ELECTRICAL STIMULATION OF AFFERENT NEURAL PATHWAYS FOR SUPPRESSION OF URETHRAL REFLEXES

by

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Electrical Stimulation of Afferent Neural Pathways for Suppression of Urethral Reflexes

Abstract

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Aberrant urethral reflexes following neurological injury or disease are a major component of voiding dysfunction, preventing micturition and leading to serious medical complications. Current neuroprostheses—such as the Brindley implant—require irreversible transection of the dorsal sacral roots (dorsal rhizotomy), permanently eliminating remaining sensation and desirable reflexes. These side effects greatly reduce the number of people choosing the approach. This project therefore addresses an unmet clinical need for rhizotomy-free suppression of aberrant urethral reflexes by employing electrical stimulation of afferent neural pathways to interrupt the unwanted responses. This afferent-based approach will improve bladder control neuroprostheses for human use.

In experiments with spinal-intact canines (Aim 1)—designed to demonstrate a fully implanted system—afferent stimulation was applied to the intradural dorsal roots. Although there was evidence of increased voiding, evoked bladder pressures were generally inadequate. Exhaustive troubleshooting suggested the need for an improved animal preparation.
A novel feline preparation with good neuroanatomical homology to the human was developed (Aim 2a). It exhibited aberrant post-spinalization urethral responses that were quantified with clinical urodynamic measures (Aim 2b). The results were consistent with these responses being reflexive and mediated by urethral stretch or flow receptors (Aim 2c). These acute reflexes were functionally similar to those occurring chronically in humans with spinal cord injury, making the experimental preparation a useful tool for developing future pharmacological or neuroprosthetic therapies.

We demonstrated reflex suppression with rhizotomy-free and surgically non-invasive afferent stimulation of sacral skin dermatomes, providing a novel proof-of-concept with significant clinical potential. Some cats exhibited reflex exacerbation. Subsequent investigation of the reflex modulation suggested parameters—such as electrode location and stimulation amplitude—that governed surface stimulation efficacy (Aim 2c). The most effective suppression occurred with sacral dermatomes stimulated at sufficiently high current amplitudes.

These results provide the key data for motivating future chronic animal and human studies, in which further optimization of the stimulation pattern will improve the suppression effect. The low cost of this innovative modality of surgically non-invasive and rhizotomy-free reflex suppression make it an attractive clinical alternative for treating voiding dysfunction that has distinct advantages over the present state of the art.
CHAPTER 1. INTRODUCTION, RATIONALE, AND SPECIFIC AIMS
Overview

Loss of bladder control is a debilitating impairment occurring in individuals with spinal cord injury (SCI), stroke, multiple sclerosis, Parkinson’s disease, and other dysfunctions of the central and peripheral nervous systems (Herbaud 1993; Fowler 1999). Numerous approaches have been developed for the restoration of bladder function, including drugs, mechanical devices, and implanted functional electrical stimulation (FES) systems. This chapter will summarize device-based treatments of incontinence and voiding dysfunction, including neuroprostheses designed for restoring voiding in people with SCI.

The long-term goal of this research is to develop an innovative neuroprosthetic approach for restoring bladder function (micturition and continence) in individuals with voiding dysfunction or incontinence by combining stimulation of afferent pathways with bladder drive. This modality would eliminate the irreversible surgical transection of sacral sensory nerve roots (dorsal rhizotomy) presently required for eliminating unwanted urethral reflexes with existing neuroprostheses. This rhizotomy-free approach would have wide application to voiding dysfunction of various etiologies. The immediate goal of the proposed research is to demonstrate feasibility of this method in animal preparations and to understand the underlying afferent pathways.

Clinical Impact of Urinary Incontinence and Voiding Dysfunction

The estimated prevalence of urinary incontinence varies by definition, but with a restriction to daily urinary incontinence, the prevalence in the United States is
approximately 17.8 million community-dwelling adults (Hu et al. 2004). This figure corresponds to an estimated 12% of women and 5% of men. The associated total costs are $19.5 billion in 2000 dollars, or over $24 billion in 2009 when adjusted for inflation.

Spinal cord injury has an incidence of 12,000 cases per year (40 cases per million population) and a prevalence of over 255,000 cases (NSCIS 2008) in the United States. Worldwide incidence ranges from 10.4 to 83 cases per million inhabitants per year, with the mean age of injury being 33 years and with men being 3.8 times more likely to be injured than women (Wyndaele and Wyndaele 2006). Similarly representative worldwide prevalence data are not available (Wyndaele and Wyndaele 2006). Lower urinary tract problems are a leading complication of SCI (Gaunt and Prochazka 2006). The total direct and indirect costs of SCI in the United States were calculated to be $5.6 billion in 1988 (Creasey and Dahlberg 2001), corresponding to over $10 billion in 2009 when adjusted for inflation. Specifically, the median individual costs for bladder care in people with SCI who received a Finetech/Brindley VOCARE implant were estimated to be $8,152 in 1998 dollars, equivalent to $10,638 in 2009 dollars. A more recent study within the Veterans Health Administration with a larger sample size found the total individual direct medical costs to be $21,450 in 2005 dollars (French et al. 2007), equivalent to $23,362 in 2009 dollars. In addition to this economic impact, there is also considerable psychological and social hardship due to the embarrassment associated with continued urine leakage especially while in public. As a result, people with SCI consider regaining bladder function to be a major recovery priority, with paraplegics ranking restoration of bladder or bowel function behind only that of sexual function as
being of highest priority for recovery (Anderson 2004). Even in quadriplegics, over 40% rated restoration of bladder or bowel function as the first or second most important functional restoration for improving quality of life—above both sexual function and the ability to walk (Anderson 2004).

However, many people without SCI—typically women—also suffer from urinary incontinence. Prevalence estimates again vary based on how incontinence is defined. A meta-review of the literature showed that up to 55% of women suffer from incontinence, with the condition divisible into various subtypes (Holroyd-Leduc et al. 2008) as will be described in the next section. In older women, the prevalence of urinary incontinence varies from 17% to 55%, while in younger women the prevalence ranges from 12% to 42%. A recent survey of women in the United States using a more stringent definition of urinary incontinence (moderate to severe leakage only) estimated the national prevalence to be 15.7% of women (Nygaard et al. 2008).

Various treatment options are available to individuals with incontinence and voiding dysfunction. Pharmacological options for treating bladder overactivity include alpha blockers, anticholinergic agents, botulinum-A toxin injections into the bladder wall, and capsaicin injections into the bladder lumen to reduce sensory feedback. Alpha blockers have had limited success while botulinum-A toxin shows more promise (Reynard et al. 2003). Anticholinergic drugs such as tolterodine (Detrol) or oxybutynin (Ditropan) can have undesirable side effects (Creasey et al. 2001). In addition, the effectiveness of drugs can decrease with time, leading to patient dissatisfaction.
Device-based treatment approaches are similarly varied. Mechanical aids include catheters and condoms for draining the bladder. Commercially available neuroprosthetic systems include the Finetech/Brindley VOCARE implant, the Medtronic InterStim implant, and the Urgent PC tibial nerve stimulator. Other neuroprosthetic approaches attempt to stimulate the peripheral nerves or bladder muscle itself. These methods are described in a recent review of device-based bladder control (Gaunt and Prochazka 2006). Their advantages and disadvantages will be further reviewed in the following sections, followed by discussions of the motivation for the present studies, the specific aims of the dissertation, and the envisioned clinical device.

Lower Urinary Tract Physiology and Pathophysiology

The lower urinary tract (LUT) consists of the bladder, urethra, periurethral muscles such as the external urethral sphincter (EUS), and accessory organs (Figure 1). In the normal individual, the LUT is responsible for two main functions: continence (urine storage until an appropriate time for voiding arises) and micturition (voiding of urine from the body). Continence reflexes are based in the spinal cord, whereas micturition is mediated by reflex arcs involving the brain (Fowler et al. 2008). The highly distensible bladder is able to store approximately 500 mL of urine, with fluid leakage prevented by bladder inhibition and automatic tonic contraction of the EUS (Park et al. 1997). The latter state, known as the guarding reflex, is maintained by reflex arcs involving the pelvic and pudendal nerves (Craggs et al. 2006). Voiding involves a series of reflexes, including relaxation of the EUS and bladder neck and contraction of the
bladder itself, sustained by a facilitative urethro-vesical reflex promoted by urine in the urethra (Craggs et al. 2006). Our understanding of the neural control of continence and micturition continues to evolve, with many discrepancies persisting in the literature (Kinder et al. 1995). Nevertheless, a simplified working model was developed by the International Continence Society and appears in Figure 2 (Morrison et al. 2005).

Micturition involves the entire neuraxis, including contributions from the central, autonomic, and somatic nervous systems. The bladder can contract autonomously without central nervous system input (Morrison et al. 2005; Finney et al. 2008), presumably due to tension mechanoreceptors in the bladder wall (Herbaut 1993). Involvement of the sacral spinal cord contributes segmental reflexes that can drive automatic bladder contractions (Barrington 1914; Barrington 1928; Barrington 1931) via C-fiber afferents (Fowler 1999; Fowler et al. 2008) and tension receptors in the bladder and urethra (Herbaut 1993). The final upper level of cortical and brainstem control is required for functional micturition and continence. In the intact system, voiding is initiated via the pontine micturition center (also known as Barrington’s nucleus) in the brainstem with involvement of other neuron populations including the periaqueductal gray, the medial prefrontal cortex (Fowler et al. 2008), and other portions of the frontal lobe (Fowler 1999). The prefrontal cortex is also implicated in a variety of social behaviors and executive processes (Dalley et al. 2004; Krajbich et al. 2009; Mariano et al. 2009a), emphasizing the importance of cognitive inputs to voiding control.

Micturition is maintained by continued detrusor contraction with reciprocal EUS inhibition to empty the bladder. During urethral flow, afferent fibers in the pelvic and
Pudendal nerves carry sensory information to the sacral spinal segments. The projection of the primary afferents onto the spinal interneurons constitutes the first step in sensorimotor transformations performed by spinal reflex systems (Levinsson et al. 2002). A population of segmental interneurons in the sacral spinal cord are involved in the control of sacral efferent outflow (de Groat et al. 2001; Shefchyk 2005). Sacral interneurons mediating inhibitory control of the sphincter motor neurons during micturition have been defined in recent years (Shefchyk 2005). A population of glycinergic inhibitory neurons may contribute to the decrease in urethral sphincter motor neuron activity during micturition (Shefchyk et al. 1998; Shefchyk 2001). Stimulation within a region of GABAergic interneurons dorsal to the central canal in the cat (Blok et al. 1997) resulted in a decrease in urethral pressure. The authors suggested that this was mediated by direct inhibition of urethral sphincter motor neurons by interneurons activated within the area (Blok et al. 1997).

Incontinence occurs when these storage and voiding capabilities are disrupted due to bladder overactivity, weakness of the periurethral muscles, or derangements of the various spinal reflex arcs involved in micturition. Following SCI at thoracic or higher levels, profound changes in bladder function occur. Reflex arcs are interrupted due largely to loss of descending input from structures such as the lateral and medial pontine nuclei, thus leading to uninhibited sympathetic reflexes (Craggs et al. 2006) that prevent reciprocal EUS inhibition; the result is aberrant and undesirable urethral reflexes that impede voiding. There is evidence that segmental interneurons mediating the excitability changes in perineal and urethral afferents during micturition are housed...
in the sacral segments and are part of the spinal micturition-generating circuitry (Shefchyk 2001). It has been shown by intracellular motor neuron recordings that pudendal and perineal afferent-evoked excitatory postsynaptic potentials were attenuated in urethral sphincter motor neurons that hyperpolarized during voiding (Fedirchuk et al. 1994).

These disruptions can cause detrusor-sphincter dyssynergia (DSD), in which the bladder and EUS contract simultaneously, preventing voiding (Figure 3), as well as detrusor hyperreflexia, in which the bladder contracts at very small volumes. DSD may result in part from detrusor hyperreflexia (Craggs et al. 2006) and can be divided into three clinical types based on electromyographic findings during urodynamic evaluation (Blaivas et al. 1981). In DSD, the inability to void urine due to the aberrant urethral reflexes can have serious clinical consequences. These sequelae include urinary tract infection, which in healthy men has been linked to large post-void residual volumes (Truzzi et al. 2008), stone formation, high bladder pressures leading to renal damage (Shingleton and Bodner 1993), and potentially life-threatening autonomic dysreflexia (Creasey et al. 2001), particularly in patients with lesions above T6 (Giannantoni et al. 1998).

Female urinary incontinence can be subdivided into several basic types for workup and diagnosis in the clinic (Holroyd-Leduc et al. 2008). Stress incontinence is urine leakage upon exertion such as sneezing or coughing. Urge incontinence is leakage due to overactive bladder that results in a sudden urge to urinate. Other incontinence types in women include overflow incontinence (often related to an obstruction) and
functional incontinence (related to cognitive or mobility difficulties). Mixed incontinence includes features of both stress and urge incontinence. Stress, urge, and mixed incontinence are the most common types diagnosed in women; stress incontinence is more prevalent in younger women and urge and mixed incontinence are more common in older women (Holroyd-Leduc et al. 2008).

Because it does not typically result from damage to or disease of the central or peripheral nervous systems, female urinary incontinence is non-neurogenic; coordination between the bladder and the EUS is preserved. In contrast, injury or disease that disrupts coordination between the bladder and the EUS leads to the aberrant urethral reflexes of DSD with their potentially deleterious sequelae.

**Mechanical Devices for Treating Voiding Dysfunction**

Use of catheters is one of the most common treatments of urinary retention. In World War II, catheterization to empty the bladder significantly reduced mortality from neurogenic bladder complications following SCI (Gaunt and Prochazka 2006). Today, clean intermittent catheterization is commonly prescribed. However, urinary tract infections remain a major problem, affecting up to 70% of patients using this method (Gaunt and Prochazka 2006). Furthermore, good hand function is required in order to insert and withdraw the catheter, which is not possible with tetraplegics or other SCI patients with limited mobility.

Urethral stents, originally developed for treatment of urethral strictures, are another common means of alleviating bladder incontinence in patients with DSD (Gaunt
and Prochazka 2006). Before their use, sphincterotomy, or the surgical cutting of the EUS, was a common—but irreversible—procedure for alleviating DSD. Although effective, many recipients of this procedure demonstrated high leak point pressures—increasing the risk of renal damage—as well as other complications including erectile dysfunction and bladder neck stenosis or stricture (Reynard et al. 2003). The urethral stent was used as an alternative; it operates by dilating the urethra mechanically to allow passage of urine. However, continence is eliminated, requiring the patient to wear a condom and bag for collection of passed urine. Possible complications include stent encrustation and migration (Reynard et al. 2003). Nonetheless, the devices have proven to be a clinically successful treatment for DSD (Chancellor et al. 1999).

Intraurethral pumps have also been developed for management of non-neurogenic chronic urinary retention in women. The pump is inserted into the urethra, held in place by fins that deploy within the bladder. A turbine impeller is magnetically coupled to an external unit for pumping action. The device is not designed for chronic use, due to reported difficulties with discomfort, infection, and possible urethral damage (Gaunt and Prochazka 2006).

Another treatment modality no longer commonly used is the “artificial sphincter,” essentially an inflatable cuff implanted around the bladder neck. The cuff is inflated or deflated by an automatically controlled pump. The system was typically used in male patients with post-prostatectomy incontinence. The device does have clinical efficacy for SCI patients, but infection is a major concern (Gaunt and Prochazka 2006).
FES Devices for Treating Voiding Dysfunction

Devices that apply electrical stimulation for restoration of bladder function have many potential input sites (Figure 4). Several of these devices are reviewed in this section.

Intraspinal Stimulation

Intraspinal stimulation, explored primarily in the 1960s and 1970s, was able to achieve efficacy in both male and female patients, although the former often had spastic EUS activity that impaired voiding (Gaunt and Prochazka 2006). However, this approach has been abandoned, due largely to the 40% failure rate of the highly invasive surgery necessary for insertion of penetrating electrodes into the spinal cord (Gaunt and Prochazka 2006). Even if the success rate of the surgery were greater, concerns about spinal cord damage, CSF leakage due to piercing the dura mater, and electrode degradation chronically would remain as significant impediments to the clinical feasibility of this approach.

Sacral Root Stimulation

The nerves in the second, third, and fourth sacral spinal roots serving both bladder and urethral sphincter can be electrically stimulated at different points along their path from the spinal canal to the bladder and EUS. Electrical stimulation for bladder control can provide improved health through prevention of complications, and greater personal independence (Appell 1998; van Kerrebroeck 1998). This can lead to
an overall reduction in the need for nursing care, support staff and physicians (Creasey et al. 2000). In attempts to achieve micturition, electrodes have been placed in the spinal cord (Nashold et al. 1973; McCreery et al. 2004), on pelvic nerves (Habib 1967), in the bladder wall (Kantrowitz and Schamaun 1963), and on sacral roots.

Sacral nerve stimulation for electrical control of bladder function was initiated by Habib in 1963 (Habib 1967). The sacral nerve roots, which may be approached dorsally by a laminectomy of selected lumbar vertebrae, are a viable site for electrode placement for micturition control. Brindley developed a technique for intradural sacral anterior root stimulation (Brindley 1977). Tallala and associates used an extradural approach to the sacral roots (Tallala et al. 1986). Tanagho et al. used sacral stimulation combined with dorsal rhizotomy and neurotomy of the pudendal supply for micturition control in paraplegics (Tanagho et al. 1989).

The first commercially-available FES device for bladder emptying in SCI patients (i.e. neurogenic bladder) was the Finetech/Brindley VOCARE device (Gaunt and Prochazka 2006). Recipients must have complete SCI at thoracic levels or above and problems with other forms of bladder management (Ragnarsson 2008). The VOCARE system (Figure 5) employs implanted electrodes and stimulator inductively coupled to an external controller unit that also provides power. The implanted electrodes stimulate the anterior (ventral) sacral roots (see also Figures 1 and 7) and can be placed either intradurally or extradurally (Gaunt and Prochazka 2006), typically on the S2 through S4 nerve roots bilaterally (Creasey et al. 2001). The extradural system is approved by the United States Food and Drug Administration (FDA) for restoring
micturition (Creasey and Dahlberg 2001). A sleeve loosely sutured around each nerve root holds the electrodes in place (Creasey et al. 2001). When coupled with surgical transection of the dorsal sensory roots—a dorsal rhizotomy—the device is quite effective. Dorsal rhizotomy interrupts the sensory (afferent) signals from the bladder that cause hyperreflexia and DSD, also eliminating the autonomic dysreflexia often triggered by large increases in bladder pressure. A study demonstrated restoration of functional micturition in 18 of 21 patients, with functional micturition defined as the ability to urinate greater than 200 mL with the aid of the implanted neuroprosthesis (Creasey et al. 2001). In addition, the costs of bladder (and bowel) management were reduced by over 80% (Creasey and Dahlberg 2001).

In addition to the invasiveness of the required surgical procedures, a major drawback of this system is the need for dorsal rhizotomy, as it eliminates reflex erection and ejaculation in men (Creasey et al. 2001) along with reflex defecation and any remaining perineal sensation (Gaunt and Prochazka 2006). The necessity for dorsal rhizotomy to achieve voiding makes the current approach to neural prosthetic restoration of bladder function undesirable for many individuals. Furthermore, the voiding pattern produced by the VOCARE device is non-physiologic as stimulation also directly activates the EUS (Brindley et al. 1982). Success of the device relies on the different time constants of relaxation for the smooth muscle of the bladder versus the skeletal muscle of the EUS. Intermittent stimulation causes contractions of both the bladder and the EUS, but the much faster relaxation of the EUS between stimulus pulses allows passage of urine in high-pressure spurts (Brindley 1977; Brindley et al. 1982).
Another device requiring less invasive surgery for implantation is the Medtronic InterStim device, intended for use with non-neurogenic bladder problems including urge incontinence and urinary retention (Gaunt and Prochazka 2006). The basic neuromodulatory principle on which the device functions is that low-frequency, low amplitude stimulation of the sacral roots can maintain EUS contraction without eliciting detrusor contraction (Gaunt and Prochazka 2006). The InterStim device is battery powered and fully implanted, so there is no need for an external controller except for initial programming of the unit for optimizing stimulation parameters for the patient. A single quadripolar electrode is inserted in the S3 foramen to lie alongside the S3 extradural spinal root (Figure 6); no laminectomy or other bone work is required.

However, the InterStim device can only be used in patients with non-neurogenic bladder conditions; trials in patients with neurogenic bladder problems from SCI have shown less efficacy (Gaunt and Prochazka 2006). Screening of patients for potential InterStim implants requires temporary placement of a percutaneous foramen electrode connected to an external stimulator. Although in sum less surgically invasive than the VOCARE implant, the need for both a screening procedure and surgical access to the sacral foramina of the vertebrae has inherent risks that might discourage potential implant recipients.

**Bladder Wall Stimulation**

Direct electrical stimulation of the bladder wall was studied in the 1960s and 1970s, but is no longer actively pursued. Although successful in dog studies, human
trials in patients with SCI showed far less efficacy. The primary difficulty was due to spreading of the stimulus current to other structures such as the EUS and pelvic floor musculature, causing unwanted contractions that prevented voiding (Gaunt and Prochazka 2006).

