular stimulation (Bruns et al. 2008; Bruns et al. 2009b).

The results in Chapter 4 show that while the size of bladder contractions was indeed dependent on the temporal pattern of pudendal afferent stimulation, no pattern significantly increased the size bladder contractions compared to regular 33 Hz. However, some patterns of stimulation with 33 Hz mean frequencies significantly reduced the size of bladder contractions evoked by stimulation, and revealed that pattern is an important determinant reflex bladder activation. Pauses, gaps and bursts in these ineffective patterns reduced the effectiveness of stimulation with 33 Hz stimulation.

Investigation of the effects of patterned stimulation on the firing rate of neurons in a model of the pudendo-vesical reflex (Chapter 6) revealed that some stimulation pattern features prevent a stimulation-evoked increased in firing rate of the neuron in
the sacral parasympathetic nucleus (SPN), the efferent control of the bladder. Pauses in pudendal afferent stimulation patterns silenced the activity of interneurons and produced a decrease in the firing rate of the SPN neuron. Bursts and periods of high frequency stimulation of the pudendal afferents failed to produce a corresponding increase in interneuron firing rate, further reducing the SPN neuron firing rate and bladder activation. Random trains of 33 Hz stimulation produced bladder contractions that were more variable in size than regular 33 Hz stimulation. This occurred because each stimulation train was composed of a different arrangement of inter-pulse-intervals (IPIs) drawn from the same distribution, and highlights the sensitivity of the neural network controlling reflex bladder activation to changes in IPI.

We found that there was no significant difference in bladder activation between our pattern of burst stimulation and three previously published burst patterns (Bruns et al. 2008), and these patterns all produced significantly smaller bladder contractions than regular 33 Hz stimulation. This result was surprising however, since burst stimulus patterns evoked greater bladder pressures than continuous stimulation by either direct PN or intraurethral stimulation of pudendal afferents (Bruns et al. 2008; Bruns et al. 2009a). This discrepancy may have been caused by differences in neural recruitment with stimulation, as previous studies reported both high and low frequency responders. For example, low frequency burst patterns of direct PN stimulation may have activated CSN fibers, which have a different frequency response profile than DNP, explaining...
why we did not see an increase in bladder activation with DNP stimulation. This also suggests that additional investigation into temporal patterns of CSN stimulation might reveal information about the mechanisms involved in that supraspinal pathway.

### 7.1.4 GABA$_A$ mediates reflex bladder inhibition by PN stimulation

The objective of this aim was to identify the neural mechanisms responsible for pudendal afferent stimulation-evoked inhibition of bladder contractions in anesthetized cats by pharmacological blockade of potentially critical neurotransmitters. Reflex inhibition of the bladder by low frequency pudendal afferent stimulation was previously thought to be mediated through adrenergic receptors and the hypogastric nerve, as this is how bladder relaxation occurs under normal neural control of the lower urinary tract. A previous study of the mechanisms of bladder excitation suggested that hypogastric and adrenergic-mechanisms were not necessary for bladder excitation and that inhibition with pudendal afferent stimulation was preserved following transection of the hypogastric nerve and administration of adrenergic antagonists (Woock et al. 2011).

In Chapter 5, we found that picrotoxin, a noncompetitive antagonist for GABA$_A$ (gamma-aminobutyric acid) receptors, blocked reflex bladder inhibition produced by 10 Hz stimulation of the compound pudendal nerve or a distal sensory branch, DNP. Blockade of stimulation-evoked bladder inhibition with picrotoxin was dose-dependent, where low doses (0.5 mg/kg) did not affect inhibition and high doses (1.5 mg/kg)
blocked inhibition. The loss of inhibition with 10 Hz stimulation with picrotoxin returned following a washout period, indicating that the reversible blockade of GABA\(_A\) receptors was responsible for the blockade of stimulation-evoked inhibition. Intrathecal administration of picrotoxin at the lumbosacral level also blocked inhibition of bladder contractions with pudendal afferent stimulation. This indicated that GABA\(_A\) in the lumbosacral spinal cord plays a critical role in bladder inhibition with low frequency pudendal afferent stimulation.