Peripheral Nerve Stimulation

The desire for a surgically non-invasive means of restoring bladder control has led to the development of another commercially-available bladder neuroprosthesis system, the Urgent PC tibial nerve stimulator—formerly known as the Urosurge SANS device—that received FDA approval in 2000 (Gaunt and Prochazka 2006). This system has no implanted components. Surface or percutaneous stimulation of the posterior tibial nerve at the medial aspect of the ankle (see Figure 4) can inhibit the detrusor, restoring continence. The tibial nerve receives innervation from the L4-S3 spinal roots (Netter et al. 2003); the significant sacral innervation is likely a major reason why this approach is successful. Although the technique of tibial nerve stimulation has been shown to be effective, the Urgent PC device itself has little clinical data to support its efficacy (Gaunt and Prochazka 2006).

Another means of surgically non-invasive bladder control is stimulation of the dorsal penile or clitoral nerve, as this can inhibit detrusor activity (Gaunt and Prochazka 2006). Both mechanical and surface electrical stimulation of this superficial branch of the pudendal nerve have been explored. Studies have demonstrated surface electrical stimulation of the dorsal penile nerve to be successful in reducing bladder hyperreflexia
in patients with SCI (Gaunt and Prochazka 2006). This method of stimulation remains an area of active research.

**Current Approaches**

Ongoing research involves the use of high frequency alternating current (HFAC) to block unwanted propagation of action potentials. In cats, extradural sacral root stimulation has been combined with HFAC block of the pudendal nerve, eliminating dyssynergic urethral contractions without the need of a dorsal rhizotomy (Boger et al. 2008). In humans, pharmacological block of the pudendal nerves bilaterally has been shown to eliminate DSD in similar fashion (Schurch et al. 1994). Therefore, application of HFAC block to the pudendal nerve of the human may produce a similar effect. Combined with sacral root drive, this approach could produce coordinated rhizotomy-free voiding without EUS co-activation.

Low frequency continuous stimulation of the sensory sacral roots has been shown to be effective in combating bladder hyperreflexia in humans. Experiments by a number of investigators have shown that surface electrical stimulation of the sensory genital branch of the pudendal nerve can abolish hyperreflexive bladder contractions in persons with spinal injury. Craggs and colleagues have implanted electrodes on the extradural sacral nerve roots (mixed motor and sensory) in three persons with spinal injury without conducting a sacral dorsal rhizotomy (Craggs and McFarlane 1999). Continuous, low frequency stimulation at an amplitude sufficient to activate large diameter sensory fibers (such as genital afferent branches of the pudendal nerve)
suppressed bladder hyperreflexia and increased bladder volumes before hyperreflexive contractions occurred in all three subjects post-operatively.

**Motivating the Specific Aims**

The major neuroprosthetic approaches discussed thus far rely on *either* efferent stimulation of the bladder (VOCARE or InterStim) *or* afferent stimulation of peripheral nerves (tibial or dorsal genital nerves). The presently implemented technique of electrical stimulation of sacral motor nerves generates effective voiding only after irreversible dorsal rhizotomy. Without rhizotomy, dyssynergic urethral reflexes prevent voiding in both humans (Kirkham et al. 2002) and animals (Bhadra et al. 2002; Bhadra et al. 2006b). Although rhizotomy eliminates these reflexes, the procedure has poor patient acceptance because it also eliminates preserved sensation and desirable reflexes such as those for sexual and bowel function (Creasey et al. 2001; Gaunt and Prochazka 2006). Therefore, a rhizotomy-free means of suppressing aberrant urethral reflexes would address a major unmet clinical need.

Bhadra and associates used current-controlled quasitrapezoidal waveforms (Grunewald et al. 1998; Bhadra et al. 2001; Bhadra et al. 2002; Bhadra et al. 2006b) to activate the small diameter (bladder) motor neurons of the sacral ventral roots without co-activating the large myelinated neurons supplying the skeletal muscle of the EUS. The quasitrapezoidal pulse’s exponentially decaying trailing phase follows the time constant of the sodium channel’s $h$ gate to prevent anodic break—thus arresting action potential initiation in large myelinated fibers (Grunewald et al. 1998; Bhadra et al. 2001; Bhadra et al. 2002; Bhadra et al. 2006b).
However, even with this selective stimulation, voiding was poor in canine experiments.

Nevertheless, under certain conditions before rhizotomy, phasic reflex EUS activity and bladder voiding occurred, suggesting that neural signals from the EUS provided afferent “feedback” to central spinal circuits to produce voiding. Such a pattern has been reported in species that use small urine spurts to mark territory (Streng et al. 2004) and in chronic spinalized animals during sacral stimulation (Walter et al. 1989). These observations led to the notion of applying quasitrapezoidal stimulation to both ventral (motor) and dorsal (sensory, afferent) roots of the canine, using the afferent stimulation to interrupt aberrant EUS reflexes and thus allow rhizotomy-free voiding (Bhadra et al. 2006c). These findings are the empirically-driven foundation of the present research.

This project will use electrical stimulation of afferent pathways with trains of rectangular pulses to obtain rhizotomy-free suppression of aberrant urethral reflexes. If successful, this novel approach could be rapidly translated to clinical use, providing an innovative method of bladder evacuation to subjects with voiding dysfunction. Additionally, this stimulation modality could be implemented with modifications of existing stimulator technology.

Due to the novelty of the proposed work, there is no extant a priori experimental framework. Therefore, a necessary part of the project will be developing appropriate animal preparations that exhibit aberrant urethral reflexes. The afferent pathways mediating these reflexes will then need to be explored at both neurophysiological and
functional levels. The specific aims have thus been designed with this wide-ranging approach in mind—necessarily reduced so that the project is tractable. Expected scientific contributions are summarized below.

- A rhizotomy-free neuroprosthetic approach to suppressing aberrant urethral reflexes will be demonstrated in the canine.
- A novel acute spinal feline preparation exhibiting aberrant urethral reflexes will be developed as a necessary experimental tool.
- Rhizotomy-free and surgically non-invasive afferent stimulation as a means of suppressing these reflexes will be achieved in the feline.
- The neurophysiology of these reflexes and their suppression will be characterized in the feline.

Together, these results will provide a segue to human clinical studies and an envisioned neuroprosthetic device.

**Specific Aims**

Sensory inputs from the lower urinary tract modulate spinal circuits that coordinate the bladder and sphincter during voiding and continence. It has been previously shown that electrical stimulation of sensory nerves can both evoke bladder contractions without EUS activation and produce voiding. This project proposes a novel and innovative approach using afferent stimulation that exploits existing spinal reflex circuitry to reduce pathological urethral reflexes impeding voiding (Figure 3). Generally, bladder contractions will be directly evoked by stimulation of the sacral ventral spinal
roots with implanted nerve-cuff electrodes placed intradurally or extradurally (Figures 1 and 4). In concert with this bladder drive, rhizotomy-free afferent stimulation will be used to reduce or eliminate aberrant urethral reflexes. Afferent drive will be applied via electrodes implanted on the sacral dorsal roots (Figure 7) or via surgically non-invasive electrical stimulation of the sacral skin dermatomes. Putative input and output parameters to the neural control of the LUT (Figure 2) will be identified. The results of this project will provide the needed foundation for developing improved, rhizotomy-free human-implantable neural prostheses capable of vastly enhancing the quality of life of individuals with voiding dysfunction.

1. Production of Voiding by Combined Sensory and Motor Root Activation in the Canine

This aim will attempt to provide an in-vivo demonstration of a fully implanted system utilizing activation of both sensory (dorsal) and motor roots to increase voiding without dorsal rhizotomy in canines. Bladder and urethral pressures will be recorded, as will be bladder voiding during combined sensory-motor root drive. Based on preliminary results (Bhadra et al. 2006c), it is anticipated that afferent stimulation of the dorsal roots will increase voiding compared to ventral drive alone. These results should demonstrate the effectiveness and clinical potential of this neuroprosthetic configuration while refining stimulus parameters for optimal results.

2. Suppression of Urethral Reflex Activity by Surface Stimulation of Sacral Dermatomes
   a. Pudendal Nerve Neuroanatomy of the Feline
Stimulation of afferent pathways in a feline animal model requires a complete and accurate understanding of underlying neuroanatomy. Discrepancies persist in the literature regarding how the pudendal nerve branches to give rise to the dorsal nerve of the penis (DNP). This aim will use gross dissection and histology to clarify this path. Discovering a better than previously appreciated neuroanatomical homology between the cat and human would further justify the suitability of the feline preparation for use in genitourinary studies.

b. **Demonstration of a Surgically Non-Invasive Proxy for Dorsal Root Stimulation in the Feline**

This aim will develop an acute spinal feline preparation exhibiting aberrant urethral reflexes, quantify the reflexes, and attempt to suppress the reflexes with surface stimulation of the sacral skin dermatomes. This non-surgical proxy for dorsal root stimulation would have significant applications to humans with voiding dysfunction. Bladder and urethral pressures will be recorded during sacral dermatome stimulation and bladder activation with extradural electrodes. It is expected that stimulation of sacral skin dermatomes will reduce aberrant urethral reflex contractions that occur during and after bladder activation.

c. **Characterizing Aberrant Urethral Reflexes and Their Modulation by Surface Stimulation in the Feline**
This aim will further validate Aim 2b’s novel experimental preparation and reflex suppression modality. The goal will be to characterize the neurophysiological mechanisms underlying the urethral reflexes and the modulatory effects of surface stimulation. Putative key parameters for reflex modulation—such as anatomical location of the surface electrode and stimulation amplitude—will also be identified. A better understanding of neurophysiological and functional control of LUT reflexes will be critical to developing an eventual clinical device.

The envisioned micturition-restoring clinical device ultimately resulting from this work could take several forms (Figure 8), all of which are revisited at the conclusion of Chapter 6. In every conception, afferent stimulation is used for reflex suppression, obviating the need for irreversible dorsal rhizotomy as occurs with VOCARE (yellow area of Figure 8). The afferent stimulation could be applied to the dorsal sacral roots, requiring a laminectomy and intradural access (Aim 1, red area of Figure 8), or less invasively by stimulating peripheral afferent nerves of the skin (Aims 2b and 2c, green area of Figure 8). Surface stimulation with self-adhesive or garment-style electrodes (Patterson and Lockwood 1993; Wiley InterScience (Online service) 2006) would not require surgery, but this method would necessitate removal and reapplication of electrodes on a regular basis. Alternatively, cutaneous wire electrodes could be used to stimulate the skin afferents, with leads tunneled underneath the skin to a superficially implanted stimulator (final column of Figure 8). The surgical invasiveness of these
afferent stimulation modalities is distinct from the loss of function incurred by dorsal rhizotomy—itself an invasive procedure.

Bladder contractions could be produced by a variety of means, also varying in degree of surgical invasiveness. Efferent stimulation of the ventral sacral roots—via either intradural or extradural electrodes—is the most invasive, and presently occurs with VOCARE (Brindley) implants. In particular, this modality requires a laminectomy, a surgical procedure that can have severe medical complications (Possover 2009).

Alternatively, reflex bladder contractions could be elicited via stimulation of peripheral nerves (Boggs et al. 2005; Bruns et al. 2008) such as the pudendal or deep perineal nerves, thus offering a less invasive surgical option. Finally, a manual method of bladder emptying such as a Credé maneuver could be used.

No one configuration of a final implanted system will be universally applicable to all patients with voiding dysfunction. Instead, this project will provide several approaches to expand the armamentarium of neuroprosthetic options available to researchers and clinicians. These modalities will furnish alternatives for restoration of bladder function that have distinct advantages over the present state of the art.

Acknowledgements

The author wishes to thank Narendra Bhadra, M.D., Ph.D. and Kenneth Gustafson, Ph.D. for their editorial contributions to this chapter.
Figure 1. Schematic representation of the main structures of the lower urinary tract and their innervation from the spinal cord.
**Figure 2.** Simplified model of the neural control of (A) urine storage and (B) voiding reflexes, as developed by the International Continence Society (Morrison et al. 2005).
Figure 3. Normal bladder-EUS coordination (left panel) is lost following spinal cord injury, leading to DSD and associated sequelae (right panel).
Figure 4. Reproduced illustration of potential implant sites for neuroprostheses (Gaunt and Prochazka 2006). The VOCARE device stimulates at (H), the InterStim device stimulates at (K), and the tibial nerve stimulator operates at (F). Other modalities include peripheral nerve stimulation at (E) or (N), intraspinal stimulation at (L), or bladder muscle stimulation at (B).
Figure 5. Reproduced illustration of the VOCARE system (Creasey et al. 2001).
**Figure 6.** Reproduced illustration of foramen needle electrode placement for the Medtronic InterStim device (Gaunt and Prochazka 2006).
Figure 7. Schematic of the presently implemented system (VOCARE) with rhizotomy on the left and the rhizotomy-free sensory-motor stimulation scheme on the right. Based on an illustration by N. Bhadra.
<table>
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<td>Not applicable</td>
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**Figure 8.** Envisioned device matrix, including the current state of the art requiring irreversible dorsal rhizotomy (yellow) and proposed afferent stimulation approaches. The afferent-based approaches examined in this project are shaded red and green.
CHAPTER 2. PRODUCTION OF VOIDING BY COMBINED SENSORY AND MOTOR ROOT ACTIVATION IN THE CANINE
Preface

The work comprising this chapter addresses Specific Aim 1.

Abstract

Aberrant contractions of the EUS are a major component of voiding dysfunction. Current neuroprosthetic approaches require irreversible sectioning of the dorsal sacral roots. The goal of the present study was to suppress such reflexes in the canine using afferent electrical stimulation of intact dorsal roots, thus improving voiding.

Eleven adult female dogs were implanted with spiral electrodes on the ventral and dorsal sacral roots under general anaesthesia; these electrodes provided bladder activation and afferent stimulation, respectively. Stimulation was with intermittent pulse trains composed of biphasic rectangular pulses. Urodynamic parameters were recorded, including bladder pressure, urethral pressure, and volume of fluid voided.

Sacral root maps were constructed for all 11 dogs. Additional CMG and voiding trials demonstrated small evoked bladder pressures. These low pressures were insufficient to produce significant voiding despite exhaustive troubleshooting and extensive experimental improvements. Nonetheless, some results were obtained to suggest an effect of afferent stimulation on voiding.

The small evoked bladder pressures and meager voiding may have resulted from subtle nerve damage that occurred during these acute experiments. Chronic animals are proposed as a more suitable experimental preparation for future studies. Their use would eliminate a potential confound and address more completely the original goal of
demonstrating a rhizotomy-free neuroprosthetic approach for improving voiding utilizing combined sensory-motor stimulation.

**Introduction**

In healthy adults, evacuation of the urinary bladder can be initiated and terminated voluntarily. In infants and young children (Fowler et al. 2008)—and in many animals—the bladder empties involuntarily via reflex activity. In some neurological disorders, coordination between the somatic and autonomic nervous systems required for bladder emptying is lost. Such loss of coordination can produce pathological reflexes that impair voiding and continence. Current treatment approaches for voiding dysfunction have marked disadvantages. The Brindley procedure’s sacral ventral (motor) root stimulation requires invasive surgery and irreversible dorsal rhizotomy, leading to undesirable side effects (Creasey et al. 2001). Without rhizotomy, however, aberrant urethral reflexes prevent voiding in both humans (Kirkham et al. 2002) and animals (Bhadra et al. 2002; Bhadra et al. 2006b).

Sensory inputs from the lower urinary tract modulate spinal circuits that coordinate the bladder and external urethral sphincter (EUS) during voiding and continence. In prior work by Narendra Bhadra, Volker Grünewald, and J. Thomas Mortimer that used quasitrapezoidal waveforms (Grunewald et al. 1998; Bhadra et al. 2001; Bhadra et al. 2002; Bhadra et al. 2006b) for selective activation of small diameter (bladder) motor neurons of the sacral ventral roots without activation of the EUS, voiding responses were poor (Bhadra et al. 2002) due to EUS reflexes. However,
bladder voiding sometimes occurred during stimulation before rhizotomy. In the
 corresponding experimental trials, there was a phasic reflex activity pattern originating
 from the EUS during voiding. This result suggested that neural signals from the EUS
 provided “feedback” to central spinal circuits via afferent nerves from the lower urinary
 tract, thus allowing voiding. Such a pattern has been reported in species that use small
 urine spurts to mark territory (Streng et al. 2004). Similar patterns have also been
 observed during sacral stimulation in chronic spinalized animals (Walter et al. 1989).
 These observations led to the notion of applying quasitrapezoidal stimulation to both
 ventral (motor) and dorsal (sensory) roots to interrupt aberrant EUS reflexes and allow
 rhizotomy-free voiding in the canine (Bhadra et al. 2006c).

 The main disadvantage of the above approach is the need for specialized
 electronics to produce the quasitrapezoidal waveform. Therefore, the goal of the
 present study was to demonstrate a putative neuroprosthetic system whereby biphasic
 rectangular pulses delivered to the ventral roots activate the bladder. Without the fiber
 selectivity of the quasitrapezoidal pulse, EUS co-activation would be expected in
 addition to any aberrant urethral contractions. Afferent stimulation in the form of
 additional biphasic rectangular pulses delivered to the dorsal roots was used in an
 attempt to suppress the undesired urethral contractions. Successful voiding with the
 simpler rectangular waveform would demonstrate a new neuroprosthetic approach for
 rhizotomy-free voiding that could exploit existing stimulator technology.
Materials and Methods

Eleven sexually and neurologically intact adult female dogs 14.0-30.0 kg were used. The canine model, widely used for voiding studies, provides the required spinal size for placement of intradural sacral root electrodes. A power analysis of urodynamic data from previously published results and early experiments was used to determine the number of animals to include in the study. Testing for means of total voided volumes (mL) different from each other with $\alpha = 0.01$, an error standard deviation of 2 mL, and a “difference to detect” of 10 mL, a sample size of eight successful animals would result in a minimum power of 0.990. With a “difference to detect” of 12 mL, eight animals would give a minimum power of 0.999. All procedures were approved by the Case Western Reserve University (CWRU) institutional animal care and use committee. After initial experiments ($n = 7$), a specific check to rule out active estrus was instituted for the final experiments ($n = 4$) by the CWRU Animal Resource Center. This effort attempted to minimize the possible confound of derangements in urodynamic responses due to the hormonal changes of the canine estrous cycle (Hamaide et al. 2005).

The surgical preparation was derived from the work of Bhadra et al. (Grunewald et al. 1998; Bhadra et al. 2002; Bhadra et al. 2006b). Briefly, animals were anaesthetized with sodium pentobarbital (30 mg/kg). Isoflurane was added as needed (dosed to effect) for surgical procedures. Blood pressure was monitored via an implanted femoral artery line in the first two dogs and a carotid line in the remaining nine dogs; the change was due to a hematoma resulting from the femoral line in the second dog. A 6 F dual lumen (DLC-6D, Life Tech, TX) or 7 F Foley (Rochester Medical,
MN) catheter was implanted in the bladder suprapubically and connected to an external transducer (Deltran I, Utah Medical Devices, UT) to monitor bladder pressure (P\text{ves}) in the initial experiments \((n = 7)\). Urethral pressure \((P\text{ur})\) was measured in these animals with an 8 F urethral perfusion catheter placed at the location of the EUS as determined by a urethral pressure profile. For the final experiments \((n = 4)\), it was decided to reduce the surgical burden for the animals by instead utilizing a triple-lumen urethral catheter (TLC-9M, Life Tech) for filling the bladder and recording both \(P\text{ves}\) and \(P\text{ur}\). Recorded responses were consistent regardless of catheter type. Neither of the catheter types used for recording \(P\text{ur}\) fully obstructed the urethra, allowing measurement of any voided fluid with an in-house volume meter based on a commercially available load cell (LCC-ESP-4 0.6 kg, Load Cell Central, PA).

With the assistance of a surgical microscope (Storz Urban US-1, Urban Engineering, Inc., CA), the sacral roots were exposed through an incision in the dura after a laminectomy. The intradural sacral anterior roots were tested with a bipolar or tripolar hook electrode connected to a stimulator (Pulsar 6bp or 6bp-as, FHC, ME; DS8000, World Precision Instruments, FL) delivering biphasic current-controlled pulses 0.75-2.0 mA \((n = 10)\) or, in the final dog, biphasic voltage-controlled pulses at 5.0 V peak-to-peak \((V\text{p-p})\). Ventral roots eliciting the greatest—though not necessarily at full tetanic recruitment—electrically evoked bladder contractions \((S1-S3,\ as\ verified\ by\ post-mortem\ dissection)\) were implanted bilaterally (Figure 1) with custom spiral tripolar electrodes, usually with two ipsilateral roots enclosed in each spiral to maximize bladder activation. The electrodes were developed by Bhadra and associates (Bhadra et al.)
Corresponding dorsal roots (typically at S2 or S3) also received electrodes for unilateral application of afferent feedback (Figure 1), generally with only one root per electrode. In one animal, after trials with combined sensory-motor stimulation, all dorsal roots at levels below those implanted with electrodes were surgically transected; a handful of trials with only ventral root stimulus then occurred.

After situating the animal so that the pelvis and abdomen were suspended above the table, a cystometrogram (CMG) was performed with saline delivered by a syringe infusion pump (Genie YA-12, Kent Scientific, CT) at 2-10 mL/min to determine the leak volume. Brindley-style electrical stimulation in the form of rectangular current-controlled (20 Hz intermittent, primarily 2 s on / 2 s off, primarily 100 μs pulse width, biphasic, cathodic-first, 800 μA – 4.0 mA, n = 9 dogs) or voltage-controlled (20 Hz intermittent, primarily 2 s on / 2 s off, 100 and 200 μs, biphasic, negative first, 2-10 V_p-p, n = 3 dogs) pulses was applied to the ventral root electrodes to evoke bladder contractions. The ninth dog received both current- and voltage-controlled stimulation. For voiding trials, ventral drive was time-limited to 10 or 20 seconds. As a result, 100% voiding was not expected, as the duration of stimulation was insufficient to allow voiding to completion. Afferent stimulation consisted of trains of rectangular current-controlled pulses (20 Hz intermittent, primarily 1 s on / 1 s off, 100 and 200 μs, biphasic, cathodic-first, 50 μA – 3.0 mA) applied to dorsal root electrodes. Resulting P_{ves} and P_{ur} values were recorded, along with any voided volumes (V_{void}). Dorsal drive was both phase-locked to (n = 6 dogs) and phase-independent of (n = 5 dogs) the bladder drive.
The data acquisition hardware was as described for previous feline studies (Boger et al. 2008) and consisted of a computer-based system (NI DAQ 6024E, National Instruments Corporation, TX) with customized software interface (LabVIEW 7.1, National Instruments Corporation). Data processing was performed with MATLAB (R2007a, The MathWorks, MA) using code developed for this study. The end of an experimental trial was taken to be when $P_{ur}$ returned to within 10% of baseline.

Extracted urodynamic pressure values were $P_{ves,ave}$ (average absolute bladder pressure), $P_{ves,max}$ (maximum absolute bladder pressure), $P_{ves,ave,evoked}$ (average evoked bladder pressure, $P_{ves,ave} - P_{ves,baseline}$), $P_{ves,max,evoked}$ (maximum evoked bladder pressure, $P_{ves,max} - P_{ves,baseline}$), $P_{ves,end}$ (end-of-stimulus bladder pressure), $P_{ves,end,norm}$ (normalized end-of-stimulus bladder pressure, $P_{ves,end} / P_{ves,ave}$), $P_{ur,ave,on}$ and $P_{ur,ave,off}$ (average urethral pressure during stimulus on and off phases, respectively), $P_{ur,max,on}$ and $P_{ur,max,off}$ (maximum urethral pressure during stimulus on and off phases, respectively), $P_{ur,end}$ (end-of-stimulus urethral pressure), and $P_{ur,end,norm}$ (normalized end-of-stimulus urethral pressure, $P_{ur,end} / P_{ur,ave}$). Additional urodynamic values extracted included $V_{volq}$ and $Q_{ave}$ (volumetric flow in mL/s). A key input parameter was $V_{inf}$, the volume of saline infused into the bladder for testing.

The data set was subdivided into three portions: (1) hook tests, (2) trials during CMGs, and (3) voiding trials. The hook test data were used to construct root maps of $P_{ves}$ and $P_{ur}$ responses. Pairwise correlations were computed for the CMG trials using Pearson product-moment correlation coefficients. Further summary statistics, correlations, and one-way analyses of variance (ANOVAs) were performed (SPSS 16.0.2.
GP, SPSS, IL; JMP 5.1, SAS Institute, NC) for the voiding trials to test for effects of dorsal root stimulation. Trial blocks were not fully randomized or counterbalanced.

**Results**

The subdivided data set comprised hook test trials in all 11 dogs (n = 172, 9-20 trials per dog), CMG trials in six of 11 dogs (n = 89, 7-20 trials per dog), and voiding trials in nine dogs (n = 203, 4-41 trials per dog). The leak volume in these dogs as determined by the CMGs ranged widely from 70 mL to 270 mL.

Current thresholds for all ventral root electrodes—obtained in eight of nine dogs—were ≤ 400 μA, except for one animal for which thresholds were ≤ 2 mA. For voltage-controlled ventral drive (n = 3 dogs), all electrodes had peak-to-peak voltage thresholds ≤ 2V_{p-p}.

**Root Maps**

A total of 90 spinal roots were tested in all 11 dogs (6-10 roots per dog). The majority (n = 74) of these roots were intradural ventral roots but small numbers of extradural (n = 11) and intradural dorsal (n = 5) roots also were tested. Absolute and evoked bladder pressures and absolute urethral pressures were used to construct the root map of Figure 2. Bladder pressures were recorded for all trials and were highest from S1 to S3, corresponding to the root levels implanted with spiral electrodes. Root test P_{ur} data were available in six animals only. The number above each bar in Figure 2 represents the n of trials performed for that measure at that level.
The asterisk after the S4 label of Figure 2 reflects an anatomical discrepancy in the literature. Veterinary anatomy texts depict only three sacral vertebral levels in both dogs (Sisson et al. 1975) and cats (McClure et al. 1973), suggesting that only three sacral spinal roots should be present. However, a dermatome mapping study in the cat describes a small S4 dermatome (Kuhn 1953), suggesting a corresponding fourth sacral root. Such a root may be small and present in only some species. This work assumes an S4 spinal root and dermatome.

**CMGs**

Significant correlations were found between $V_{\text{inf}}$ and $P_{\text{ves,ave}}$ ($r = -0.4354$, $p < 0.0001$), $P_{\text{ur,ave,on}}$ ($r = 0.2324$, $p < 0.03$), $P_{\text{ur,ave,off}}$ ($r = 0.2875$, $p < 0.02$), and $P_{\text{ur,max,off}}$ ($r = 0.6437$, $p < 0.0001$). Other significant correlations occurred between $P_{\text{ves,max}}$ and $P_{\text{ur,ave,on}}$ ($r = 0.5391$, $p < 0.0001$) and $P_{\text{ur,max,on}}$ ($r = 0.5867$, $p < 0.0001$). The number of trials used to compute these correlations varied from 68 to 89 due to missing values. Omitted are expected correlations such as those between average and maximum $P_{\text{ur}}$ or $P_{\text{ves}}$ values or between $P_{\text{ur}}$ values during on and off phases of intermittent stimulus. All $P_{\text{ves}}$ values included in this correlation analysis are absolute pressures.

**Voiding**

Overall, the mean ± standard error of the mean (SEM) for $P_{\text{ves,ave,evoked}}$ was 13.29 ± 0.59 cm $H_2O$, the average $P_{\text{ves,max,evoked}}$ was 25.12 ± 1.17 cm $H_2O$, the average $P_{\text{ves,ave}}$ was 19.97 ± 0.84 cm $H_2O$, and the average $P_{\text{ves,max}}$ was 31.80 ± 1.16 cm $H_2O$. The
average $V_{\text{void}}$ was $5.0 \pm 0.4$ mL and the average $Q_{\text{ave}}$ was $0.22 \pm 0.02$ mL/s. These values were calculated for $n = 203$ trials. The post-stimulus voids ($n = 20$ trials) were larger, averaging $17.7 \pm 4.1$ mL. Altogether, these voids corresponded to voiding efficiencies no greater than 61% and generally below 20%.

Significant correlations (all $p < 0.0001$) were found between $V_{\text{void}}$ and $P_{\text{ves,ave}}$ ($r = 0.3520$), $P_{\text{ves,max}}$ ($r = 0.6344$), $P_{\text{ves,ave,evoked}}$ ($r = 0.5317$), $P_{\text{ves,max,evoked}}$ ($r = 0.6472$), $P_{\text{ur,ave,on}}$ ($r = 0.4466$), and $P_{\text{ur,max,on}}$ ($r = 0.4265$). $Q_{\text{ave}}$ was significantly correlated (all $p < 0.0001$) with $P_{\text{ves,ave}}$ ($r = 0.2905$), $P_{\text{ves,max}}$ ($r = 0.6040$), $P_{\text{ves,ave,evoked}}$ ($r = 0.5119$), $P_{\text{ves,max,evoked}}$ ($r = 0.6512$), $P_{\text{ur,ave,on}}$ ($r = 0.4420$), $P_{\text{ur,max,on}}$ ($r = 0.4207$), and $V_{\text{void}}$ ($r = 0.9878$). $P_{\text{ur,max,on}}$ was significantly correlated with $P_{\text{ves,ave}}$ ($r = 0.1604, p < 0.025$), $P_{\text{ves,max}}$ ($r = 0.1528, p < 0.031$), and $P_{\text{ves,ave,evoked}}$ ($r = 0.1449, p < 0.041$). Correlations were computed with either 201 or 203 trials due to missing values. There was a handful of additional trials ($n = 20$) in which post-stimulus voiding occurred (e.g. the bladder contracted after ventral drive ceased). This $V_{\text{void,post}}$ was significantly correlated with $P_{\text{ves,ave}}$ ($r = -0.7249, p < 0.0005$), $P_{\text{ves,max}}$ ($r = -0.7210, p < 0.0005$), $P_{\text{ves,ave,evoked}}$ ($r = 0.4864, p < 0.03$), and $P_{\text{ur,max,on}}$ ($r = -0.4496, p < 0.047$). As with the CMG data, expected correlations have been omitted. Figure 3 summarizes these results with a color map of the correlation coefficients.

One-way ANOVAs were performed to search for any effect of afferent (dorsal) root stimulation on these urodynamic values. There was a very weak trend of decrease in $P_{\text{ves,ave,evoked}}$ with the application of afferent stimulation, but it was not significant ($F = 1.8365, p = 0.1769$). There was no significant effect of afferent stimulation on $V_{\text{void}}$. 
Dorsal rhizotomy in one dog had no measurable effect.

There is some minor evidence (Figure 4) in one dog to suggest a slight (2.3 mL) increase in $V_{\text{void}}$ with afferent stimulation when the bladder was filled to 100 mL or 150 mL ($n = 22$ randomized counterbalanced trials, $p < 0.01$, independent samples $t$-test). Insufficient for a statistical test are a handful of trials ($n = 20$) with post-stimulus voiding showing that afferent stimulation somewhat delayed the onset of the reflex voiding contractions (Figure 5). A guarding reflex is evident in the corresponding $P_{ur}$ traces during urethral flow.

**Discussion**

Overall, the elicited bladder pressures were insufficiently high, resulting in poor voiding. Dorsal root stimulation therefore had no significant effect on generated pressures or voiding. Although marginal evidence suggests a slight effect of afferent stimulation (Figures 4 and 5), poor repeatability resulted in too few trials obtained. The voiding increase of Figure 4 was *statistically* significant, but the corresponding voiding efficiency was below 15% and therefore not of *clinical* significance.

*Generated Bladder Pressures*

Amounts voided were significantly correlated to elicited bladder pressures, suggesting that low maximum evoked bladder pressures (averaging $25.12 \pm 1.17$ cm H$_2$O) were the proximate cause of the meager voiding (averaging $5.0 \pm 0.4$ mL and
generally below 20% voiding efficiency). Indeed, in one dog with larger generated bladder contractions (average $P_{\text{ves, max, evoked}}$ of $54.04 \pm 2.20$ cm H$_2$O), greater voiding subsequently occurred (average $V_{\text{void}}$ of $12.4 \pm 1.3$ mL and a voiding efficiency of 61%).

Three prior canine studies by Bhadra and associates that used similar methods were examined for comparison. The first of these (Grunewald et al. 1998) had evoked $P_{\text{ves}}$ values of 45 cm H$_2$O ($n = 19$ females, rectangular drive). The second (Bhadra et al. 2002) had evoked $P_{\text{ves}}$ values of 65 cm H$_2$O ($n = 8$ females, intermittent drive). The third comparison study (Bhadra et al. 2006b) had evoked $P_{\text{ves}}$ values of 70-90 cm H$_2$O ($n = 3$ females, $n = 2$ males). In all studies, evoked bladder pressures were generally two or three times greater than those of the present study. Comparing these published results with the present data, an evoked $P_{\text{ves}}$ of 50 cm H$_2$O appears to be the minimum threshold for overcoming urethral resistance and generating significant voiding.

Troubleshooting

A thoroughgoing review of confounding factors potentially responsible for the poor evoked $P_{\text{ves}}$ and $V_{\text{void}}$ values was conducted. Extensive troubleshooting then occurred throughout the course of the experiments.

Electrodes were implanted on the correct roots for maximal bladder activation. As discussed in the Methods and as indicated by the root maps (Figure 2), stimulation of roots S1-S3 (with levels verified by post-mortem dissection) elicited the largest bladder contractions and therefore received spiral electrodes. These sacral root levels agree with prior work by Bhadra et al. in analogous preparations (Grunewald et al. 1998;
Bhadra et al. 2001; Bhadra et al. 2002; Bhadra et al. 2006b). Additionally, the bladder and urethral response thresholds for the electrodes used in the present study were low. It is therefore unlikely that incorrect electrode placement or excessively high nerve/electrode interface impedances were responsible for the meager $P_{ves}$ responses. Nevertheless, electrode design was improved during the study with introduction of a tighter spiral cuff having a lower cuff-to-nerve ratio. Modifications to the stimulation regime also occurred, including phase-locking the ventral and dorsal stimulation channels, employing voltage-controlled stimulation, and replacing stimulator hardware due to software glitches discovered with the DS8000. None of these changes appreciably affected urodynamic responses.

Surgical complications were noted in the first (bladder line remediation, laminectomy at incorrect levels, spinal cord damage), second (femoral arterial line hematoma, bladder line remediation), seventh (cord contusion), and ninth (carotid line hematoma) dogs. Continual refinements were therefore made in the animal preparation and surgical technique to minimize trauma. As discussed in the Methods, the femoral line was switched to a carotid line in response to an observed hematoma. Also as discussed in the Methods, the suprapubic bladder line was replaced with a triple-lumen urethral catheter, thus eliminating an entire surgical procedure. The 9 F triple-lumen catheter was 3 mm in diameter, close to the 2.67 mm diameter of the 8 F single-lumen urethral catheter previously used and around which fluid can flow (Bhadra et al. 2002; Bhadra et al. 2006b). Although these efforts improved the stability of the animal preparations, urodynamic responses were unimproved.
Minor potential confounds were also addressed. Vaginal bleeding, suggesting active estrus, was noted in three dogs. Estrus can impair urodynamic responses in the female canine (Hamaide et al. 2005). Therefore, the last four dogs were monitored for estrus as indicated in the Methods, with a standing order to abort an experiment if any clinical signs of menstruation were observed. No such signs were observed in these dogs. Additionally, the laboratory animal supplier was switched from a random source (LBL) to a purpose-bred (Covance) facility for the final three dogs. As before, these efforts did not improve the obtained urodynamic responses.

**Summary and Proposed Additional Improvements**

In sum, the overriding difficulty of this study was generation of bladder pressures insufficiently great to produce significant voiding. None of the extensive troubleshooting measures undertaken resulted in material improvements in the urodynamic responses.

It is possible that despite the great care taken during surgical procedures, subtle damage occurred to the sacral roots or spinal cord itself and that this damage precluded recovery of responses in the limited timeframe of an acute experiment. Therefore, in future experiments, chronic spinal intact dogs might provide a superior experimental alternative. Unlike in an acute preparation, recovery from minor nerve injury during cuff implantation is possible with a chronic animal, thus removing a major potential experimental confound. More testing sessions are also possible in a chronic animal, thus reducing the number of animals needed for a study. Use of male dogs for these
chronic experiments would eliminate the further complication of estrus. However, special preparation is required for maintaining aseptic technique during chronic surgical procedures and for providing animal husbandry for long durations. Chronic spinal dogs would further permit observation of post-spinalization reflex behavior approximating the DSD seen in humans with SCI, but additional animal welfare and husbandry concerns would necessarily arise.

Yet another future option would be to attempt the experiments in acute cats with intradural electrodes, given this species’ greater experimental robustness (see Chapter 4). However, due to the much smaller size of intradural roots in the cat, there is a reduced ability to manufacture cuffs with an appropriate cuff-to-nerve ratio and a greater risk of nerve injury during electrode implantation.

Given the lack of success with the acute female canine preparation despite exhaustive troubleshooting, it was deemed not possible to justify—scientifically or ethically—additional experiments beyond the eleven chronicled here. It is anticipated that at least one of the proposed future approaches will yield better experimental results able to address with the original device demonstration goal.

**Conclusions**

Ultimately, it was not possible to duplicate the successful preparation of Bhadra and associates. Despite methodical experimental review and comprehensive troubleshooting, bladder pressures remained too low for appreciable voiding. It was therefore not scientifically or ethically justifiable to continue with the experiments.
Nevertheless, the difficulties with the experimental preparation do not invalidate the intradural approach for delivering afferent stimulation. An improved experimental paradigm could use chronic spinal-intact male dogs to eliminate the possible confounds of nerve injury and estrus, while also allowing more testing sessions per animal. Other alternatives for future studies include chronic spinalized dogs or acute cats with intradural electrodes. These modified approaches would be more likely to achieve the device demonstration originally intended with this series of experiments.

If successful in animals, the combined dorsal and ventral intradural stimulation modality could be tested in human intraoperative subjects. Such clinical tests could use a modified three-channel Brindley implant, as has been previously attempted with limited efficacy (Kirkham et al. 2002). If successful, these tests would provide a proof-of-concept of a neuroprosthetic approach that could be rapidly deployed in the clinic.

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Figure 1. Experimental schematic for intradural canine sensory-motor root stimulation, illustrating implantation sites for intradural spiral electrodes. Not depicted are the pressure and volume recording instrumentation, which is described in the text.
Figure 2. Root maps for all dogs ($n = 11$, 9-20 trials per dog). In total, 90 roots were tested in all 11 dogs (6-10 roots per dog). The number above each bar represents the $n$ of trials performed for that measure at that level. The asterisk after the S4 label reflects an anatomical discrepancy in the literature; anatomical texts (McClure et al. 1973; Sisson et al. 1975) only describe three sacral spinal segments but literature on dermatome mapping (Kuhn 1953) suggests that there is a small S4 segment and root. The latter interpretation is followed here.
Figure 3. Color map of Pearson product-moment correlation coefficients for all voiding trials \( (n = 203) \). Post-stimulus voiding was observed in only a subset of trials \( (n = 20) \). Note the expected diagonal symmetry of the correlation matrix. Significant correlations \( (p < 0.05) \) are discussed in the text.
Figure 4. Randomized and counterbalanced trials ($n = 20$) in one dog showed evidence of a statistically significant ($p < 0.01$)—but not clinically significant—increase in $V_{\text{void}}$ with the application of afferent stimulation. Error bars indicate $\pm 1$ standard error of the mean (SEM).
Figure 5. Representative raw trials showing evidence of post-stimulus voiding delay (right panel) with application of afferent stimulation (thick black horizontal trace). Ventral root stimulation is indicated by the intermittent waveform trace. As labeled, the black trace is $P_{urt}$, the dark gray trace is $P_{ves}$, and the light gray trace is $V_{void,post}$. Note the slightly different horizontal axis scaling. The urethral reflexes observed during flow in both panels are the guarding reflex.
CHAPTER 3. THE FELINE DORSAL NERVE OF THE PENIS ARISES FROM THE DEEP PERINEAL NERVE AND NOT THE SENSORY AFFERENT BRANCH
Preface

This chapter addresses Specific Aim 2a. It is currently a journal article in print (Mariano et al. 2008b).

Abstract

The cat has been used extensively as an animal model for urogenital studies involving the pudendal nerve (PN). However, discrepancies persist in the literature regarding the origin of the dorsal nerve of the penis (DNP). The present study used gross dissections and serial histological cross sections to demonstrate that the DNP arises from the deep perineal nerve (DPN) and not the sensory afferent branch as previously reported. This finding indicates a better than previously appreciated neuroanatomical homology between the cat and human.

Introduction

The PN and its branches are critical to proper urogenital function and are a primary research target for understanding and treating bladder sphincter dyssynergia resulting from lower spinal cord injury (Boggs et al. 2005; Bhadra et al. 2006a). The cat has been a widely used model for these systems level neural studies due to similarities between the feline and human nervous systems. Previous experience in our laboratory has shown the DPN giving rise to the DNP, an observation that differs from extant literature such as Martin et al’s “typical” feline sacral plexus, which showed the dorsal
genital nerves arising from a dedicated sensory afferent (AFF) branch of the PN (Martin et al. 1974).

The purpose of the present study was to clarify the course of the DPN and DNP as they arose from the PN in the male cat and compare this pattern to known human anatomy. Closer neuroanatomical homology between cat and human has potential clinical repercussions, as it would extend the suitability of the feline as an electrophysiological model for human neurourological studies.

**Materials and Methods**

Dissections were performed on cadavers of sexually intact adult male cats ($n = 8$) with body weight 3.3 to 5.5 kg (Harlan Sprague Dawley, Madison, WI USA; Liberty Research, Waverly, NY USA). The PNs (left: $n = 8$; right: $n = 1$) had been exposed premortem for an electrophysiological study by surgical section and lateral reflection of the gluteus superficialis and gluteofemoralis muscles using methods previously described (Boggs et al. 2005). Cadavers were then refrigerated or frozen for between three and 32 days until dissection. Blunt dissection was used to trace the PN and its branches as distally as possible, usually including much of the penile shaft. The PN was traced rostrally in all cadavers—and in one to the sacral roots. In an additional cadaver, the pubic symphysis was removed to reveal ventral pelvic structures. Dissection was aided by use of a surgical microscope (Storz Urban US-1; Urban Engineering, Inc., Burbank, CA, USA) and head mounted magnifier (OptiVISOR DA-3; Donegan Optical
Company, Inc., Lenexa, KS, USA). Digital photographs were taken using a 3.3 megapixel camera (Canon PowerShot G1; Canon, Inc., Tokyo, Japan).

Six PNs (left: \( n = 5 \); right: \( n = 1 \)) and their branches were then excised from five cadavers with the branches transected as far distally as possible and the main trunk as far rostrally as possible. The harvested segments were photographed, placed in 10% buffered formalin, and refrigerated at approximately 7° C.