As well, other pharmacological antagonists were used to evaluate the role of other neurotransmitters in bladder inhibition by pudendal afferent stimulation. Administration of strychnine (glycine antagonist), naloxone (opioid antagonist), phentolamine (\(\alpha\)-adrenergic antagonist), and propranolol (\(\beta\)-adrenergic antagonist), and transection of the hypogastric nerve all failed to block bladder inhibition with low frequency pudendal afferent stimulation. This work was important because it provided a clear mechanism of action for bladder inhibition and clarified the contributions by neurotransmitters that had been previously debated. For example, many prior studies reported variable effects of blocking opioid receptors on stimulation-evoked bladder inhibition (Chen et al. 2010; Hotta et al. 2012; Tai et al. 2012; Mally et al. 2013).

These experiments demonstrated that GABA\(_A\) in the lumbosacral spinal cord is necessary for bladder inhibition by pudendal afferent stimulation, and that glycineric, adrenergic, and opioidergic mechanisms may not be necessary. These results have
important implications for understanding the mechanisms of neuromodulation with PNS and SNS with Interstim®, which activates afferents from both the pelvic and pudendal nerves. Bladder inhibition, particularly after SCI, likely utilizes a sacral spinal network that is mediated by GABA. These results also indicate that pharmacological interventions, such as GABA agonists for overactive bladder, should be investigated to improve the efficacy of existing neuromodulation techniques.

7.1.5 Pudendo-vesical reflexes can be explained by network interactions in the spinal cord

The objective of this aim was to develop a computational model of the sacral spinal network underlying the pudendo-vesical reflex that controls bladder activation or inhibition by pudendal afferent stimulation. Prior to this work, our understanding of the mechanisms of how pudendal afferent stimulation produced bladder activation or inhibition was limited. Few computational models of neural control of the bladder existed, and those that did focused on the muscular mechanics of bladder or urethra or lacked quantitative representation of the neural circuits or contributions of pudendal afferents.

In Chapter 6, we developed a network of linear integrate and fire (LIF) neurons based on electrophysiological and anatomical tracing studies of neurons in the spinal cord and those that innervate the lower urinary tract. The model generated realistic patterns of neural firing activity and bladder pressures, as well as reproduced responses
to various frequencies and patterns of pudendal afferent stimulation. The anatomical structure of the model of excitatory and inhibitory interneurons in the network was critical to both the volume-dependence of stimulation-evoked bladder contractions and frequency-dependence of stimulation on bladder activity.

This work demonstrated that the frequency and volume-dependent features of the pudendo-vesical reflex can be explained by network interactions in the lumbosacral spinal cord. Bladder activation or inhibition is the result of a network of excitatory and inhibitory interneurons in the spinal cord that modulate SPN firing rate based on afferent inputs. Stimulation with high frequencies, around 33-40 Hz, produced a high firing rate of the excitatory interneurons and SPN neuron, increasing bladder pressure. Low frequencies of stimulation or patterns with pauses reduced the firing rate of interneurons in the network and prevented an increase in firing rate of the SPN. Bursts of high frequency stimulation fail to evoke similar increases in the firing rates of interneurons or an increase in SPN firing rate.