Histological processing for confirmation of gross dissection findings was done by the Histology Core Facility, University Hospitals, Cleveland, OH USA. Three complete specimens were labeled with histopathology tissue marking dyes and cut at 5-10 mm intervals with a razor blade. The segments were enclosed in Histo-Wrap© (Obex Industries, Buffalo, NY USA), placed in tissue cassettes, again stored in 10% formalin, and refrigerated. Samples were embedded in paraffin, cut in 5 µm transverse sections, stained with hematoxylin and eosin, and mounted on glass slides. Digital photomicrographs were taken with a binocular microscope (Axioskop D-7082; Carl Zeiss Jena GmbH, Jena, Germany) and a camera (SPOT 1.1.0) connected to a PC running commercial image acquisition software (SPOT 4.0.9). The resulting images were rotated or flipped as necessary with software (GIMP 2.2.6) to establish consistent image orientation using careful examination of fascicular structure and arrangement.

**Results**

The PN was found to arise primarily from the S1 and S2 sacral roots. In all cases, the origin of the DPN was identified visually by noting the bifurcation of the PN into a
branch supplying the external anal sphincter (EAS) and another giving rise to the DPN. The AFF branch was most clearly observed in three cats—later processed histologically—arising 10-15 mm proximal to the PN bifurcation. The EAS branch (or inferior rectal nerve) was easily identified by noting ramifications disappearing into the EAS muscle. In all cats, blunt dissection showed the DPN running inferior to the ipsilateral inferior pubic ramus; in five cats, the dissection was continued distally to show the DPN giving rise to the DNP as it coursed along the dorsal surface of the penis beneath a thick fascia layer analogous to Buck’s fascia in the human (Yang and Bradley 1998c). For the cadaver in which the pubic symphysis was removed, the DPN was also observed sending a branch that innervated the external urethral sphincter (EUS) muscle (Figure 1). A representative specimen appears in Figures 2 and 3.

In Figure 2a, the DPN was clearly identified as arising distal to the AFF branch at a bifurcation of the PN with the other fork supplying the EAS branch. Two candidates for the ischiocavernosus (ISCH) muscle’s innervation are also shown. The EUS was not identified in this cadaver; the small projection arising between cuts (l) and (m) of Figure 2 was found not to contain nerve tissue upon histological examination. Figure 2b diagrams the typical PN branching pattern based on all dissections in the present study, with special attention paid to the origins of the DNP.

Figure 3 shows serial cross sections corresponding to the gross image in Figure 2a and was representative of the other two histologically processed specimens. In the proximal cross sections, fascicles were found to have a mixed role as axons supplying multiple distal structures were enclosed within one perineurial sheath. The AFF branch
arose from dedicated fascicles in two specimens (including that depicted in Figure 3). In each specimen, fascicles giving rise to the DPN in turn divided into smaller fascicles supplying the DNP and EUS branches, mirroring the observed gross anatomy of Figure 1. Moving distally along the penis, the DNP divided into many small fascicles as seen in (o) of Figure 3. The precise origin of the EUS branch could not be identified in any of the histologically processed samples but is expected to be near (n) as this was the approximate location of the EUS muscle during gross dissection. In multiple slides, large voids were observed between the perineurial sheathes and neuron cell bodies, suggesting shrinkage or mechanical damage during preparation. As a result of these artifacts, cross sectional areas of individual fascicles could not be reliably obtained.

**Discussion**

The human DNP arises from the PN usually distal to the EAS branch but separately from the perineal nerve (Juenemann et al. 1988; Shafik et al. 1995; Schraffordt et al. 2004; Gustafson et al. 2005). This is largely analogous to the described feline neuroanatomy, in which the DNP arises distal to the EAS and AFF branches but is itself a distal continuation of the DPN as opposed to a separate terminal branch of the PN. The distal human urethra is innervated by the DNP (Yang and Bradley 1998a; Yang and Bradley 1998c), a finding that can be extended to the feline based on urethral stimulation results (Bradley et al. 1973) when taken in light of the present study.

The serial histological cross sections also confirm the results of the gross dissections, demonstrating that the DPN supplies both the DNP and EUS, thus
suggesting a mixed afferent and efferent function of the feline DPN. This finding is inconsistent with the Martin et al. branching pattern, as it showed a dedicated sensory PN branch giving rise to the DNP and a dedicated motor branch supplying the DPN (Martin et al. 1974). However, Martin et al. examined the distribution of myelinated fibers in the DPN and DNP and found the former to be 46% afferents, a result consistent with it giving rise to a large afferent nerve such as the DNP—itself found to be 96% afferents. Therefore, segregating the male feline PN into anatomically separate sensory and motor branches is an oversimplification.

Conclusions

The findings of the present study extend the validity of the feline as an appropriate model for human neurourological studies. Furthering our knowledge of sensory innervation of the penis could aid in restoration of micturition and sexual function especially given the proposed role of penile afferents in bladder excitation and ejaculation (Yang and Bradley 1998b; Gustafson et al. 2004).

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Figure 1. Dorsolateral view of the left PN (pud. n.) and major branches (scale in cm). The dissection involved a ventral approach to remove the pubic symphysis, exposing branches of the left DPN that supplied the EUS muscle and the DNP, and was continued proximally. The left testicle was removed and the perineum divided to demonstrate the left PN giving rise to the left DPN.
Figure 2. (a) Gross image of a nerve sample from a different cat than that of Figure 1 (scale in cm). The EUS was not identified. All other branches have been labeled. Transverse sections taken at marks (a)-(o) correspond to the panels of Figure 3. (b) Revised PN branching diagram showing the DPN giving rise to the DNP. Figure adapted from Martin et al., 1974.
Figure 3. Serial histological cross sections taken as indicated in Figure 2a. Black bars in each panel indicate 100 µm. Note that three distinct scales are used to best show structural detail: panels (a)-(f) are 1700 µm wide in total, panels (g)-(n) are 900 µm wide in total, and panel (o) is 2550 µm wide in total. In slices (k)-(n), the precise division
between DNP and EUS fascicles is unclear. The small projection seen between (l) and (m) in Figure 2a was found not to contain nerve tissue and is not represented here. Slice (o) shows only DNP fascicles due to its distal location.
CHAPTER 4. SUPPRESSION OF REFLEX URETHRAL RESPONSES BY SACRAL DERMATOME STIMULATION IN AN ACUTE SPINALIZED FELINE MODEL
Preface

This chapter addresses Specific Aim 2b. It is currently a journal article in print (Mariano et al. 2009b). The work was presented in poster form at the Society for Neuroscience Annual Meeting (Mariano et al. 2007; Mariano et al. 2008a) and is also included in a submitted United States patent application (Bhadra et al. 2009).

Abstract

Reflex contractions of the external urethral sphincter (EUS) are a major component of voiding dysfunction after neurological injury or disease. Aberrant urethral reflexes can prevent voiding and cause serious medical complications. Characterizing these urethral reflexes during genitourinary studies is necessary for evaluating novel pharmacological or neuroprosthetic approaches. The objectives of the present study were to generate urethral reflexes in the acute spinal feline, to quantify these reflexes, and to suppress them with electrical stimulation of the sacral dermatomes.

This study comprised eight male cats. Anaesthesia was maintained with alpha-chloralose or sodium pentobarbital. The spinal cord was transected between T10 and T12, and nerve cuff electrodes were placed on the extradural S2 sacral roots to provide bladder activation. Bladder and urethral pressures were recorded during and after bladder contractions. Electrical stimulation was applied non-invasively to the sacral dermatomes with commercial surface electrodes.
Urethral reflexes were elicited consistently in six cats. The corresponding urethral pressure spikes were quantified. Putative metrics of urethral reflex activity such as the rate and average magnitude of reflex pressure spikes correlated significantly with standard urodynamic variables. Electrical stimulation of the sacral dermatomes suppressed urethral reflexes in three cats.

These findings in an acute spinal feline preparation demonstrate a non-invasive means of suppressing undesirable urethral reflexes. Translation of this work to clinical use could improve neuroprostheses for restoring bladder function and enhance treatment of aberrant urethral reflexes in humans.

**Introduction**

In healthy adults, evacuation of the urinary bladder can be initiated and terminated voluntarily. In infants and young children (Fowler et al. 2008) and in many animals, the bladder empties involuntarily by reflex activity. In some neurological disorders, coordination between the somatic and autonomic nervous systems required for bladder emptying is lost. Such loss of coordination can produce pathological reflexes that impair voiding and continence.

Normally, voiding occurs when the smooth muscle of the bladder contracts and the skeletal muscle of the external urethral sphincter (EUS) relaxes. Loss of descending cortical input can lead to unopposed parasympathetic tone and uncoordinated visero-somatic reflexes of the lower urinary tract (Craggs et al. 2006). These aberrant reflexes cause detrusor-sphincter dyssynergia (DSD), in which the bladder and EUS contract
simultaneously, preventing voiding (Watanabe et al. 1996). Bladder hyperreflexia may also occur (Craggs et al. 2006). Chronically, these effects lead to clinically significant problems including urine reflux into the ureters and kidneys and life-threatening autonomic dysreflexia (Creasey et al. 2001). Individuals with spinal cord injury (SCI) place great emphasis on restoration of bladder function as a recovery priority (Anderson 2004) for this costly condition (Creasey and Dahlberg 2001).

Current treatment approaches for voiding dysfunction have marked disadvantages. The Brindley procedure using sacral anterior root stimulation requires invasive surgery and irreversible dorsal rhizotomy, which has undesirable side effects (Creasey et al. 2001). Without rhizotomy, urethral reflexes prevent voiding in both humans (Kirkham et al. 2002) and animals (Bhadra et al. 2002; Bhadra et al. 2006b).

However, under certain conditions without rhizotomy in the canine, phasic reflex EUS activity and bladder voiding were observed, suggesting that neural signals from the EUS provided “feedback” to central spinal circuits to produce voiding. This observation led to application of afferent stimulation to interrupt the aberrant EUS reflexes and allow voiding without rhizotomy in the canine (Bhadra et al. 2006c).

Recent work in the feline demonstrates that electrical (Gustafson et al. 2003; Boggs et al. 2005; Boggs et al. 2006a; Boggs et al. 2006b) and perigenital mechanical (Fowler et al. 2008; Tai et al. 2008) afferent stimulation can produce bladder excitation via a spinal micturition reflex. Peripheral afferent stimulation can also produce reflex bladder contractions in cats (Shefchyk and Buss 1998) and humans (Gustafson et al. 2004). In addition, neuromodulation by stimulating afferent pathways has been
explored in other contexts, including alleviating congenital nystagmus in humans (Sheth et al. 1995) and reducing extensor spasticity during treadmill walking in incomplete SCI patients (Bajd et al. 2002).

This study had three main goals. An acute spinal feline preparation exhibiting aberrant urethral reflexes was developed. A method for quantifying the urethral reflex contractions was devised, allowing correlation of the reflexes to standard urodynamic variables. Finally, these urethral reflexes were suppressed by non-invasive electrical stimulation of the sacral dermatomes. Our approach for quantifying and suppressing these undesirable urethral reflexes will aid comparison of emerging therapies, fulfilling an unmet clinical need.

Materials and Methods

Eight sexually and neurologically intact adult male cats 3.7-5.4 kg were used. All procedures were approved by the Case Western Reserve University institutional animal care and use committee. Cats are sufficiently complex to serve as a neurophysiological model of the urogenital system with better than previously appreciated neuroanatomical homology to the human (Mariano et al. 2008b). The surgical preparation was substantially similar to that previously described (Boger et al. 2008). Briefly, surgery was performed under isoflurane anaesthesia (dosed to effect). Experimental testing occurred under either α-chloralose (65 mg/kg IV, n = 6 cats) or sodium pentobarbital (25 mg/kg IV, n = 2 cats) anaesthesia. A 6 F dual lumen (DLC-6D, Life Tech, TX) or 7 F Foley (Rochester Medical, MN) catheter was implanted in the
bladder suprapubically and connected to an external transducer (Deltran I, Utah Medical Devices, UT) to monitor bladder pressure ($P_{ves}$). Urethral pressure ($P_{ur}$) was measured with a 3.5 F urethral perfusion catheter or a 2 F or 4 F microtransducer-mounted catheter (FT-21C-ABOG, Scisense, ON; CTC/4F-2, Gaeltec, UK) placed at the location of the EUS as determined by a urethral pressure profile. Recorded responses were consistent regardless of catheter type. This catheter obstructed the urethra, preventing direct voiding measurements. A laminectomy of vertebral levels T10-T12 was then performed, and the exposed spinal cord was transected with micro-scissors and the aid of a surgical microscope (Storz Urban US-1, Urban Engineering, Inc., CA) usually after instillation of 0.1 mL bupivacaine. Post-mortem dissection verified spinalization between T10 and T12 with two of eight spinalizations incomplete. One of these cats was a non-responder excluded from the analysis. The other animal (Cat 1) exhibited urethral reflexes despite the ventral 20% of the white matter being intact. A sacral laminectomy allowed implantation of custom tripolar cuff electrodes bilaterally on the extradural sacral roots (S1 or S2) eliciting the greatest electrically evoked bladder contractions.

The acute phase of spinal shock resolves quickly in cats (McCouch et al. 1935) and passed after approximately one hour in this study, signaled by return of the anal stretch or bulbospongiosus reflexes (or both). Experimental testing began after an additional 3.5 hours, allowing significant recovery of evoked lower urinary tract and bladder responses (Boggs et al. 2005). The bladder was then filled incrementally with warm saline from a syringe infusion pump (Genie YA-12, Kent Scientific, CT) at 0.5-3
mL/min to 15-45 mL; filling was ended when the bladder became reflexive. Electrical stimulation (continuous 20 Hz, 100 μs biphasic, cathodic-first current-controlled rectangular pulses, 600 μA – 3 mA) was then applied to the extradural sacral roots (Pulsar 6bp or 6bp-as, FHC, ME; DS8000, World Precision Instruments, FL) to elicit bladder contractions. Urethral pressures were monitored for post-stimulus reflex activity.

Intermittent surface stimulation (primarily 20 Hz, 0.5 s on / 0.5 s off, 200 μs monophasic, cathodic current-controlled rectangular pulses, 4 – 20 mA) was applied (DS7A, Digitimer, UK) to shaved, depilated, and cleaned skin of the S1-S3 sacral dermatomes (Kuhn 1953; Brown and Fuchs 1975) through 5 cm by 5 cm self-adhesive electrode pads (Re-Ply 655, Tyco/Uni-Patch, MN). An identical return electrode was placed on the upper back or ipsilateral thigh.

To ensure that observed urethral reflexes were not an artifact of bladder stimulation modality—wherein both sensory and motor roots were activated by the extradural electrodes— an additional male cat (3.5 kg) was tested. This animal only received stimulation of the left pudendal nerve (primarily 33 Hz 100 μs biphasic cathodic-first current-controlled rectangular pulses, 1–5 mA), which produced bladder contractions via an afferent-mediated micturition reflex (Boggs et al. 2005). No sacral root access or stimulation occurred. The remainder of the experimental setup was as described for the sacral drive animals.

The data acquisition hardware was as previously described (Boger et al. 2008), consisting of a computer-based system (NI DAQ 6024E, National Instruments...
Corporation, TX) with customized software interface (LabVIEW 7.1, National Instruments Corporation). Data processing was performed with MATLAB (R2007a, The MathWorks, MA) using code developed for this study. The end of an experimental trial was taken to be when $P_{ur}$ returned to within 10% of baseline.

The algorithm extracted the urethral reflex metrics $N_{spk}$ (reflex pressure spike count), $P_{spk,ave}$ (average spike amplitude), and $P_{spk,ave,norm}$ (normalized average spike amplitude, $P_{spk,ave} / P_{ur,ave}$). Both are useful as $P_{spk,ave}$ is a primary measure and the normalization of $P_{spk,ave,norm}$ is more robust for comparing across cats. $N_{spk}$ was calculated per trial and was therefore biased due to varying trial length and to pooling $N_{spk}$ data across cats, as trial blocks were not balanced. Therefore, an additional measure, the spike rate (events per second) was calculated as $R_{spk} = N_{spk}/[(\text{trial duration}) – (\text{bladder stimulus duration})]$, with all durations in seconds.

Extracted urodynamic predictor variables were $P_{ves,ave}$ (average bladder pressure), $P_{ves,end}$ (end-of-stimulus bladder pressure), $P_{ves,end,norm}$ (normalized end-of-stimulus bladder pressure, $P_{ves,end} / P_{ves,ave}$), $P_{ur,ave}$ (average urethral pressure), $P_{ur,max}$ (maximum urethral pressure), $P_{ur,end}$ (end-of-stimulus urethral pressure), and $P_{ur,end,norm}$ (normalized end-of-stimulus urethral pressure, $P_{ur,end} / P_{ur,ave}$).

Correlation matrices were computed using Pearson product-moment correlation coefficients and two-tailed significances. Analyses of variance (ANOVAs) and t-tests were also performed (SPSS 16.0.2 GP, SPSS, IL and JMP 5.1, SAS Institute, NC). Separately fit two-way ANOVAs and independent samples t-tests were chosen because trial blocks were unbalanced in this preliminary study.
Results

Twitch- or spike-like urethral reflexes were produced and quantified in an acute spinal feline model. Surface stimulation of the sacral dermatomes significantly affected these urethral reflexes, suppressing them in three of six animals.

Generation of Urethral Reflexes

Six of eight cats (designated as Cats 1-6) exhibited consistent and robust post-spinalization urethral reflexes that were observed after direct stimulation ended (Figure 1). The other two cats exhibited only occasional slow sustained rises in $P_{ur}$ and therefore were not analyzed further. In total, 214 trials occurred without sacral dermatome surface stimulation applied (3-42 trials per cat), and 127 trials occurred with surface stimulation (2-35 trials per cat). Cat 1 with incomplete spinalization yielded the fewest trials. In each trial, urethral reflex activity typically ceased within 45-60 seconds, except for Cat 4, in which urethral reflex activity extended for approximately three minutes.

Quantification of Urethral Reflexes

To assess the quantitative predictors and metrics, correlations were computed for trials without sacral dermatome surface stimulation, pooled across all six responder cats (Table I). This matrix included $n = 214$ trials, except for $P_{ves,ave}$ and $P_{ves,end,norm}$ ($n = 211$). The urethral reflex metrics $R_{spk}$, $P_{spk,ave}$, and $P_{spk,ave,norm}$ exhibited the greatest
number of significant correlations to urodynamic predictor variables representing bladder and urethral pressures. These metrics were therefore included in the ANOVAs.

**Suppression of Urethral Reflexes**

Surface stimulation of the sacral dermatomes affected urethral reflex activity in all six cats. Surface stimulation significantly reduced urethral reflex activity in three cats, increased $R_{spk}$ in one cat (Cat 4), and increased reflex activity in two cats. An example from Cat 4 of suppression with extended reflex duration appears in Figure 2.

The analyzed metrics ($R_{spk}$, $P_{spk,ave}$, and $P_{spk,ave,norm}$) were examined for each cat, both without and with sacral dermatome surface stimulation (Figure 3a-3c). Animals were categorized as (1) positive responders (Cats 1-3), in which surface stimulation decreased urethral reflex activity, and (2) negative responders (Cats 4-6), in which surface stimulation exacerbated these reflexes (though only for $R_{spk}$ with Cat 4). The positive responder group comprised 60 trials without surface stimulation (3-42 trials per cat) and 37 trials with surface stimulation (2-26 trials per cat). The negative responder group comprised 154 trials without surface stimulation (23-73 trials per cat) and 90 trials with surface stimulation (27-35 trials per cat). Panels d-f of Figure 3 present the same data as directly calculated differences between averages of pooled trials without and with surface stimulation (dark bars). The lighter bars are a calculated measure that weights each cat equally to eliminate pooling bias from unbalanced trial blocks.

One-way ANOVAs assessed reflex suppression in the positive responders, the negative responders, and all cats (Table II). In the positive responder group, $R_{spk}$, $P_{spk,ave}$,
and $P_{\text{spk,ave,norm}}$ were significantly reduced (all $F > 7$, all $p < 0.01$) when sacral dermatome surface stimulation was applied. In the negative responder group, all metrics increased significantly (all $F > 9$, all $p < 0.0025$) with surface stimulation. When Cats 1-6 were analyzed together, no significant differences were found (all $F < 2.25$, all $p > 0.10$).

To account for the multiple comparisons inherent in one-way ANOVA, a series of two-way ANOVAs was performed with separate model fits for each urethral reflex metric (Table II). For positive responder cats (Cats 1-3), there was a significant main effect of the between-subjects factor of cat for all metrics (all $F > 4$, all $p < 0.02$), but no significant interactions. Only the within-subjects factor of $P_{\text{spk,ave}}$ showed a significant reduction ($F = 7.1429$, $p < 0.01$) when sacral dermatome surface stimulation was applied. For negative responder cats (Cats 4-6), all metrics showed a significant main effect of cat (all $F > 20$, all $p < 0.0001$), but no significant interactions. Surface stimulation significantly increased all reflex metrics (all $F > 13$, all $p < 0.0004$). For all cats, there again was a significant main effect of cat for all reflex metrics (all $F > 19$, all $p < 0.0001$) but no significant interactions. Both $R_{\text{spk}}$ and $P_{\text{spk,ave,norm}}$ showed significant increases (all $F > 5$, all $p < 0.03$).