Elimination of interneurons in the network model disrupted the frequency and volume-dependent effects of pudendal afferent stimulation seen in animal experiments, validating the structure of the network model. Removing the medial excitatory and inhibitory interneurons in the network produced a thresholded, monotonically increasing bladder pressure response to increasing frequencies of pudendal afferent stimulation. Previous studies suggested that the volume-dependence of pudendal
afferent stimulation-evoked bladder contractions was the result of a convergence of pudendal and pelvic afferent inputs (Woock et al. 2011). Manipulation of the model corroborated this notion, as the INo, which receives both pelvic and pudendal afferent inputs, produced the volume-dependence. Further comparison of model results to results seen in vivo allowed for the dissection of the underlying mechanisms of the pudendo-vesical reflex. Reduction of the synaptic weights of GABAergic inhibitory interneurons, mimicking blockade of GABAergic receptors with picrotoxin, resulted in a loss of bladder inhibition with 10 Hz stimulation, as seen in the cat in Chapter 5.

This model demonstrates that the characteristic features of the pudendo-vesical reflex can be explained by neural network interactions in the lumbosacral spinal cord, matching what has been suggested by pre-clinical and clinical studies where the effects of stimulation persist after spinal cord transection or injury. This model represents a significant advance in computational models of the neural control of the LUT, and is the only one that uses a network of synaptically-connected LIF neurons to produce realistic spiking behavior and demonstrate the mechanisms behind of pudendal afferent stimulation. This model of the network reproduces results from experiments in animals and may be used to identify specific mechanisms of action and develop novel, more effective electrical stimulation techniques for bladder control.

7.2 Future Directions

This dissertation produced significant advances in methods to improve bladder
activation with PNS and improved our understanding of the mechanisms of pudendo-vesical reflexes. However, additional work in this area is necessary to determine the clinical feasibility and utility of potential applications of PNS. The following section describes additional experiments which could be performed to further study and improve PNS.

7.2.1 Pre-clinical, Animal Experiments

7.2.1.1 Methods of identification and selective stimulation or recording of neurons

Previous anatomical tracing studies of the pudendal and pelvic nerves revealed projections to various spinal cord nuclei in animals (Morgan et al. 1981; Roppolo et al. 1985). However, additional studies should be performed to clarify the specific nuclei that receive projections by branches or fascicles of these nerves, such as the CSN and DNP for the pudendal nerve, or urethral afferents of the pelvic nerve. This information could inform potential mechanistic differences underlying the effects on bladder activation seen with stimulation of different branches. Tracing studies would also reveal the extent of spinal and supraspinal projections in the CSN and DNP pathways, as these have been attributed to predominately supraspinal and spinal reflexes (Woock et al. 2008; Yoo et al. 2008a), respectively. Further, fascicles in the pudendal nerve that innervate a desired target nucleus, e.g., the dorsal gray commissure (an important spinal nucleus in our model), could be stimulated with novel selective electrodes (Tyler and Durand 2002; Mathews et al. 2014) to improve reflex bladder control.
7.2.1.2 Optogenetic selective stimulation of peripheral nerves

We demonstrated that selective co-stimulation of pudendal afferents, via electrode cuffs on distal afferent branches of the pudendal nerve, improved reflex bladder activation and voiding efficiency. Selective stimulation (or co-stimulation) of afferents could also be achieved with optogenetic methods. The use of light to activate ion channels of specific neurons could lead to more selective control of afferents; a recent study successfully transfected peripheral nerves via intramuscular injection of adeno-associated virus serotype 6, which enabled expression of channelrhodopsin (ChR2) in the motor neurons innervating the injected muscle (Towne et al. 2013). Similar methods could be employed to target the DGN and CSN; for example, injecting viruses into the distal and proximal urethra with spectrally separated channelrhodopsins (e.g., ChR2, which absorbs blue light, and ReaChR, which absorbs red light), would result in differential expression for each nerve. This would mean that light stimulation of the compound nerve could be designed to produce selective activation of the fascicles in each branch. Other uses for optogenetic methods could be envisioned for application for bladder control, such as selective blockade of efferent fibers which innervate the EUS and are responsible for dyssynergia.

7.2.1.3 Additional pharmacological studies of the mechanisms of PNS

In Chapter 5 we demonstrated that picrotoxin reversibly and dose-dependently blocked bladder inhibition with 10 Hz pudendal afferent stimulation during
isovolumetric bladder contractions. Cystometry following administration of pharmacological antagonists would reveal whether low frequency stimulation-evoked inhibition during filling is mediated by the same mechanisms as inhibition of distension-evoked contractions under isovolumetric conditions. It is known that different mechanisms may mediate bladder activity at low and high bladder volumes (Fall et al. 1977), and a previous study of PNS inhibition during cystometry with low-dose picrotoxin (Xiao et al. 2014a) was consistent with the results from our isovolumetric experiments. It is expected that high-dose picrotoxin will block PNS-evoked increases in bladder capacity, but these experiments will quantify the mechanisms of inhibition during filling for improved continence.

As a follow-up to the experiments evaluating the mechanisms of bladder inhibition with 10 Hz PNS, experiments should be performed to quantify the role of GABA\(_B\). While many spinal cord neurons have both GABA\(_A\) and GABA\(_B\) receptors, these neurotransmitters play different roles in neural circuits. It is important to identify the role of GABA\(_B\) in PNS-evoked inhibition, or if GABA\(_A\) alone is responsible for the inhibitory effects of low frequency stimulation. However, glycine is also known to be colocalized with GABA on many spinal cord neurons, but was not necessary for PNS inhibition in Chapter 5.

Because bladder inhibition with low frequency stimulation was mediated by GABA\(_A\), experiments should be performed to explore how GABA\(_A\)ergic agonists could
be used to enhance the effects of low frequency stimulation. This could be done with animal experiments using intrathecal or intravenous delivery of GABA\textsubscript{A} agonists, such as muscimol, to determine whether bladder inhibition is increased with the administration of the drugs. Strong bladder inhibition during isovolumetric bladder conditions or increased low frequency stimulation-evoked bladder capacities would be expected. Since bladder excitation with high frequency stimulation was not mediated by GABA\textsubscript{A} blockade, we would expect that high frequency stimulation-evoked bladder activation would be preserved following the administration of a GABA\textsubscript{A} agonist. If this agonist approach was successful, a new device might combine low frequency PNS and local, spinal administration of GABAergic agonists to produce long-lasting continence and high frequency PNS to efficiently empty the bladder. However, the dose and site of administration of the GABAergic agonist would be critical to prevent harmful side effects, like those seen with baclofen pumps: drowsiness, weakness in the lower extremities, and seizures.

Additional experiments should also be performed to evaluate further the mechanisms of bladder activation with high frequency stimulation. This was investigated by Woock et al. (Woock et al. 2011), who found that stimulation-evoked activation was generated by pelvic efferent activation via the pelvic ganglion. Pharmacological blockade of AMPA or NMDA glutamate receptors with intrathecal administration of CNQX or AP5, respectively, during high frequency stimulation of
pudendal afferent stimulation would clarify the contribution these neurotransmitters play in the excitatory pudendo-vesical reflex in the spinal cord. Mechanistic differences may exist between pathways activated by stimulation of different branches or fascicles of the pudendal nerve, and identifying these differences would reveal how each pathway participates in the pudendo-vesical reflex.

### 7.2.2 Clinical Studies

In addition to the animal experiments mentioned in the previous section, many clinical studies remain necessary to determine the effectiveness of PNS for bladder control in SCI and other applications. Because of the neural, and potentially mechanistic, differences between animal models and humans, new PNS approaches must be validated to confirm their effects in humans. This section describes studies that should be performed to demonstrate the utility of PNS in the clinical setting.