**Pudendal Nerve Stimulation**

In the additional animal that received bladder drive via only the pudendal nerve, urethral reflexes similar to those elicited with sacral drive occurred (Figure 1b). In 42 pooled trials without ($n = 32$) and with ($n = 10$) surface stimulation, independent
samples t-tests revealed that sacral dermatome surface stimulation significantly reduced $P_{spk,ave}$ ($p = 0.034$, equal variances assumed) and $P_{spk,ave,norm}$ ($p < 0.005$).

**Discussion**

This study demonstrated production of aberrant urethral reflexes in an acute spinal feline preparation. These data are the first demonstration of suppression of such reflexes with surface stimulation of the sacral dermatomes. The approach has significant clinical potential for reducing undesirable urethral reflex activity in people with SCI or other neurological disorders, and it could be incorporated into future bladder neural prostheses.

Urethral reflexes following acute spinal transection may not be neurophysiologically equivalent to detrusor-sphincter dyssynergia (DSD) resulting from chronic neurological changes after SCI. Therefore, although this acute preparation has considerable experimental utility, further study in chronic preparations is required to demonstrate effectiveness for DSD.

*Generation of Urethral Reflexes*

In this study, an acute SCI feline preparation was developed that exhibited robust post-spinalization urethral reflexes. Although post-SCI urethral reflexes are usually seen chronically and may result from urethral flow (Galeano et al. 1986), acute SCI excitatory afferent models have been used to examine spinal neural circuits and to
obtain insight (Boggs et al. 2005; Boggs et al. 2006a; Boggs et al. 2006b) before validation in chronic SCI (Tai et al. 2008) and human genitourinary studies.

Urethral reflexes could not be observed during bladder drive due to simultaneous direct sphincter activation from extradural sacral root stimulation. Therefore, urethral reflexes were observed only after bladder pressure was decreasing. Nonetheless, these reflexes are similar to DSD. The reflexive EUS activity observed after sacral stimulation (Figure 1), presumably due to urethral fluid flow, is similar to that observed in humans with chronic SCI after sacral stimulation (Kirkham et al. 2002). In humans, this post-stimulus reflexive EUS activity (DSD) prevents voiding and requires a dorsal rhizotomy to attain voiding. Canine studies have shown a similar result (Bhadra et al. 2006b). The observed urethral reflexes are also consistent with urethral-flow-activated dyssynergia.

Bladder excitation modality did not affect urethral reflexes; they could also be elicited with pudendal nerve mediated bladder contractions and then suppressed with surface stimulation. Therefore, the urethral reflexes were not an artifact during extradural sacral root activation, which could indirectly activate afferent pathways. Anaesthetic agents also did not materially affect responses. The reflexes were absent in spinal-intact animals and occurred at physiologically normal bladder volumes. It is possible that sacral root and pudendal stimulation activated other reflex pathways that contributed to the urethral activity.

Urethral pressure responses include non-EUS activity from the pelvic floor and urethral smooth muscle. However, the time constant of the repeated, rapid, spike-like
contractions is consistent with skeletal muscle. Pelvic floor contractions were also observed in synchrony with urethral pressure spikes. Future studies could eliminate smooth muscle effects with blocking agents. Nonetheless, the non-EUS contributions in the present study are functionally relevant as they could also impede voiding.

**Quantification of Urethral Reflexes**

The choice of recorded values for analysis was motivated by urodynamic variables that would be easily obtainable in a clinical setting with existing equipment and techniques, although these quantities usually are not examined in this fashion. Quantification was consistent and unbiased using an automated computer algorithm. The resulting correlation matrix indicated that the best urethral reflex metrics were $R_{\text{spk}}$, $P_{\text{spk,ave}}$ and $P_{\text{spk,ave,norm}}$. Similarly, the best urodynamic predictors of urethral reflex activity were $P_{\text{ur,ave}}$ and $P_{\text{ur,max}}$ (Table I).

**Suppression of Urethral Reflexes**

Electrical surface stimulation delivered non-invasively to the sacral dermatomes suppressed urethral reflex contractions in three of six cats with sacral bladder excitation and in the one cat with pudendal nerve bladder excitation. $R_{\text{spk}}$, $P_{\text{spk,ave}}$ and $P_{\text{spk,ave,norm}}$ were sensitive metrics as they showed significant decreases and increases in the positive and negative responders (with the exceptions of $R_{\text{spk}}$ and $P_{\text{spk,ave,norm}}$ in the positive responder two-way ANOVA). Even in the negative responders, there was a statistically significant increase in all urethral reflex metrics. Although the opposite of
what occurred in Cats 1-3, the statistical significance of these results demonstrates that surface stimulation nevertheless modulated the neurophysiologic response in these animals. It is hypothesized that these effects could be afferent-mediated, although additional control tests would be necessary to rule out other influences.

The parameter space for surface stimulation of the sacral dermatomes was not fully explored; therefore, the observed reflex suppression effect was not optimized. Some variation of surface stimulation duty cycle did occur, although the majority of trials followed the 0.5 s on / 0.5 s off intermittent paradigm. Surface stimulation amplitude also varied but for most trials remained below the threshold for visible muscle fasciculations in the tested animal. Higher stimulation amplitudes increased the urethral reflexes—perhaps by an afferent-mediated reflex—and would therefore impede voiding. Although feline dermatomes are not well defined and may overlap (Kuhn 1953; Brown and Fuchs 1975), there is evidence to suggest a dermatomal specificity for the suppression effect. In Cat 6, a control test was performed, comprising a series of 12 trials in which only surface stimulation was applied at random to two identical electrodes placed over lumbar (L3-L4) or sacral (S1-S3) dermatomes. For each trial, the stimulus amplitude was slowly increased from 2 mA to 15 mA, and the amplitude at which urethral responses first appeared was noted. This control test showed that the lumbar dermatomes required greater stimulation (2.4 times the visible muscle fasciculation threshold of 5.6 mA) to evoke urethral responses than did the sacral dermatomes (1.4 times the fasciculation threshold of 8.5 mA), suggesting a greater excitability of the sacral dermatomes to surface stimulation than of the lumbar
dermatomes. Despite the limited parameter variation in the present study, the results demonstrate functional improvement using a non-invasive stimulation modality.

Clinical Relevance

A non-surgical and effective means of suppressing DSD could address a major unmet clinical need. Urinary neuroprostheses can improve voiding (Gaunt and Prochazka 2006) and reduce the costs of post-SCI bladder care (Creasey and Dahlberg 2001), but often require irreversible dorsal rhizotomy that causes undesirable side effects (Creasey et al. 2001). Modification of these existing devices to add sacral dermatome stimulation could improve effectiveness and clinical acceptance. For example, a rhizotomy-free Brindley approach using surface stimulation to reduce aberrant EUS reflexes and afferent inhibitory stimulation to provide continence could increase the number of individuals choosing this management approach.

Conclusions

Urethral reflexes were generated in an acute spinal feline preparation. Non-invasive electrical stimulation of the sacral dermatomes could suppress these aberrant reflexes. Although further work is needed to optimize the suppression effect in chronic spinalized animals, the results of the present study have clear clinical implications. Enhanced neuroprostheses incorporating this non-invasive approach could alleviate DSD in humans with voiding dysfunction from injury, stroke, or disease with a reduced surgical burden, increasing patient acceptance and vastly improving quality of life.
Acknowledgements

The authors thank Adam Boger, Ph.D., Tim Bruns, Ph.D., Tina Emancipator, Yanina Grinberg, Obinna Nwanna, and Petar Bajic for technical assistance. This work was supported by NIH DK077089, Department of Veterans Affairs RR&D B3675R, and Department of Education GAANN P200A040207. The first author received prior partial support from NIH T32 GM007250 (CWRU MSTP).
<table>
<thead>
<tr>
<th>Urodynamic Predictors</th>
<th>Metrics</th>
<th>( N_{spk} )</th>
<th>( R_{spk} )</th>
<th>( P_{spk,ave} )</th>
<th>( P_{spk,ave,norm} )</th>
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</thead>
<tbody>
<tr>
<td>( P_{ur,ave} )</td>
<td></td>
<td>-0.156</td>
<td>-0.211</td>
<td>0.358</td>
<td>-0.226</td>
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<tr>
<td>( P_{ur,max} )</td>
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<td>-0.274</td>
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<tr>
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<td>0.238</td>
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<td>0.031</td>
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<td>0.008</td>
<td>-0.178</td>
<td>0.076</td>
<td>-0.202</td>
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</table>

\( p < 0.05 \)

**Table I.** The correlation matrix was computed for trials without surface stimulation, pooled from Cats 1-6. Cell values are Pearson product-moment correlation coefficients; cell shading indicates two-tailed significances of \( p < 0.05 \).
Table II. The degree of reflex suppression was assessed with one- and two-way ANOVAs. The factors for the two-way ANOVA were individual animal and application of surface stimulation. The number of trials for each condition is indicated at the top.

<table>
<thead>
<tr>
<th>n of trials</th>
<th>Positive Responders (C1-C3)</th>
<th>Negative Responders (C4-C6)</th>
<th>All Cats (C1-C6)</th>
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<tr>
<td></td>
<td>No Surface</td>
<td>Surface</td>
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<tr>
<td></td>
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<td>154</td>
<td>214</td>
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<td></td>
<td>37</td>
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<tr>
<td></td>
<td>$p$</td>
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<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>$P_{spk,ave}$ (cm H$_2$O)</td>
<td>$F$</td>
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<td></td>
<td>$p$</td>
<td>0.0001</td>
<td>0.0023</td>
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<td></td>
<td>$P_{spk,ave,norm}$</td>
<td>$F$</td>
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<td>$p$</td>
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<td>$p$</td>
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<td>$P_{spk,ave,norm}$</td>
<td>$F$</td>
<td>1.5990</td>
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<tr>
<td></td>
<td>$p$</td>
<td>&gt; 0.20</td>
<td>&lt; 0.0001</td>
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Figure 1. (a) Representative trial from Cat 5 exhibiting aberrant urethral reflex pressure spikes following sacral root bladder drive. The horizontal black bar indicates the duration of application of sacral drive. (b) Representative trial with reflex spikes derived from the cat with reflex bladder drive by pudendal nerve stimulation. $P_{ur}$ is urethral pressure and $P_{ves}$ is bladder pressure. Note the different y-axis scaling.
Figure 2. Dermatome stimulation reduced urethral reflex activity. The top panel is from a trial with sacral root bladder drive and no surface stimulation; the bottom panel is a repeat trial with surface stimulation commencing at 0 sec. Both trials were taken from Cat 4. $P_{ur}$ is urethral pressure and $P_{ves}$ is bladder pressure.
Figure 3. Histograms for urethral reflex metrics (given as a mean ± 1 SEM error bars), presented for individual cats, positive responders (Cats 1-3), and negative responders (Cats 4-6). The top panels (a-c) indicate non-normalized average reflex spike amplitude, normalized average spike amplitude, and spike rate, respectively. The numbers above the bars for individual cats represent the $n$ of trials for each condition (without or with surface stimulation applied). The lower panels (d-f) represent the same data using directly calculated differences between averaged pooled trials without and with surface stimulation (dark bars). The lighter bars are a measure that weights each cat equally to eliminate pooling bias caused by unbalanced trial blocks. Error bars represent ± 1 SEM.
CHAPTER 5. CHARACTERIZING ABERRANT URETHRAL REFLEXES AND THEIR MODULATION BY SURFACE STIMULATION IN THE ACUTE SPINAL FELINE
Preface

This chapter addresses Specific Aim 2c and is currently a manuscript in preparation.

Abstract

Aberrant urethral reflexes after neurological injury or disease are a major component of voiding dysfunction, preventing functional micturition and continence. In our prior work (Mariano et al. 2009b), we developed a novel acute spinal feline preparation in which spike-like aberrant urethral reflexes were generated, quantified, and suppressed—the latter without rhizotomy and by non-surgical surface stimulation of the sacral dermatomes. The present study extends this work by characterizing the neurophysiology underlying the reflexes and suggesting key parameters for effective reflex modulation.

Eight sexually-intact adult male cats received lower thoracic spinalizations and extradural stimulation of the S2 roots bilaterally to excite the bladder. Experimental trials occurred under α-chloralose anaesthesia. Electrical stimulation was applied without surgery to lumbosacral dermatomes through commercially-available self-adhesive electrodes. Neurophysiological characterization included analyzing correlations between bladder volume, urethral pressure, and aberrant urethral reflex metrics. Tests under isoflurane anaesthesia assessed the nature of the observed urethral responses. To investigate reflex modulation, electrode location and size were varied, as were stimulation amplitude and duty cycle.
Aberrant post-spinalization urethral responses were elicited in all eight cats and modulated by surface stimulation in two. Isoflurane tests were consistent with these responses being reflexive. Pressure data suggested that these reflexes are mediated by stretch or flow receptor afferents in the urethra. Dermatomal location of surface stimulation and stimulation current amplitude were important parameters for successful reflex modulation.

Neurophysiological characterization and parameter identification were performed in an acute spinal model exhibiting suppressible aberrant urethral reflexes. These findings will guide the design of future neuroprosthetic or pharmacological approaches for treating voiding dysfunction in patients wishing to avoid irreversible dorsal rhizotomy. In particular, the inexpensive and surgically non-invasive nature of the surface stimulation modality has wide applications—even if efficacious in only a minority of patients.

**Introduction**

Healthy adults can initiate and terminate evacuation of the urinary bladder voluntarily. In certain neurological disorders, the coordination between the somatic and autonomic nervous systems required for bladder emptying is lost, producing pathological reflexes that impair micturition and continence.

Normally, voiding occurs when the smooth muscle of the bladder contracts and the skeletal muscle of the external urethral sphincter (EUS) relaxes. Loss of descending cortical input can lead to unopposed parasympathetic tone and uncoordinated viscero-
somatic reflexes of the lower urinary tract (Barrington 1914; Craggs et al. 2006). These aberrant reflexes cause detrusor-sphincter dyssynergia (DSD), in which the bladder and EUS contract simultaneously, thus preventing voiding (Watanabe et al. 1996; Kirkham et al. 2002). Chronically, these effects lead to clinically significant problems including reflux of urine into the ureters and kidneys and life-threatening autonomic dysreflexia (Creasey et al. 2001). Current treatment approaches have marked disadvantages, due largely to the undesirable side effects of the dorsal rhizotomy procedure (Creasey et al. 2001) necessary for eliminating DSD. Individuals with spinal cord injury (SCI) place great emphasis on restoration of bladder function as a recovery priority (Anderson 2004) for this costly condition (Creasey and Dahlberg 2001; French et al. 2007).

In the canine, preliminary work showed that afferent stimulation could interrupt aberrant urethral reflexes in rhizotomy-free spinal-intact animals (Bhadra et al. 2006c). Subsequently, an acute spinal feline preparation was developed that exhibited aberrant urethral reflexes (Mariano et al. 2009b) independent of bladder activation method, as they occurred with both direct drive of the sacral roots or reflex (Boggs et al. 2005) pudendal nerve drive. The urethral reflexes were suppressed by electrical surface stimulation of the sacral dermatomes. This proof-of-concept study demonstrated reflex suppression in four of seven cats and reflex exacerbation in two cats. The dichotomy of responses underscored that these aberrant reflexes and their suppression require further characterization.

Therefore, the goal of the present study was to investigate the neurophysiological mechanisms underlying these reflexes by analyzing pressure data
and performing an isoflurane test. In our previous study (Mariano et al. 2009b), a “control test” with only current-controlled surface stimulation suggested that sacral dermatomes (S1-S3) were more sensitive to electrical stimulation than were lumbar dermatomes (L3-L4). The present study directly compared the reflex modulation effect of lumbar and sacral dermatomes. Also examined were the importance of electrode size, stimulation amplitude, and stimulation duty cycle. Characterizing these reflexes and their means of suppression will guide development of new pharmacological or neuroprosthetic therapies—addressing a major unmet clinical need.

Materials and Methods

Eight sexually and neurologically intact adult male cats 3.7-4.7 kg were used. All procedures were approved by the Case Western Reserve University institutional animal care and use committee. Cats are sufficiently complex to serve as a neurophysiological model of the urogenital system and have better than previously appreciated neuroanatomical homology to the human (Mariano et al. 2008b).

The surgical preparation was as described previously in Chapter 4 (Boger et al. 2008; Mariano et al. 2009b). Briefly, all surgical procedures occurred under isoflurane anaesthesia (dosed to effect). Experimental testing occurred under α-chloralose anaesthesia (65 mg/kg IV) with buprenorpine added for analgesia due to α-chloralose’s debatable anaesthetic qualities (Holzgrefe et al. 1987). The α-chloralose was administered at the conclusion of surgeries in the first two cats and during surgeries in the remaining six cats. A 6 F dual lumen catheter (DLC-6D, Life Tech, TX) was implanted
in the bladder suprapublically and connected to an external transducer (Deltran I, Utah Medical Devices, UT) to monitor bladder pressure ($P_{\text{ves}}$). Urethral pressure ($P_{\text{ur}}$) was measured with a 3.5 F perfusion catheter placed at the location of the EUS (generally 3.5 cm to 4.5 cm proximal to the meatus) as determined by a urethral pressure profile. A laminectomy of lower thoracic vertebral levels was then performed, and the exposed spinal cord was transected with micro-scissors and the aid of a surgical microscope (Storz Urban US-1, Urban Engineering, Inc., CA) after instillation of 0.1 mL bupivacaine. Spinalization levels ranged from T9 to the T12/T13 junction with no observable effect on the urethral reflexes later obtained. Post-mortem dissection indicated that spinalization was complete in four animals (Cats 1, 4, 5, and 6 below) and 85%-95% complete (based on cross-sectional area) in the remaining four cats. A sacral laminectomy allowed implantation of custom tripolar cuff electrodes bilaterally on the extradural sacral roots eliciting the greatest electrically evoked bladder contractions, which were determined to be S2 in all cats based on post-mortem dissection.

Acute spinal shock resolves quickly in cats (McCouch et al. 1935; Boggs et al. 2005; Mariano et al. 2009b). In this study, anal reflexes returned within 1.5 hours in all cats except one (Cat 2 below); that cat exhibited aberrant urethral reflexes no more than 5.5 hours post-spinalization. After several more hours, the bladder was filled incrementally with warm saline from a syringe infusion pump (Genie YA-12, Kent Scientific, CT) at 1-2 mL/min to determine the bladder volume at which urethral reflexes were observed to be most robust secondary to bladder drive. Subsequent experimental trials occurred at this “test volume.”
Electrical stimulation (continuous 20 Hz, 100 μs biphasic, cathodic-first current-controlled rectangular pulses, 0.5-3 mA) was applied to the extradural sacral roots (DS8000, World Precision Instruments, FL) to elicit bladder contractions. Urethral pressures were monitored for post-stimulus reflex activity.

Intermittent surface stimulation (20 Hz, 100 μs monophasic, cathodic current-controlled rectangular pulses, 2.3-15.8 mA) was applied (DS7A, Digitimer, UK) to shaved, depilated, and cleaned skin. The duty cycle was generally 50% (0.5 s on / 0.5 s off), but 10% (0.1 s on / 0.9 s off) and 90% (0.9 s on / 0.1 s off) duty cycles were also employed to assess the importance of this parameter on reflex modulation. Using available feline dermatome maps (Kuhn 1953; Brown and Fuchs 1975), electrodes of two different sizes were placed on the lumbosacral dermatomes. Larger 5 cm by 5 cm patch electrodes (Re-Ply 655, Tyco/Uni-Patch, MN) with a 25.81 cm² contact surface area were placed on either the L4-L7 or the S1-S3 dermatomes to allow a coarse comparison of dermatomal differences in reflex suppression; randomized and counterbalanced trials were obtained. Smaller 19 mm diameter disk electrodes (Nicolet Biomedical, WI) with a 2.84 cm² contact surface area were placed on non-adjacent L5, L7, S2, and S4 dermatomes (Figure 1) for a finer-resolution examination of dermatomal differences in reflex suppression; randomized and counterbalanced trials were again obtained. A 5 cm by 5 cm return electrode was placed on the ipsilateral mid-lumbar region (L3-L5 dermatomes) or ipsilateral thigh (L4-L7 dermatomes). For the stimulating electrodes, thresholds for visible muscle fasciculations and urethral responses were recorded; stimulation was generally set at 0.1 mA below the visible muscle fasciculation threshold.
Some trials occurred with stimulation amplitude deliberately halved to assess the importance of amplitude as a parameter for reflex modulation. These trials were randomized and counterbalanced with “full” stimulation amplitude trials and control (no stimulation) trials.

In one animal responding to surface stimulation, the skin of the sacral dermatomes was anaesthetized with application of a 5% lidocaine patch (Lidoderm, Endo Pharmaceuticals, PA) for 10 minutes; surface stimulation was then resumed. In another cat, isoflurane was administered at 1.5% to 2.0% and the time-dependent effects on the aberrant urethral responses monitored.

The data acquisition hardware was as used previously (Boger et al. 2008; Mariano et al. 2009b). A customized software interface (LabVIEW 7.1, National Instruments Corporation) recorded data; these data were then processed by an algorithm (MATLAB R2007a, The MathWorks, MA) written by the author. The algorithm considered the end of an experimental trial to be when \( P_{ur} \) returned to within 10% of baseline.