#### 7.2.2.1 Long-term, clinical feasibility studies of PNS

Additional well-designed, randomized and blinded studies should be performed in patients with SCI to identify the long-term effects of PNS, and compare these to other stimulation modalities, like SARS and SNS. PNS received a CE mark in 2008 (Bock et al. 2010), after a study demonstrating the feasibility of chronic PNS for neurogenic detrusor overactivity revealed that implanted patients showed a significant improvement in maximum cystometric capacity (Spinelli et al. 2005). However, additional work is needed to evaluate the long-term efficacy of stimulation and
determine optimal clinical stimulation parameters and implantation techniques. Our work in Chapter 3 is consistent with other studies (Boggs et al. 2006b; Tai et al. 2008; Woock et al. 2008) that suggest that stimulation frequency significantly affects the bladder response. In particular, our results showed that some combinations of high frequencies of co-stimulation produced large bladder contractions, while 10 Hz distal urethral stimulation failed to evoke contractions in all subjects tested, producing inhibition in some. Therefore, high frequencies (20-40 Hz) should be used to evoke bladder contractions and emptying, and low frequencies (5-10 Hz) should increase bladder capacities, consistent with results from preliminary clinical trials (Spinelli et al. 2005; Kennelly et al. 2010; Kennelly et al. 2011).

7.2.2.2 **Detrusor-sphincter dyssynergia and PNS**

Detrusor sphincter dyssynergia (DSD) is a serious problem for patients with SCI. Our animal experiments show that high frequency stimulation of dorsal genital afferents in the pudendal nerve limits reflex activation of the EUS and EAS. However, it is unclear whether blockade of EUS activation from DSD will also be produced in humans, as recent results with sacral dermatomal stimulation did not suppress EUS activation (McCoin et al. 2014). This could be examined with transcutaneous or intraurethral stimulation of the dorsal genital nerve during episodes of DSD. This would reveal if certain frequencies of pudendal afferent stimulation block DSD and indicate potential benefits of PNS over SARS. However, as we saw in Chapter 3, in studies such as these, it
would be important to use reflex responses to ensure that pudendal, not pelvic, afferents are activated by stimulation; pelvic nerve stimulation or stimulation of proximal urethral afferents have been shown to produce an increase in sphincter activation (Yoo et al. 2011).

Other techniques to prevent or reduce the effects of DSD include the use of co-stimulation of the sacral roots and/or pudendal nerve. Co-stimulation of the sacral roots or compound pudendal nerve to generate bladder contractions and high frequency nerve block of the efferent, motor fibers to the EUS would produce bladder activation without DSD from aberrant reflexes or stimulation (Boger et al. 2008). High frequency block of the PN coupled with stimulation of sacral nerve or proximal PN in animals produced significant improvements in voiding efficiency compared to sacral nerve stimulation alone (Shaker et al. 1998; Boger et al. 2008). However, high-frequency block stimulation waveforms and protocols must be carefully designed to avoid nerve damage from long-term exposure to stimulation that produces electro-chemical reactions at the electrode-tissue interface (Agnew and McCreery 1990; Merrill et al. 2005), and avoid onset activation of the EUS (Bhadra et al. 2006; Vrabec et al. 2013), which could increase outlet resistance and impair voiding efficiency. High frequency block was well tolerated in chronically implanted animals without anesthesia (Gaunt and Prochazka 2009), demonstrating the clinical value of such an approach, particularly since our results in Chapter 3 indicate that patient discomfort can limit subject participation.
7.2.2.3 Neural innervation of lower urinary tract in humans

Ultimately, novel methods of PNS implantation will require better identification of the neural supply of the pelvic floor and urethra, suggested by our work in Chapter 3 to receive contributions from pelvic (proximal) and pudendal (distal) nerves. A previous study of the fascicular anatomy and surgical access of the pudendal nerve in humans found that placement of a nerve cuff on the PN is both anatomically and surgically feasible (Gustafson et al. 2005), but did not evaluate access of distal branches or the urethral innervation. The heterogeneity of reflex responses evoked by intraurethral stimulation in Chapter 3 further suggest that urethral innervation may differ substantially; e.g., some subjects with SCI had short latency, pudendal-mediated reflex responses to stimulation in the proximal urethra. Identifying differences in innervation between patients or across sexes could be performed through novel nerve tracing methods such as Clarity (Chung and Deisseroth 2013; Yang et al. 2014). With respect to the results from Chapter 3, it would also be valuable to perform a comprehensive study of the reflexes and corresponding intraurethral stimulation locations to explore how functional innervation differs across patients. This information, coupled with the results of anatomical studies would confirm that different latencies measured in Chapter 3 are the result of stimulation of pudendal and pelvic pathways.