The MATLAB algorithm extracted the urethral reflex metrics \( N_{spk} \) (reflex pressure spike count), \( P_{spk,ave} \) (average spike amplitude), and \( P_{spk,ave,norm} \) (normalized average spike amplitude, \( P_{spk,ave} / P_{ur,ave} \)). \( P_{spk,ave} \) is a primary measure and the normalization of \( P_{spk,ave,norm} \) is more robust for comparing across cats. \( N_{spk} \) was calculated per trial and was therefore biased due to varying trial length. A more robust derived measure, the spike rate (events per second), was therefore calculated as \( R_{spk} = N_{spk} / [(\text{trial duration in seconds}) - (\text{bladder stimulus duration in seconds})] \). Extracted urodynamic predictor
variables were $P_{ves,ave}$ (average bladder pressure), $P_{ves,max}$ (maximum bladder pressure), $P_{ur,ave}$ (average urethral pressure), and $P_{ur,max}$ (maximum urethral pressure).

Statistical analysis (SPSS 16.0.2 GP, SPSS, IL) included computation of correlations using Pearson product-moment correlation coefficients and two-tailed significances. Additional analyses of variance (ANOVAs) and independent-samples $t$-tests were also computed.

**Results**

Twitch or spike-like aberrant urethral reflexes were obtained in all eight acute spinal feline preparations (designated as Cast 1-8) of this study. Surface stimulation modulated urethral reflex activity in two cats. The results suggested that sacral dermatomes must be stimulated at sufficiently high current amplitudes to obtain effective reflex suppression. In all, 215 trials without surface stimulation (3-67 trials per cat) and 352 trials with surface stimulation (12-80 trials per cat) were analyzed. The number of trials per cat varied from 15 (Cat 2) to 134 (Cat 6).

**Neurophysiological Characterization**

Aberrant urethral reflexes were obtained in all eight cats following bilateral stimulation of the extradural S2 roots. In our prior study (Mariano et al. 2009b), urethral reflexes occurred in seven of nine cats. In all animals except Cat 2 of the present study, anal reflexes returned within 1.5 hours of spinalization; these reflexes were not observed in Cat 2. *Urethral* reflexes were not checked until the start of
experimental testing some hours later; these were first observed—after either urethral catheter insertion or bladder stimulation—between 5.5 and 8.5 hours post-spinalization and were present in all cats.

For the entire data set \((n = 567)\) trials, the mean ± SEM (standard error of the mean) of the infused bladder volume at testing was 13.3 ± 0.6 mL, underscoring the generally low volumes at which reflexes occurred. Considered as a percentage of the CMG leak volume, which ranged from 30 mL to over 80 mL, the test volumes were estimated to be 3% to 64% of \(V_{\text{leak}}\) with a mean of 26%. A few drops of urine leakage often accompanied experimental trials.

For the control trials without surface stimulation \((n = 215)\), the generated bladder contractions were robust, with a mean \(P_{\text{ves,ave}}\) of 22.45 ± 1.53 cm H2O and a mean \(P_{\text{ves,max}}\) of 57.49 ± 3.92 cm H2O. Generated urethral pressures were also strong, with a mean \(P_{\text{ur,ave}}\) of 148.17 ± 10.11 cm H2O and a mean \(P_{\text{ur,max}}\) of 214.55 ± 14.63 cm H2O. Aberrant urethral reflex “spikes” were generally robust, with a mean \(P_{\text{spk,ave}}\) of 44.41 ± 3.03 cm H2O—or approximately one-third of \(P_{\text{ur,ave}}\). The mean \(R_{\text{spk}}\) was 0.222 ± 0.023 spikes/sec. The reflex “spikes” typically ceased within approximately 30-75 seconds, although reflex durations as short as 10 s and as long as 2-3 minutes were also noted (Figure 2). There was a mean basal urethral tone \(P_{\text{ur,base}}\) of 17.27 ± 1.18 cm H2O. The mean \(P_{\text{ves,base}}\) was consistently low at 5.32 ± 0.24 cm H2O.

For the control trials, significant positive correlations were found between the bladder test volume and reflex metrics \(P_{\text{spk,ave}}\) and \(R_{\text{spk}}\) (all \(p < 0.002\)). Volume was also strongly positively correlated to \(P_{\text{ur,ave}}\) and \(P_{\text{ur,max}}\) (all \(p < 0.0005\)). Bladder pressure had
fewer significant correlations, although $P_{ves,ave}$ was positively correlated to $R_{spk}$ ($p < 0.02$) and $P_{ves,max}$ was negatively correlated to $R_{spk}$ ($p < 0.0005$). The urethral pressure metrics $P_{ur,ave}$ and $P_{ur,max}$, however, were significantly positively correlated to $P_{spk,ave}$ (all $p < 0.006$) and significantly negatively correlated to $P_{spk,ave,norm}$ (all $p < 0.0001$). This latter effect is an artifact of the normalization achieved by dividing the reasonably constant $P_{spk,ave}$ values by increasingly large $P_{ur,ave}$ values. $P_{ur,ave}$ and $P_{ur,max}$ were significantly negatively correlated to $R_{spk}$ (all $p < 0.007$).

The isoflurane test was performed on an animal (Cat 8) with robust responses. The results of Figure 3 show a progressive diminishment—and eventual elimination—of urethral responses with administration of isoflurane at 1.5% to 2.0%. Withdrawal of the general anaesthetic allowed progressive recovery of urethral responses in a similar timeframe. Duration of isoflurane administration, percent concentration, and corresponding mean arterial pressure (MAP) as measured by an automatic sphygmomanometer are indicated in the figure.

*Reflex Modulation*

Of the eight cats tested, Cat 6 was a “positive responder” exhibiting reflex suppression with surface stimulation at 20 Hz, 0.9 s on / 0.1 s off. Conversely, Cat 8 was a “negative responder” exhibiting reflex exacerbation when surface stimulation was applied at 0.5 s on / 0.5 s off. The other six cats exhibited urethral reflexes but there was no clear modulation by surface stimulation. Therefore, a two-way ANOVA performed on the entire data set showed no significant difference in any reflex metric
(i.e. $P_{\text{spk,ave}}, P_{\text{spk,ave,norm}}, R_{\text{spk}}$) when surface stimulation was applied (all $F < 1.9$, all $p > 0.17$). Analyzing each cat individually with independent-samples $t$-tests confirmed that surface stimulation significantly reduced $P_{\text{spk,ave}}$ and $P_{\text{spk,ave,norm}}$ (all $p < 0.035$) but not $R_{\text{spk}}$ ($p > 0.6$) in Cat 6 ($n = 134$ trials). In Cat 8 ($n = 65$ trials), surface stimulation significantly increased $P_{\text{spk,ave}}$ and $R_{\text{spk}}$ (all $p < 0.03$) but not $P_{\text{spk,ave,norm}}$ ($p = 0.077$).

Because Cats 6 and 8 responded oppositely to surface stimulation, a simple pooling of their trials for separate analysis was not instructive. Instead, the difference in each reflex metric due to application of surface stimulation was calculated on a per-trial basis; this was possible because testing blocks were randomized and counterbalanced in these animals. The absolute values of the calculated differences were then used for statistical analysis. A one-sample $t$-test revealed that $|\Delta P_{\text{spk,ave}}|$, $|\Delta P_{\text{spk,ave,norm}}|$, and $|\Delta R_{\text{spk}}|$ were significantly different from a test value of zero (all $p < 0.0005$).

Cats 1-4 all received 19 mm disk electrodes on non-adjacent dermatomes L5, L7, S2, and S4 for surface stimulation. Two-way ANOVA on this subset of the data ($n = 205$ trials) revealed no significant effect of any reflex metric (all $F < 1.3$, all $p > 0.25$). Pooling all surface stimulation trials ($n = 173$)—including a handful obtained with a 5 cm by 5 cm electrode on the S1-S3 dermatomes—and comparing to the control trials ($n = 50$) with an independent samples $t$-test showed only a weak trend of decrease in $R_{\text{spk}}$ ($p = 0.265$).

However, in both the positive responder (Cat 6) and the negative responder (Cat 8), both lumbar dermatomes (L4-L7, 5 cm by 5 cm electrodes) and sacral dermatomes (S1-S3, 5 cm by 5 cm electrodes or S2, 19 mm diameter disk electrode) were stimulated. Two-way ANOVA for pooled trials ($n = 40$) from both cats was not particularly instructive
due to the opposite responder types and revealed a significant effect of cat on all reflex metrics (all $F > 5.5$, all $p < 0.03$) but a significant effect of electrode location only on $P_{\text{pk,ave,norm}}$ ($F = 3.758$, $p = 0.034$). Tukey’s HSD post-hoc tests confirmed this difference to be between lumbar and sacral surface stimulation ($p = 0.025$). Separate one-way ANOVAs on each cat revealed a significant effect of dermatome for only the positive responder on $P_{\text{pk,ave}}$ and $P_{\text{pk,ave,norm}}$ ($F = 16.770$, $p < 0.0005$). Figure 4 illustrates this result with $P_{\text{pk,ave}}$ data, as that metric had the greatest number of significant positive correlations to infused bladder volume and generated pressures. Tukey’s HSD post-hoc tests confirmed that these effects were due to sacral stimulation being significantly better than either lumbar stimulation or no stimulation (all $p < 0.01$), with lumbar and no stimulation being statistically equivalent ($p > 0.9$) in their reflex suppression efficacy.

In the positive responder, both the 5 cm by 5 cm patch electrode (on the S1-S3 dermatomes) and the 19 mm disk electrode (on the S2 dermatome) were effective. A one-way ANOVA ($n = 46$ trials) showed a significant effect of electrode size on all urethral reflex metrics (all $F > 3$, all $p \leq 0.05$). Post-hoc tests with Tukey’s HSD confirmed that the two electrode sizes were equivalent in their reflex suppression efficacy (all $p > 0.07$). Each patch significantly reduced $P_{\text{pk,ave}}$ and $P_{\text{pk,ave,norm}}$ relative to no surface stimulation (all $p < 0.002$). For $R_{\text{pk}}$, only the larger patch had a significant effect relative to no stimulation ($p = 0.041$). Separately analyzing the disk electrode trials ($n = 24$) with an independent samples $t$-test, $P_{\text{pk,ave}}$ and $P_{\text{pk,ave,norm}}$ were both significantly reduced (all $p < 0.01$), as was the case when a $t$-test performed on all Cat 6 trials.
In the positive responder, the current amplitude of sacral surface stimulation (S1-S3, 5 cm by 5 cm electrodes or S2, 19 mm diameter disk electrode) was also varied \((n = 68\) trials). A one-way ANOVA revealed a significant effect of amplitude on \(P_{\text{spk,ave}}\) and \(P_{\text{spk,ave,norm}}\) \((all \ F > 25, all \ p < 0.0005)\), illustrated for \(P_{\text{spk,ave}}\) in Figure 5. Tukey’s HSD post-hoc tests confirmed that “full” surface stimulation amplitude \((0.1\ mA \text{ below the visible muscle fasciculation or urethral response thresholds})\) was superior to either “half” amplitude stimulation or no stimulation \((all \ p < 0.0005)\). Indeed, “half” stimulation and no stimulation were statistically equivalent \((all \ p > 0.8)\).

Duty cycle was varied in randomized and counterbalanced fashion only in the negative responder between no surface stimulation \((0\% \text{ duty cycle})\), 10\% duty cycle \((0.1\ s \text{ on} / 0.9\ s \text{ off})\), 50\% duty cycle \((0.5\ s \text{ on} / 0.5\ s \text{ off})\), and 90\% duty cycle \((0.9\ s \text{ on} / 0.1\ s \text{ off})\). However, the small number of trials \((n = 16\) total) did not provide statistical robustness. As a result, a one-way ANOVA showed no significant effect on any reflex metric \((all \ F < 1.9, all \ p > 0.19)\).

In the positive responder, a 5\% lidocaine patch applied for 10 minutes to the portions of the S1-S3 dermatomes receiving surface stimulation with a 5 cm by 5 cm patch electrode did not affect urethral reflex suppression. It was not possible, however, to determine how much lidocaine was actually absorbed by the skin during this brief time of application.
Discussion

This empirically-driven study provides additional experimental support of the novel acute spinal feline preparation previously developed (Mariano et al. 2009b) for Aim 2b (Chapter 4). The present results partially characterize the neurophysiology underlying these reflexes and suggest key parameters governing the observed reflex modulation. Fewer cats responded to surface stimulation in this study, emphasizing the vastness of the parameter space and the need for additional acute and chronic animal experiments—as well as eventual human studies.

Neurophysiological Characterization

All eight cats exhibited urethral reflexes following bladder stimulation as compared to seven of nine cats in our previous study—making 15 of 17 cats overall. Observation of aberrant urethral reflexes was possible because felines recover rapidly from the acute phase of spinal shock (McCouch et al. 1935; Boggs et al. 2005; Mariano et al. 2009b). The reflexes were dependent on bladder volume and occurred at a fraction of $V_{\text{leak}}$. The best urethral reflex metrics were $P_{\text{spk,ave}}$ and $R_{\text{spk}}$ and the best urodynamic predictors of reflex activity were $P_{\text{ur,ave}}$ and $P_{\text{ur,max}}$, generally consistent with our prior findings (Mariano et al. 2009b).

The reflexes could not be observed during bladder drive due to simultaneous direct activation of the EUS by the extradural sacral root stimulation. The observed acute reflexes are not necessarily neurophysiologically equivalent to chronic DSD, although acute SCI excitatory models have been used as an experimental tool (Boggs et
al. 2005; Boggs et al. 2006a; Boggs et al. 2006b) prior to chronic SCI (Tai et al. 2008) or human studies. The acute reflexes are functionally similar to those seen in humans with chronic SCI after sacral stimulation (Kirkham et al. 2002).

Prior work has suggested that chronic post-SCI urethral reflexes in the feline may be flow-mediated (Galeano et al. 1986). Evidence for flow or stretch receptors in the urethra exists (Thor and Muhlhauser 1999), and urethral afferents have been implicated in coordinating bladder and EUS activity (Barrington 1928; Barrington 1941; Shefchyk and Buss 1998). The positive correlations between bladder test volume, urethral pressures, and $P_{spk,ave}$ are consistent with this notion; cats with higher test volumes (i.e. greater infused volumes at which urethral reflexes were most robust) had higher urethral pressures and therefore larger reflex “spikes.” Bladder contractions driving fluid into the urethra, thereby activating urethral stretch or flow receptors that mediate an urethro-urethral reflex arc is one plausible mechanism supported by the data. The negative correlations between urethral pressures and $R_{spk}$ additionally suggest that these reflexes reduce in frequency—but rise in amplitude—as urethral pressure increases. Similar results were found in our prior study (Mariano et al. 2009b), providing further corroboration.

The reversible abolishment by isoflurane of the post-spinalization urethral responses is consistent with them being reflexive. It is already known (Mariano et al. 2009b) that these reflexes are not an artifact of the bladder stimulation modality—as they occur after both direct sacral drive and reflex bladder activation with pudendal nerve drive (Shefchyk and Buss 1998; Boggs et al. 2005)—and were present under both
α-chloralose and sodium pentobarbital anaesthesia. Future neurophysiological studies aimed at non-traumatically disconnecting the bladder from the urethra—such as by a small balloon catheter with additional lumina for bladder and urethral pressure management—should reveal further details of these LUT reflex circuits. A non-traumatic method of urethro-vesical disconnection would be a marked improvement over prior experiments that have utilized a ligature or clamp (Galeano et al. 1986) and may therefore have damaged the nervous supply of urethral structures (Martin et al. 1974; Mariano et al. 2008b).

Reflex Modulation

Reflex modulation was obtained in two of eight cats—of which only one was a positive responder. Previously in Chapter 4 (Mariano et al. 2009b), four cats were positive responders. Experimental techniques were standardized and kept identical to those used in the previous study. Therefore, considering the seven cats of that study that exhibited urethral reflexes in addition to the eight of this study, reflex suppression was obtained in five of a total 15 cats. Even with a surface stimulation efficacy of 33% for suppressing urethral reflexes, an eventual clinical implementation remains feasible, because the proposed stimulation modality is inexpensive and easily implemented without surgery. For example, stimulation of sacral dermatomes with commercially-available patch electrodes or cutaneous wire electrodes could be combined with suprapubic tapping for generation of bladder contractions in individuals with SCI.
The present results are consistent with the previous “control test” (Mariano et al. 2009b) suggesting that sacral dermatomes were more excitable to surface stimulation than were lumbar dermatomes. The greatest reflex suppression occurred with electrodes placed on the sacral dermatomes. In the positive responder, reflex modulation did not depend on electrode size, with a 19 mm diameter disk electrode on the S2 dermatome essentially as effective as a 5 cm by 5 cm electrode on the S1-S3 dermatomes. However, feline dermatomes overlap (Kuhn 1953; Brown and Fuchs 1975), as do those of humans (Greenberg 2003; Lee et al. 2008). This imprecision may partially explain the lack of definitive reflex modulation with stimulation of multiple non-adjacent dermatomes (L5, L7, S2, and S4) in Cats 1-4. Dermatome mapping was not done, as this surgically invasive and time-consuming process would have precluded other experimentation. Nonetheless, future examination of feline lumbosacral dermatomes to construct a “best case” dermatome map would be beneficial.

It is possible that the noted sacral dermatome stimulation effect was due to activation of pudendal nerve branches as the placement of the surface electrode was superficial to the approximate course of the nerve (Martin et al. 1974; Mariano et al. 2008b). However, dissection underneath the electrode location noted only fat and no superficial nerve fibers, suggesting that sensory nerves of the skin provided the afferent pathway responsible for reflex suppression. Although the lidocaine patches failed to degrade suppression efficacy—as would be expected if such suppression were mediated by afferent nerves of the skin—this may have been due to the pharmacokinetics of transdermal lidocaine absorption, given that the patches were designed for slow release
in treating chronic pain in humans. Injectable local anaesthetic was not considered as an alternative since the skin would need to be pierced multiple times to obtain analgesia of a large area, thus risking unwanted nociceptive input before the drug took effect. A better option would be a topical anaesthetic gel, similar to that used in pediatrics.

Another key factor for successful reflex suppression was use of sufficiently high stimulation current amplitudes, as the “low” amplitude was equivalent to no stimulation in the positive responder. These thresholds did have a tendency to drift upwards slowly as the experiment progressed, necessitating periodic adjustment of the stimulation amplitude. Despite the care taken to use fresh electrodes firmly applied to shaved and depilated skin, slow loss of electrode adhesion—with concomitant change in electrode impedance—was the likely cause of threshold rises. This drift has implications for an eventual clinical system. Although surface electrodes may be optimal for a short-term clinical test, a more permanent solution might employ cutaneous wire electrodes with leads tunneled to an implanted stimulator. Such a configuration would still be far less invasive than current systems requiring laminectomy and irreversible dorsal rhizotomy.

Although brief tests suggested that the 90% duty cycle (0.9 s on / 0.1 s off) was more effective in the positive responder—as opposed to the 50% duty cycle typically used—an insufficient number of trials were performed to allow a full examination. It is expected that the stimulation pattern will be a key parameter, as is the case for reflex bladder activation (Bruns et al. 2008; Bruns et al. 2009). The optimal stimulation pattern is also expected to vary from species to species. The present results narrow the
parameter space regarding electrode location, size, and stimulation amplitude, but work remains for optimizing the pattern.

**Conclusions**

The present study was observational in nature, arising from initial canine experiments (Bhadra et al. 2006c) and our work with the acute spinal feline preparation (Mariano et al. 2009b). The present findings provide additional evidence in support of this novel preparation, eliciting aberrant urethral reflexes in another series of feline experiments. The neurophysiological mechanisms underlying these reflexes were studied. The results are consistent with these responses being reflexive and suggest that they are mediated by urethral afferents sensing fluid pressure or flow.

The reflex modulation results also suggest key parameters likely governing the efficacy of surface stimulation, such as dermatomal location and stimulation amplitude. Conversely, electrode size does not seem to be an important factor. Thorough knowledge of these key parameters will be critical to refining the human anatomical target in future clinical studies and to informing the design of new neuroprosthetic or pharmacological therapies.

The reduced efficacy of surface stimulation in this study underscores the need for additional studies—both to reveal further the neurophysiological mechanisms underlying the reflexes and to optimize the surface stimulation pattern for reflex suppression. Acute spinal studies with a decerebrate feline preparation would eliminate possible confounds resulting from anaesthesia, especially since α-chloralose may
adversely affect LUT function (Rudy et al. 1991). Chronic feline studies would permit comparison of acute and chronic post-SCI aberrant urethral reflexes. Such future experiments would improve the efficacy of surface stimulation for suppressing aberrant urethral reflexes and ultimately improving voiding. Given the low cost and minimally invasive nature of this modality, clinical applications are vast even if efficacious in only a minority of patients.