7.2.3 Computational Modeling

In Chapter 6, we developed a computational model of the spinal neural network
that governs the reflex bladder response to PNS. This model was a significant advance in models of the LUT and demonstrated that the anatomical structure of excitatory and inhibitory interneurons in the network is critical to both the volume-dependence of stimulation-evoked bladder contractions and frequency-dependence of stimulation on bladder activity. While this model improved our understanding of the neural mechanisms underlying the pudendo-vesical reflex, further modification may lead to the development of novel approaches for LUT control.

7.2.3.1 Improved supraspinal network modeling

Additional detail in the modeling of the ascending and descending projections to higher order centers would produce a more comprehensive model of lower urinary tract control. In the current model these signals are represented by a simple function that produces an excitatory output after the afferent signals representing bladder activity and volume surpass a threshold. A recent study proposed a model of the switching circuit in the brainstem that controls the descending continence and micturition signals (de Groat and Wickens 2013). The structure of such a network could be incorporated with our model to produce a complete picture of the entire neural network.

7.2.3.2 Modeling bladder dysfunction caused by disease or injury

Specific injury or disease state changes could be incorporated into the model. This would allow exploration of novel treatment paradigms and investigation into the mechanisms of action of disease on changes in bladder function. For example, afferent
fibers may be sensitized in an inflammatory state produced in conditions of overactive bladder (de Groat 1997; Steers 2002), and the relationship between bladder pressure and afferent firing could be modeled to reflect increased firing rates produced by hyperexcitatory bladder conditions measured in vivo (Zvara et al. 2010). This would allow testing of whether pudendal afferent stimulation can reduce SPN firing rate in conditions of overactive bladder. Further, including multiple neurons in each afferent pathway (for example including Aβ- and C-fibers or fibers that fire and low and high bladder distension thresholds (Sengupta and Gebhart 1994; Daly et al. 2007)), would improve our ability to explore which neurons are responsible for dysfunction in certain disease states and which should be targeted by electrical stimulation. Atrophy or neural degeneration could be modeled by decreasing the efferent drive of bladder (by modifying the relationship between efferent firing rate and bladder pressure) or decreasing the sensitivity of afferent fibers to determine if pudendal afferent stimulation can generate bladder contractions and treat other types of bladder dysfunction. In SCI, aberrant reflex pathways may develop from plastic reorganization in the spinal cord (de Groat et al. 1998), so inclusion of additional neurons that change their behavior when the descending supraspinal inputs are removed would allow further exploration of the neural mechanisms of bladder dysfunction in SCI.

7.2.3.3 Modeling the pudendo-pudendal reflex

Inclusion of the neural network that controls Onuf’s nucleus, the pudendal
motor nucleus and efferent control of the EUS, would represent a significant addition to our model, and allow us to understand how these networks work together to control the entire LUT. Although neurons in the pudendo-vesical reflex likely overlap with those in the pudendo-pudendal reflex, additional interneurons are likely involved (Shefchyk 2001).

Inclusion of Onuf’s nucleus would allow investigation of sphincter activation during stimulation or cases of DSD. This would be particularly interesting if it were also coupled with a physical model of the bladder and urethra (Bastiaanssen et al. 1996), because it would allow for quantitative analysis of the effects of stimulation or pathological neural activity on bladder and urethral pressure. A complete model of the neural and muscular control of the LUT would also allow exploration of stimulation methods that produce robust bladder contractions and sphincter relaxation to improve voiding efficiency.