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Figure 1. Photograph of a cat with dermatome boundaries marked and surface electrodes applied. The black and red solid lines delineate the L5 and L7 dermatomes, whereas the dashed black and red lines indicate the S2 and S4 dermatome boundaries (Kuhn 1953). Nineteen-millimeter-diameter stimulating electrodes have been placed on the L5, L7, S2, and S4 dermatomes. A 5 cm by 5 cm return electrode is located on the L3 and L4 dermatomes.
Figure 2. Representative trials from Cat 4 (top panel) and Cat 6 (bottom panel) illustrating shorter and longer durations of aberrant urethral reflexes. In both panels, the black trace is $P_{ur}$, the gray trace is $P_{ves}$, and the horizontal black bar indicates application of sacral root stimulation. Note the slightly different horizontal axis scaling.
Figure 3. The isoflurane test performed in Cat 6 was consistent with the aberrant urethral responses being reflexive. Duration of isoflurane administration, percent concentration, and corresponding mean arterial pressure (MAP) as measured by an automatic sphygmomanometer are indicated. Note the progressive diminishment of urethral reflexes with isoflurane administration and the progressive recovery after isoflurane withdrawal.
**Figure 4.** The effect of dermatome on reflex suppression in the positive responder (Cat 6). The lumbar electrode was 5 cm by 5 cm, placed on the L4-L7 dermatomes. The sacral electrode was either a 5 cm by 5 cm patch on the S1-S3 dermatomes or a 19 mm diameter disk on the S2 dermatome. A 5 cm by 5 cm return electrode was placed on the L4-L5 dermatomes. All electrodes were ipsilateral. Highly significant ($p < 0.002$) post-hoc tests (Tukey) are indicated (**). Error bars represent ± 1 SEM (standard error of the mean).
Figure 5. The effect of surface stimulation amplitude on reflex suppression in the positive responder (Cat 6). “Full” amplitude was 0.1 mA below the visible muscle fasciculation or urethral response thresholds; “half” amplitude was one-half this level. The stimulating electrode was either a 5 cm by 5 cm patch covering portions of the S1-S3 dermatomes or a 19 mm diameter disk on the S2 dermatome. The ipsilateral 5 cm by 5 cm return electrode was located on either the thigh or the L4-L5 dermatomes. Highly significant ($p < 0.0005$) post-hoc tests (Tukey) are indicated (**). Error bars again represent ± 1 SEM.
CHAPTER 6. GENERAL DISCUSSION AND CONCLUSIONS
Overview

Aberrant urethral reflexes following neurological injury or disease are a chief component of voiding dysfunction, preventing micturition and leading to serious medical complications. Current neuroprostheses—such as the Brindley implant—require irreversible dorsal rhizotomy, permanently eliminating remaining perineal sensation and desirable reflexes (Creasey et al. 2001; Gaunt and Prochazka 2006).

This project has therefore striven to use stimulation of afferent pathways to reduce aberrant urethral reflexes in animal preparations. Aim 1 attempted to demonstrate in canines stimulation of the dorsal sacral roots combined with efferent stimulation of the ventral roots for bladder excitation. In Aim 2, improved neuroanatomical homology between feline and human was noted (Aim 2a), supporting development of a novel acute spinal feline preparation exhibiting aberrant post-sperinalization urethral responses (Aim 2b) functionally similar to those seen in humans with chronic SCI (Kirkham et al. 2002). The data were consistent with these responses being reflexive and mediated by urethral stretch or flow afferents (Aim 2c). The aberrant reflexes occurred with both extradural sacral root and reflex pudendal nerve (Boggs et al. 2005) bladder excitation (Aim 2b). Non-surgical afferent stimulation of the sacral dermatomes reversibly suppressed these reflexes, with electrode location and stimulation amplitude being putative governing variables (Aims 2b and 2c). Further optimization of the stimulation pattern in both animals and humans will improve reflex suppression efficacy and thus restore voiding. The low cost of this innovative modality
of non-invasive and rhizotomy-free reflex suppression make it an attractive clinical alternative for treatment of voiding dysfunction.

The present chapter begins with sequential reviews of each of the four specific aims or sub-aims. Integrative conclusions then segue to future work, including a proposed human study and discussion of various configurations of an envisioned clinical device.

**Aim 1: Production of Voiding by Sensory-Motor Root Activation in the Canine**

Overall, the elicited bladder pressures were insufficiently high to drive significant voiding. As a result, dorsal root stimulation had no significant effect on generated pressures or voiding. Although there is marginal evidence suggesting a slight effect of afferent stimulation (Figures 4 and 5 of Chapter 2), poor repeatability resulted in too few trials obtained. The voiding increase seen was *statistically* significant, but the corresponding voiding efficiency was below 15% and therefore not of *clinical* significance.

Amounts voided were significantly correlated to elicited bladder pressures, suggesting that low maximum evoked bladder pressures (averaging $25.12 \pm 1.17$ cm H$_2$O) were the proximate cause of the meager voiding (averaging $5.0 \pm 0.4$ mL and generally below 20% voiding efficiency). Indeed, in one dog with larger generated bladder contractions (average $P_{ves,max,evoked}$ of $54.04 \pm 2.20$ cm H$_2$O), greater voiding subsequently occurred (average $V_{void}$ of $12.4 \pm 1.3$ mL and a voiding efficiency of 61%).
Three prior canine studies by Bhadra and associates that used similar methods were examined for comparison. The first of these (Grunewald et al. 1998) had evoked $P_{ves}$ values of 45 cm H$_2$O ($n = 19$ females, rectangular drive). The second (Bhadra et al. 2002) had evoked $P_{ves}$ values of 65 cm H$_2$O ($n = 8$ females, intermittent drive). The third comparison study (Bhadra et al. 2006b) had evoked $P_{ves}$ values of 70-90 cm H$_2$O ($n = 3$ females, $n = 2$ males). All studies had dogs in which evoked bladder pressures were generally two or three times greater than those of Chapter 2, suggesting that an evoked $P_{ves}$ of 50 cm H$_2$O appears to be the minimum threshold for overcoming urethral resistance and generating significant voiding.

A thoroughgoing review of confounding factors potentially responsible for the poor evoked $P_{ves}$ and $V_{void}$ values was conducted. Extensive troubleshooting then occurred throughout the course of the experiments.

Electrodes were implanted on the correct roots for eliciting maximal bladder activation. Stimulation of roots S1-S3 (with levels verified by post-mortem dissection) elicited the largest bladder contractions and therefore received spiral electrodes. These sacral root levels agree with prior work by Bhadra et al. in analogous preparations (Grunewald et al. 1998; Bhadra et al. 2001; Bhadra et al. 2002; Bhadra et al. 2006b). Additionally, the bladder and urethral response thresholds for the electrodes used in the present study were low. It is therefore unlikely that incorrect electrode placement or excessively high nerve/electrode interface impedances were responsible for the meager $P_{ves}$ responses. Nevertheless, electrode design was improved during the study with introduction of a tighter spiral cuff having a lower cuff-to-nerve ratio.
Modifications to the stimulation regime also occurred, including phase-locking the ventral and dorsal stimulation channels, employing voltage-controlled stimulation, and replacing stimulator hardware due to software glitches discovered with the DS8000. None of these changes appreciably affected urodynamic responses.

Surgical complications were noted in the first (bladder line remediation, laminectomy at incorrect levels, spinal cord damage), second (femoral arterial line hematoma, bladder line remediation), seventh (cord contusion), and ninth (carotid line hematoma) dogs. Continual refinements were therefore made in the animal preparation and surgical technique to minimize trauma. The femoral line was switched to a carotid line in response to an observed hematoma. The suprapubic bladder line was replaced with a triple-lumen urethral catheter, thus eliminating an entire surgical procedure. The 9 F triple-lumen catheter was 3 mm in diameter, close to the 2.67 mm diameter of the 8 F single-lumen urethral catheter previously used and around which fluid can flow (Bhadra et al. 2002; Bhadra et al. 2006b). Although these efforts improved the stability of the animal preparations, urodynamic responses were unimproved.

Minor potential confounds were also addressed. Vaginal bleeding, suggesting active estrus, was noted in three dogs. Estrus can impair urodynamic responses in the female canine (Hamaide et al. 2005). Therefore, the last four dogs were monitored for estrus, but no clinical signs of menstruation were observed. Additionally, the laboratory animal supplier was switched from a random source (LBL) to a purpose-bred (Covance)
facility for the final three dogs. As before, these efforts did not improve the obtained urodynamic responses.

In sum, the overriding difficulty of this study was generation of bladder pressures insufficiently great to produce significant voiding. None of the extensive troubleshooting measures undertaken resulted in material improvements in the urodynamic responses.

It is possible that despite the great care taken during surgical procedures, subtle damage occurred to the sacral roots or spinal cord itself and that this damage precluded recovery of responses in the limited timeframe of an acute experiment. Therefore, in future experiments, chronic spinal intact dogs might provide a superior experimental alternative. Unlike in an acute preparation, recovery from minor nerve injury during cuff implantation is possible with a chronic animal, thus removing a major potential experimental confound. More testing sessions are also possible in a chronic animal, thus reducing the number of animals needed for a study. Use of male dogs for these chronic experiments would eliminate the further complication of estrus. However, special preparation is required for maintaining aseptic technique during chronic surgical procedures and for providing animal husbandry for long durations. Chronic spinal dogs would further permit observation of post-spinalization reflex behavior approximating the DSD seen in humans with SCI, but additional animal welfare and husbandry concerns would necessarily arise.

Yet another future option would be to attempt the experiments in acute cats with intradural electrodes, given this species’ greater experimental robustness (see
Chapter 4). However, due to the much smaller size of intradural roots in the cat, there is a reduced ability to manufacture cuffs with an appropriate cuff-to-nerve ratio and a greater risk of nerve injury during electrode implantation.

Given the lack of success with the acute female canine preparation despite exhaustive troubleshooting, it was not possible to justify—scientifically or ethically—additional experiments beyond the initial eleven. It is anticipated that at least one of the proposed future approaches will yield better experimental results able to address with the original device demonstration goal.

**Aim 2a: Pudendal Nerve Neuroanatomy of the Feline**

The human DNP usually arises from the PN distal to the EAS (inferior rectal) branch but separately from the perineal nerve (Juenemann et al. 1988; Shafik et al. 1995; Schraffordt et al. 2004; Gustafson et al. 2005). This is largely analogous to the described feline neuroanatomy, in which the DNP arises distal to the EAS and AFF branches but is itself a distal continuation of the DPN as opposed to a separate terminal branch of the PN. The distal human urethra is innervated by the DNP (Yang and Bradley 1998a; Yang and Bradley 1998c), a finding that can be extended to the feline based on urethral stimulation results (Bradley et al. 1973) when taken in light of our results.

Serial histological cross sections confirmed the results of the gross dissections, demonstrating that the DPN supplies both the DNP and EUS, thus suggesting a mixed afferent and efferent function of the feline DPN. This finding is in contrast to prior work showing a dedicated sensory PN branch giving rise to the DNP and a dedicated motor
branch supplying the DPN (Martin et al. 1974). However, in this prior work, the
distribution of myelinated fibers in the DPN and DNP was found to be 46% afferents
(Martin et al. 1974), a result consistent with it giving rise to a large afferent nerve such
as the DNP—itsel found to be 96% afferents. Therefore, segregating the male feline PN
into anatomically separate sensory and motor branches is an oversimplification that
does not fully acknowledge the homology. These results offer further proof of the
suitability of the feline as a neuroanatomical model for human genitourinary studies.

Aim 2b: Surgically Non-Invasive Urethral Reflex Suppression in the Acute Spinal Feline

This study demonstrated production of aberrant urethral reflexes in an acute
spinal feline preparation. These robust reflexes were then suppressed by electrical
surface stimulation of the sacral dermatomes. This non-surgical approach has
significant clinical potential for reducing undesirable urethral reflex activity in people
with SCI or other neurological disorders, and it could be incorporated into future
bladder neuroprostheses.

Urethral reflexes following acute spinal transection may not be
neurophysiologically equivalent to detrusor-sphincter dyssynergia (DSD) resulting from
chronic neurological changes after SCI. Therefore, although this acute preparation has
considerable experimental utility, further study in chronic animal preparations is
necessary to demonstrate effectiveness for DSD.

Although post-SCI urethral reflexes are usually seen chronically and may result
from urethral flow (Galeano et al. 1986), acute SCI excitatory afferent models have been
used to examine spinal neural circuits and to obtain insight (Boggs et al. 2005; Boggs et al. 2006a; Boggs et al. 2006b) before validation in chronic SCI (Tai et al. 2008) and human genitourinary studies.

Urethral reflexes could not be observed during bladder drive due to simultaneous direct sphincter activation from the extradural sacral root stimulation. Therefore, urethral reflexes were observed only after bladder pressure was decreasing. Nonetheless, these reflexes are similar to DSD. The reflexive EUS activity observed after sacral stimulation (Figure 1 of Chapter 4), presumably due to urethral fluid flow, is similar to that observed in humans with chronic SCI receiving intermittent sacral stimulation (Kirkham et al. 2002). In humans, this post-stimulus reflexive EUS activity (DSD) prevents voiding and requires a dorsal rhizotomy to attain voiding. Canine studies have shown a similar result (Bhadra et al. 2006b).

Bladder excitation modality did not affect urethral reflexes, as they could also be elicited with pudendal nerve mediated bladder contractions and then suppressed with surface stimulation. Therefore, the urethral reflexes were not an artifact of extradural sacral root activation, which could indirectly activate afferent pathways. Anaesthetic agents also did not materially affect responses. The reflexes were absent in spinal-intact animals and occurred at physiologically normal bladder volumes.

Urethral pressure responses include non-EUS activity from the pelvic floor and urethral smooth muscle. However, the time constant of the repeated, rapid, spike-like contractions is consistent with skeletal muscle. Pelvic floor contractions were also observed in synchrony with urethral pressure spikes. Future studies could eliminate
smooth muscle effects with blocking agents. Nonetheless, the non-EUS contributions in the present study are functionally relevant as they could also impede voiding.

The choice of recorded values for analysis was motivated by urodynamic variables that would be easily obtainable in a clinical setting with existing equipment and techniques, although these quantities usually are not examined in this fashion. Quantification was consistent and unbiased using an automated computer algorithm. Statistical analysis indicated that the best urethral reflex metrics were $R_{spk}$, $P_{spk,ave}$ and $P_{spk,ave,norm}$ and the best urodynamic predictors of urethral reflex activity were $P_{ur,ave}$ and $P_{ur,max}$.

Urethral reflex contractions were suppressed by surface stimulation in three of six cats with sacral bladder excitation and in the one cat with bladder excitation via the pudendal nerve. $R_{spk}$, $P_{spk,ave}$ and $P_{spk,ave,norm}$ were sensitive metrics as they showed significant decreases and increases in the positive and negative responders (with the exceptions of $R_{spk}$ and $P_{spk,ave,norm}$ in the positive responder two-way ANOVA). Even in the negative responders, there was a statistically significant increase in all urethral reflex metrics. Although the opposite of what occurred in Cats 1-3, the statistical significance of these results demonstrates that surface stimulation nevertheless modulated the neurophysiologic response in these animals. It is hypothesized that these effects could be afferent-mediated, although additional control tests would be necessary to rule out other influences.

The parameter space for surface stimulation of the sacral dermatomes was not fully explored; therefore, the observed reflex suppression effect was not optimized.
Some variation of surface stimulation duty cycle did occur, although the majority of
trials followed the 0.5 s on / 0.5 s off intermittent paradigm. Surface stimulation
amplitude also varied but for most trials remained below the threshold for visible
muscle fasciculations in the tested animal. Higher stimulation amplitudes increased the
urethral reflexes—perhaps by an afferent-mediated reflex—and would therefore
impede voiding.

Feline dermatomes are not well defined and may overlap (Kuhn 1953; Brown
and Fuchs 1975); similar variability has been noted in humans (Greenberg 2003; Lee et
al. 2008). However, there is evidence to suggest a dermatomal specificity for the
suppression effect. In Cat 6, a control test was performed, comprising a series of 12
trials in which only surface stimulation was applied at random to two identical
electrodes placed over lumbar (L3-L4) or sacral (S1-S3) dermatomes. For each trial, the
stimulus amplitude was slowly increased from 2 mA to 15 mA, and the amplitude at
which urethral responses first appeared was noted. This control test showed that the
lumbar dermatomes required greater stimulation (2.4 times the visible muscle
fascication threshold of 5.6 mA) to evoke urethral responses than did the sacral
dermatomes (1.4 times the fascication threshold of 8.5 mA), suggesting a greater
excitability of the sacral dermatomes to surface stimulation than of the lumbar
dermatomes. Despite the limited parameter variation in the present study, the results
demonstrate functional improvement using a non-surgical and rhizotomy-free
stimulation modality.
Aim 2c: Characterizing Aberrant Urethral Reflexes and Their Modulation

This empirically-driven study provides additional experimental support of the novel acute spinal feline preparation previously developed (Mariano et al. 2009b) for Aim 2b (Chapter 4). The present results partially characterize the neurophysiology underlying these reflexes and suggest key parameters governing the observed reflex modulation. Because fewer cats responded to surface stimulation, the neurophysiological characterization is the more compelling result and is intended to be a stand-alone manuscript.

Neurophysiological Characterization

All eight cats exhibited urethral reflexes following bladder stimulation as compared to seven of nine cats in our previous study—making 15 of 17 cats overall. Observation of aberrant urethral reflexes was possible because felines recover rapidly from the acute phase of spinal shock (McCouch et al. 1935; Boggs et al. 2005; Mariano et al. 2009b). The reflexes were dependent on bladder volume and occurred at a fraction of V_{leak}. The best urethral reflex metrics were \( P_{spk,ave} \) and \( R_{spk} \) and the best urodynamic predictors of reflex activity were \( P_{ur,ave} \) and \( P_{ur,max} \) generally consistent with Aim 2b.

The reflexes could not be observed during bladder drive due to simultaneous direct activation of the EUS by the extradural sacral root stimulation. The observed acute reflexes are not necessarily equivalent to chronic DSD, although acute SCI excitatory models have been used as an experimental tool (Boggs et al. 2005; Boggs et
al. 2006a; Boggs et al. 2006b) prior to chronic SCI (Tai et al. 2008) or human studies. Furthermore, the acute reflexes are functionally similar to those seen in humans with chronic SCI after sacral stimulation (Kirkham et al. 2002).

Prior work has suggested that chronic post-SCI urethral reflexes in the feline may be flow-mediated (Galeano et al. 1986). Evidence for flow or stretch receptors in the urethra exists (Thor and Muhlhauser 1999), and urethral afferents have been implicated in coordinating bladder and EUS activity (Barrington 1928; Barrington 1941; Shefchyk and Buss 1998). The positive correlations between bladder test volume, urethral pressures, and $P_{spk,ave}$ are consistent with this notion; cats with higher test volumes (i.e. greater infused volumes at which urethral reflexes were most robust) had higher urethral pressures and therefore larger reflex “spikes.” Bladder contractions driving fluid into the urethra, thereby activating urethral stretch or flow receptors that mediate an urethro-urethral reflex arc is one plausible mechanism supported by the data. The negative correlations between urethral pressures and $R_{spk}$ additionally suggest that these reflexes reduce in frequency—but rise in amplitude—as urethral pressure increases. Similar results were found in Aim 2b.

The reversible abolishment by isoflurane of the post-spinalization urethral responses is consistent with them being reflexive. Aim 2b demonstrated that these reflexes are not an artifact of the bladder stimulation modality—occurring after both direct sacral drive and reflex bladder activation with pudendal nerve drive (Shefchyk and Buss 1998; Boggs et al. 2005)—and occur under both $\alpha$-chloralose and sodium pentobarbital anaesthesia. Future neurophysiological studies aimed at non-
traumatically disconnecting the bladder from the urethra—such as by a small balloon catheter with additional lumina for bladder and urethral pressure management—should reveal further details of these LUT reflex circuits. A non-traumatic method of urethrov-vesical disconnection would be a marked improvement over prior experiments that have utilized a ligature or clamp (Galeano et al. 1986) and may therefore have damaged the nervous supply of urethral structures (Martin et al. 1974; Mariano et al. 2008b).

**Reflex Modulation**

Reflex modulation was obtained in two of eight cats—of which only one was a positive responder. Experimental techniques were standardized and kept identical to those of Aim 2b, which had four positive responders. Therefore, considering the seven Aim 2b cats that exhibited urethral reflexes in addition to the eight of this study, reflex suppression was obtained in five of a total 15 cats. Even with a surface stimulation efficacy of 33% for suppressing urethral reflexes, an eventual clinical implementation remains feasible, because the proposed stimulation modality is inexpensive and requires minimal surgery. For example, stimulation of sacral dermatomes with commercially-available patch electrodes or cutaneous wire electrodes could be combined with suprapubic tapping for generation of bladder contractions in individuals with SCI.

The present results were consistent with Aim 2b’s “control test” suggesting that sacral dermatomes were more excitable to surface stimulation than were lumbar dermatomes. The greatest reflex suppression occurred with electrodes placed on the
sacral dermatomes. Reflex modulation in the positive responder did not depend on electrode size, with a 19 mm diameter disk electrode on the S2 dermatome essentially as effective as a 5 cm by 5 cm electrode on the S1-S3 dermatomes. However, feline dermatomes overlap (Kuhn 1953; Brown and Fuchs 1975), as do those of humans (Greenberg 2003; Lee et al. 2008). This imprecision may partially explain the lack of definitive reflex modulation with stimulation of multiple non-adjacent dermatomes (L5, L7, S2, and S4) in Cats 1-4. Dermatome mapping was not done, as the surgically invasive and time-consuming process would have precluded other experimentation. Nonetheless, future examination of feline lumbosacral dermatomes to construct a “best case” dermatome map would be beneficial.

It is possible that the noted sacral dermatome stimulation effect was due to activation of pudendal nerve branches as the placement of the surface electrode was superficial to the approximate course of the nerve (Martin et al. 1974; Mariano et al. 2008b). However, dissection underneath the electrode location found no superficial nerve fibers, suggesting that sensory nerves of the skin provided the afferent pathway responsible for reflex suppression. Although the lidocaine patches failed to degrade suppression efficacy—as would be expected if such suppression were mediated by afferent nerves of the skin—this may have been due to the pharmacokinetics of transdermal lidocaine absorption, given that the patches were designed for slow release in treating chronic pain in humans. Injectable local anaesthetic was not considered as an alternative since the skin would need to be pierced multiple times to obtain analgesia of a large area, thus risking unwanted nociceptive input before the drug took effect.
Another key factor for successful reflex suppression was use of sufficiently high stimulation current amplitudes, as the “low” amplitude was equivalent to no stimulation in the positive responder. These thresholds did have a tendency to drift upwards slowly as the experiment progressed, necessitating periodic adjustment of the stimulation amplitude. Despite the care taken to use fresh electrodes firmly applied to shaved and depilated skin, slow loss of electrode adhesion—with concomitant change in electrode impedance—was the likely cause of threshold rises.