Alternatively, understanding the control of the EUS could lead to the development of novel temporal patterns of stimulation that produce bladder inhibition and strong EUS contraction, which would improve continence by increasing bladder capacity and preventing leakage past the sphincter. For example, patterns similar to those in Chapter 4 with bursts and pauses that failed to produce bladder activation could be designed to produce inhibition of bladder contractions and contractions of the EUS, as some patterns of paired-pulse stimulation facilitate the pudendo-pudendal
reflex and sphincter contraction.

7.2.3.4 Design of novel stimulation patterns and approaches

Our work in Chapter 4 showed that the temporal pattern of stimulation significantly affected bladder activation, and the computational model (Chapter 6) reproduced the effects of stimulation pattern on bladder pressure. A genetic algorithm, or other search heuristic, could be used to reveal temporal patterns of stimulation that produce the best results based on a pre-defined cost function (Wongsarnpigoon and Grill 2010). For example, the algorithm could be programmed to identify stimulation patterns that maximize the size of bladder contractions or SPN firing rate. The new temporal patterns of stimulation could then be tested in animals to evaluate bladder activation and test the predictive power of the model. Current methods of pudendal afferent stimulation are based on arbitrary frequencies that were effective in previous animal studies. The use of our model and a genetic algorithm would represent a significant innovation in methods to develop new paradigms for bladder control based on our new understanding of neural mechanisms that mediate the pudendo-vesical reflex.

7.3 Conclusion

The findings in this dissertation demonstrated the effects of novel spatial and temporal patterns of electrical stimulation of the pudendal nerve and sought to explain the mechanisms behind bladder responses evoked by pudendal afferent stimulation. We
found that spatial patterns of stimulation of pudendal afferents improved bladder activation and voiding in the cat and significantly influenced the size of bladder contractions in humans with SCI. In addition to frequency, the temporal pattern of stimulation is a critical determinant of the effects of PNS.

This dissertation also significantly advanced our understanding of the spinal neural circuits which mediate the bladder response to PNS. Low frequency stimulation-evoked bladder inhibition was blocked by picrotoxin, revealing that this requires a lumbosacral spinal GABAergic mechanism, and that glycnergic, adrenergic, or opioidergic mechanisms were not necessary. Further, a computational model of the spinal neural network underlying the pudendo-vesical reflex was developed that matched experimental results from previous studies. The frequency and pattern-dependent effects of PNS were determined by changes in firing rate of spinal interneurons in the model, suggesting that neural network interactions at the lumbosacral level can mediate the bladder response to different frequencies or temporal patterns of pudendal afferent stimulation.
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Biography

Meredith Jones McGee was born on June 18, 1987 in Raleigh, NC. She attended North Carolina State University in Raleigh between 2005 and 2009. While at NC State, she was active in a number of service organizations, social clubs, and honorary societies. She was inducted as a member of Tau Beta Pi Engineering Honor Society, Phi Kappa Phi, and Gamma Beta Phi Honor Society, and participated in Engineering World Health, Society of Women Engineers, and Sigma Kappa Sorority. In 2008-2009 she served as president of the BME Club and secretary of Tau Beta Pi. Each summer from 2006 to 2008, she worked as an applications lab intern at a local biotechnology company, NCSRT, Inc. in Apex, NC. During her last two years at NC State she received an award for undergraduate research, working with Stephen B. Knisley at the University of Chapel Hill on diagnostic methods to guide ablation for atrial fibrillation. In May 2009, Meredith graduated summa cum laude with a BS in Biomedical Engineering and minor in French. Her graduate research with Dr. Warren Grill at Duke University focused on the effects of novel methods of pudendal nerve stimulation and the mechanisms of bladder control with stimulation. She received a Student Travel Award for Problems at the Interface for the 2013 IEEE Neural Engineering Conference and Diversity Travel Award for Neural Interfaces Conference 2012. She received the PhD in May 2015.
Publications