Although brief tests suggested that the 90% duty cycle (0.9 s on / 0.1 s off) was more effective in the positive responder—as opposed to the 50% duty cycle typically used—an insufficient number of trials were performed for a full examination. It is expected that the stimulation pattern will be a key parameter, as is the case for reflex bladder activation (Bruns et al. 2008; Bruns et al. 2009). The optimal stimulation pattern is also expected to vary from species to species. The present results narrow the parameter space regarding electrode location, size, and stimulation amplitude, but more work remains for optimizing reflex suppression efficacy.

**Putative Mechanism of Urethral Reflex Modulation**

Our data are consistent with a urethro-urethral spinal reflex arc (Galeano et al. 1986) mediated by urethral stretch or flow receptors (Thor and Muhlhauser 1999) being responsible for the aberrant urethral reflexes of Aims 2b and 2c. Figure 1 depicts a possible mechanism by which these reflexes occur and by which surface stimulation modulates them; it integrates the experimental results of this project with prior findings
in the literature. The upper left panel depicts aberrant post-spinalization urethral reflexes that result from an absence of descending control, much like Figure 3 of Chapter 1. Flow of urine into the urethra—such as that following a bladder contraction—activates stretch or flow receptors (gray oval) in the urethral wall, which could be similar in structure to Pacinian corpuscles. Afferent signals from these receptors then travel along a sensory nerve, through the dorsal root ganglion, and back to the spinal cord, where they activate a spinal reflex circuit—presumed to be polysynaptic—that provides excitatory efferent drive (green). This drive results in action potentials that exit the spinal cord via the ventral root and travel down a motor nerve synapsing on the EUS. The action potentials cause the EUS to contract, thus preventing voiding.

In the upper right panel, afferent electrical stimulation is applied to the sacral dermatomes at a particular frequency \( f \) and duty cycle \( D \), exploiting the spinal reflex circuit just described so that aberrant urethral reflexes are suppressed. In essence, the patterned afferent stimulus dominates over the sensory signal generated by urethral stretch or flow receptors. With appropriately chosen \( f \) and \( D \), the reflex circuit provides an overall inhibitory drive (red) that reduces the number of action potentials arriving at the EUS, relaxing the muscle and allowing the passage of fluid. Poorly chosen \( f \) and \( D \) could fail to suppress urethral reflexes (1) by not dominating over the sensory signal from urethral receptors or (2) by amplifying or actively enhancing the sensory signal from those receptors to create an overall excitatory drive that results in stronger EUS contractions.
It is proposed that the difference between a positive responder, a negative responder, or a non-responder is due to the stimulation pattern applied to the sacral dermatomes; the pattern was not optimized for this project. Prior feline work has shown that different stimulation patterns delivered to the same afferent pathway can have disparate—or even opposite—physiological effects on reflex circuits (Boggs et al. 2005; Wenzel et al. 2006; Bruns et al. 2008; Tai et al. 2008). It is therefore not unreasonable to expect a similar dependence for aberrant urethral reflexes.

The bottom panel of Figure 1 illustrates a possible explanation. For a given cat, there may be several regions of the stimulation parameter space; this space has been collapsed to one horizontal dimension representing both f and D in Figure 1 for simplicity. One region may produce reflex exacerbation (solid green distribution) and the other reflex suppression (dashed red distribution), with an ineffective region between them. If the unoptimized stimulation parameters correspond to the blue arrow, then in this particular hypothetical cat, the chosen f and D fall just within the region of reflex suppression. However, if other cats have exacerbation or suppression regions that are slightly shifted (gray arrows), then the chosen f and D may instead fall between the two regions (consistent with a non-responder) or within a region of reflex exacerbation (consistent with a negative responder).

The preceding explanation is fully consistent with the experimental findings of Aims 2b and 2c and prior feline studies. However, the stimulation parameter space remains largely unexplored. Testing the plausibility of the proposed mechanism of reflex modulation would be best achieved through future acute or chronic animal
studies. In one series of experiments, controlled interruptions of the presumed reflex arcs of Figure 1 could be used to clarify the neural circuitry involved. Additional experiments could focus on varying the frequency and duty cycle of surface stimulation to determine if multiple parameter regions exist and if such regions vary between individual cats.

**Integrative Conclusions**

Current urinary neuroprostheses such as the Brindley system are able to improve voiding (Gaunt and Prochazka 2006) and reduce the costs of post-SCI bladder care (Creasey and Dahlberg 2001). However, they require irreversible dorsal rhizotomy, a procedure that causes highly undesirable side effects (Creasey et al. 2001). The animal studies comprising this project demonstrate a rhizotomy-free afferent-based approach for reducing the aberrant urethral reflexes that impede voiding. In particular, non-surgical surface stimulation of the sacral dermatomes suppressed urethral reflexes occurring after acute spinalization. Future human studies will assess clinical validity and refine stimulation particulars for voiding improvement.

Ultimately, it was not possible to duplicate the successful preparation of Bhadra and associates for Aim 1. Despite methodical experimental review and comprehensive troubleshooting, bladder pressures remained too low for appreciable voiding. Nevertheless, the difficulties with the experimental preparation do not invalidate the intradural approach for delivering afferent stimulation. An improved experimental paradigm could use chronic spinal-intact or spinalized male dogs. The species has the
required spinal size for placement of intradural electrodes and the chronic preparation eliminates possible confounds of nerve injury and estrus. Additionally, more testing sessions are possible in a chronic animal. These modified approaches would be more likely to achieve the device demonstration originally intended. If successful in animals, combined sensory and motor intradural stimulation could be tested in human intraoperative subjects. These tests could employ a modified three-channel Brindley implant, as has been previously attempted with limited efficacy (Kirkham et al. 2002), and ultimately lead to demonstration of a fully-implanted neuroprosthetic approach.

The feline studies extend the validity of this species as an appropriate model for human neurourological studies (Aim 2a), making it useful for studying both micturition and sexual function—especially given the proposed role of penile afferents in bladder excitation and ejaculation (Yang and Bradley 1998b; Gustafson et al. 2004). Aims 2b and 2c were empirically-motivated, arising from initial experiments (Bhadra et al. 2006c). The novel acute spinal preparation subsequently developed is a highly useful experimental tool that could aid development of future pharmacological or neuroprosthetic therapies for treating voiding dysfunction. The feline exhibits a rapid recovery from the acute phase of spinal shock. Subsequently, aberrant urethral reflexes occur that are functionally—but not necessarily neurophysiologically—equivalent to DSD observed in human subjects with chronic SCI after intermittent (Brindley) bladder stimulation (Kirkham et al. 2002). The work of Aims 2b and 2c also addresses the neurophysiological mechanisms underlying the observed aberrant urethral reflexes. The results are consistent with the aberrant urethral responses being reflexes and not
artifacts of bladder drive. Additionally, the results suggest that the reflexes are mediated by urethral afferents sensing fluid pressure or flow (Galeano et al. 1986; Thor and Muhlhauser 1999).

**Surgically non-invasive electrical stimulation of the sacral dermatomes was able to suppress these urethral reflexes** reversibly in a third of the cats tested. Beyond this proof-of-concept, the results suggest putative key parameters—such as dermatomal location and stimulation amplitude—governing the modulation of urethral reflexes by surface stimulation.

Knowledge of such parameters will be critical to refining the human anatomical targets in future clinical studies and for informing the design of an envisioned neuroprosthesis that can restore functional bladder voiding without dorsal rhizotomy. Although this project developed a novel feline acute spinal preparation, demonstrated urethral reflex suppression by surface stimulation, explored the associated neurophysiology, and began investigating the surface stimulation parameter space, much work remains to be done—particularly in optimizing the stimulation *pattern*. Acute spinal studies with a decerebrate feline preparation would allow conducting experiments without anaesthesia and thus eliminate a possible confound, especially as α-chloralose may have adverse effects on LUT function (Rudy et al. 1991). However, chronic feline studies are the preferred next step, as they would permit experiments in awake behaving animals, allow comparison of acute and chronic post-SCI responses, and afford opportunities for conducting voiding trials. Such studies would be expected to
improve the efficacy of reflex suppression and voiding while allowing cross-species comparison to the similar chronic studies proposed in dogs.

Despite the limitations, the present results show the significant clinical potential for treating voiding dysfunction afforded by this novel means of reflex suppression. The approach is feasible even if efficacious in a minority of SCI subjects due to the technical simplicity and less surgically invasive nature when compared to the current state of the art. However, the drifting stimulation thresholds noted with surface electrodes could prove inconvenient for a clinical device. Therefore, although surface electrodes may be optimal for a short-term clinical test, a more permanent solution might employ cutaneous wire electrodes with leads tunneled to a superficially implanted stimulator. Such a device would still require far less surgery than do current devices.

A rhizotomy-free, minimally-invasive, and clinically effective means of suppressing DSD and thus restoring micturition would address a major unmet need. Modification of existing devices to add sacral dermatome stimulation would improve device effectiveness and increase clinical acceptance by patients. For example, a rhizotomy-free Brindley implant using surface or cutaneous stimulation to reduce aberrant EUS reflexes and afferent inhibitory stimulation to provide continence could increase the number of individuals choosing such a management approach. Enhanced neuroprostheses incorporating stimulation without rhizotomy or invasive surgery would thus have wide application in alleviation of DSD in humans suffering from voiding dysfunction caused by injury, stroke, or disease—all of which might be achieved by augmenting existing stimulator technology. Such an approach is expected to result in
more rapid clinical deployment, greatly reduced surgical burden, and improvement of patients’ quality of life.

An envisioned rhizotomy-free neuroprosthetic device will rely on future animal and human studies. Such studies will be extensions of the main scientific contributions of this project, which are summarized below.

- A better than previously appreciated neuroanatomical homology was noted between feline and human.
- A novel acute spinal feline preparation exhibiting aberrant urethral reflexes was developed.
- Rhizotomy-free and surgically non-invasive afferent stimulation reversibly suppressed these reflexes in the feline.
- Initial characterization of the neurophysiology of these reflexes and their modulation was achieved in the feline.

**Future Human Studies**

As already discussed, future animal studies should focus on chronic spinal preparations to eliminate confounds resulting from acute damage, anaesthesia effects (Rudy et al. 1991), or lack of neurophysiological equivalence between acute and chronic urethral reflexes. Experimental protocols similar to those used in the preceding chapters could be adapted to chronic experiments, thus maximizing the ability to compare data obtained from acute and chronic preparations.
Continued success with urethral reflex suppression in animals would be strong evidence suggesting that the approach might work clinically for voiding improvement, thus motivating human subjects testing. An experimental setup (Figure 2) and protocol have been prepared for this purpose, with subject recruitment pending. Inclusion criteria are age between 18 and 60 years old, spinal cord injury above T12 (clinically labeled as motor and sensory complete), functional detrusor motor innervation as assessed by cystometry (a 50 cm H2O pressure reflex contraction of the bladder should be observed during slow filling), and signed consent form. Exclusion criteria include low capacity fibrotic bladder (≤ 200 mL), ureteric reflux, prior sphincterotomy, urethral stent, urethral stricture, prostatectomy, presence of pressure sores, or pregnancy.

For testing, a sterile 9 F triple-lumen catheter (TLC-9M, Life Tech, TX) would be inserted into the urethra to allow bladder filling and the measurement of both bladder pressure and urethral pressure at the EUS. Electrical surface stimulation would be delivered by a constant current stimulator. The stimulating electrode would be placed on the S2 dermatome with a return electrode located on the thigh.

The bladder would be filled until a distention-evoked reflex bladder contraction occurred. During filling, randomly selected periods of stimulation would be applied to the surface electrodes. The subjects would then be asked to attempt to induce voiding by suprapubic tapping or pressure with the urethral catheter in place. Such pressure commonly results in contraction of the EUS (the Credé effect), an increase in urethral pressure, and maintained closure of the bladder neck (Barbalias et al. 1983).
procedure would be applied both with and without dermatome stimulation in randomized and counterbalanced fashion while monitoring pressures and any fluid flow.

Effectiveness of surface stimulation would be assessed by urodynamic responses during repeated cystometrograms. The resulting data set would consist of average and maximum bladder and urethral pressures, as well as volume and flow rate of any fluid voided. Independent parameters would include bladder volume, stimulus frequency, and stimulus amplitude. ANOVA would be used to determine the effect of surface stimulation on the measured urodynamic variables.

Above threshold bladder volumes, reflex bladder and EUS contractions would be expected. At lower bladder volumes, a progressive increase in EUS or pelvic floor activity during bladder filling would be anticipated. Based on the foregoing animal studies, recorded urethral pressures should decrease upon stimulation of the sacral dermatomes during reflex-evoked contractions, thus increasing the amount of fluid voided. However, the optimal surface stimulation parameters in humans may differ from those in the feline, requiring tuning of the stimulation pattern. Demonstration of improved voiding in even a single human subject would be a significant result. Such a clinical proof-of-concept would justify the development of an envisioned neuroprosthesis for restoring rhizotomy-free micturition to individuals with SCI.

**Envisioned Clinical Device**

The clinical device for restoring micturition ultimately resulting from this work could take several forms (Figure 3). In all conceptions, afferent stimulation is used for
urethral reflex suppression, obviating the need for irreversible dorsal rhizotomy that accompanies the Brindley approach (first column of Figure 3). The surgical invasiveness of these afferent stimulation modalities is distinct from the loss of function incurred by dorsal rhizotomy—itself an invasive procedure.

The afferent stimulation could be applied at several locations. In Aim 1 (Chapter 2), the afferent pathways were accessed at the level of the dorsal sacral roots, requiring a laminectomy and intradural access (red area of Figure 3). Although the particular experimental preparation was not successful, the scientific approach for an intradural device demonstration remains feasible. Aim 2b (Chapter 4) and Aim 2c (Chapter 5) demonstrated a novel, rhizotomy-free, and surgically non-invasive means of afferent stimulation using self-adhesive electrodes placed on the sacral skin dermatomes (green areas of Figure 3). For a clinical implementation, these electrodes could be configured either as self-adhesive patches or as garment-style electrodes (Patterson and Lockwood 1993; Wiley InterScience (Online service) 2006). Surface stimulation does have disadvantages, however, such as the need for frequent removal and reapplication as well as the necessary readjustment of stimulation amplitudes due to threshold drifts stemming from changes in the electrode-to-skin interface impedance. Therefore, some patients might desire a lower-maintenance approach utilizing cutaneous wire electrodes with leads tunneled beneath the skin to a superficially implanted stimulator (final column of Figure 3). In such patients, the surface stimulation approach would still have use as a screening or diagnostic tool for assessing the expected efficacy of the cutaneous wire approach.
Bladder contractions could be produced by a variety of means, also varying in degree of surgical invasiveness. Efferent stimulation of the ventral sacral roots—via either intradural or extradural electrodes—is the most invasive, and currently occurs with Brindley implants. This bladder drive modality would be most feasible if delivered intradurally and combined with afferent stimulation of the dorsal sacral roots. If opening the dura mater is not desired, then an extradural bladder drive approach could be combined with surface electrode or cutaneous wire afferent stimulation. However, all of these configurations would require the invasive surgical procedure of a laminectomy, which can introduce severe medical complications (Possover 2009).

For patients wishing to avoid a laminectomy, reflex bladder contractions could be elicited via stimulation of peripheral nerves (Boggs et al. 2005; Bruns et al. 2008) such as the pudendal or deep perineal nerves. This bladder drive modality would be most feasible if combined with an afferent stimulation approach requiring little or no surgical burden, such as that offered by using surface or cutaneous electrodes. However, a configuration using dorsal sacral root stimulation would also be technically possible if desired.

Bladder contractions could also be generated manually through suprapubic tapping (e.g. the Credé maneuver). For patients wishing to avoid surgical procedures and minimize the amount of implanted hardware, use of surface electrodes for urethral-reflex-suppressing afferent drive would be the optimal configuration. However, due to the aforementioned drawbacks of surface electrodes, cutaneous wire electrodes and a small implanted stimulator might be a preferable lower-maintenance solution.
No single configuration of an envisioned neuroprosthetic system will apply to all patients with voiding dysfunction, and this project has not attempted to develop a universal solution. Instead, the preceding work suggests numerous rhizotomy-free approaches worthy of additional experimental development and eventual clinical deployment. The proposed modalities for suppressing the aberrant urethral reflexes that impair voiding will greatly expand the armamentarium of neuroprosthetic options available to both researchers and clinicians, providing alternatives with distinct advantages over the present state of the art.
**Figure 1.** Putative mechanism of aberrant urethral reflex modulation. Aberrant urethral reflexes (upper left panel) are depicted as being activated by a urethro-urethral spinal reflex arc mediated by urethral stretch or flow receptors (gray oval). Urethral reflex suppression (upper right panel) occurs when afferent stimulation—such as surface stimulation of the sacral dermatomes—dominates over sensory input from urethral receptors; the result is decreased motor drive of the EUS and concomitant relaxation of the muscle. The lower panel depicts a parameter space (collapsed to a single horizontal dimension for simplicity) with regions of reflex exacerbation (solid green distribution) and reflex suppression (dashed red distribution), with an ineffective region between them. Stimulation at a frequency \((f)\) and duty cycle \((D)\) just within the region of reflex suppression (blue arrow) in one cat may fall within the ineffective region or within the region of reflex exacerbation in other cats due to variability (gray arrows) in the locations of these regions between cats.
Figure 2. Experimental setup for human subjects testing. The equipment is tested and ready for recruitment of an appropriate human subject. Adapted from a diagram by T. Bruns.
### Urethral Reflex Suppression Modality

<table>
<thead>
<tr>
<th></th>
<th>Existing</th>
<th>Afferent Stimulation</th>
<th>Future Afferent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dorsal Rhizotomy</strong></td>
<td><strong>Dorsal Sacral Root</strong></td>
<td><strong>Large Surface Electrode</strong></td>
<td><strong>Cutaneous Wire Electrode</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bladder Drive Modality</th>
<th>Direct (Ventral Sacral Root)</th>
<th>VOCARE (current state of the art, invasive)</th>
<th>Invasive fully implanted system</th>
<th>Less invasive mixed internal and external system</th>
<th>Less invasive implanted system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral (Pudendal Reflex)</strong></td>
<td>Not applicable</td>
<td>Invasive fully implanted system</td>
<td>Less invasive mixed internal and external system</td>
<td>Less invasive implanted system</td>
<td></td>
</tr>
<tr>
<td><strong>Manual (Credé Maneuver)</strong></td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Non-invasive external system</td>
<td>Less invasive mixed internal and external system</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.** Envisioned device matrix, including the current state of the art requiring irreversible dorsal rhizotomy (yellow), the unsuccessful approach of Aim 1 (red), and the successful novel approach of Aims 2b and 2c (green).
APPENDIX. MATLAB DATA PROCESSING ALGORITHM
Overview

This MATLAB algorithm extracts and parses raw data files for experiments with either continuous (i.e. cat) or intermittent (i.e. dog) experiments. Calibrations and zero offsets made in the LabVIEW data acquisition interface are automatically applied to the recorded data. The MATLAB code performs analysis and reports numerous summary values for later statistical analysis. Constants at the beginning of the main extract_data.m file can be changed to tweak parameters for event detection. The extract_data.m file calls the processing_basic.m file (adapted from the original version authored by Adam Boger); both are necessary for proper function.

Functional Description

The code initially prompts the user to input whether bladder drive is intermittent or continuous. The start of a trial is taken to be when bladder stimulation starts. The code handles intermittent stimuli by assuming that discrete stimulus events belong to trials separated by at least intermit_sep seconds. The end of a trial is taken to be when $P_{ur}$ (with a running average taken to remove artifacts) falls within a known fraction, frac_base, of baseline pressure. This baseline is calculated by averaging over the deltaT seconds immediately prior to the start of a trial—but only after refractory seconds have elapsed—in order to prevent missing $P_{ur}$ reflex spikes due to $P_{ur}$ dropping off too soon. Using a criterion involving $P_{ur}$ falling to within $n$ standard deviations (SDs) of baseline did not provide a satisfactory result due to the very small
calculated SDs. If trials are too closely spaced, the end of the current trial is taken as the start of the next trial.

The code finds end-of-stimulus $P_{\text{ves}}$ and $P_{ur}$ values and their difference. The code also quantifies $P_{ur}$ with the cumulative sum (deprecated) and power spectral density (using the `pwelch` function). The code also quantifies $P_{ur}$ by counting the reflex spikes occurring after bladder stimulation ends and calculating their average maximum amplitude. For continuous bladder drive, the code begins searching for spikes at the end of stimulus plus delay seconds to prevent counting any post-stimulus $P_{ur}$ fluid tracking artifact as a spike. Reflex spikes occurring during a trial are detected by high-pass filtering (1 Hz cutoff) the $P_{ur}$ trace and applying a threshold that is ten times the mean of the absolute value of the filtered data vector. The rationale is that this threshold is related to signal power instead of being arbitrary. For intermittent bladder drive, spikes occurring during stimulus off-phases (i.e. between burst cycles) after delay_int seconds are also processed. The delay again combats erroneous reflex spike counting due to any $P_{ur}$ fluid-tracking artifact.

For continuous drive, the average and maximum $P_{ur}$ values are determined for the period during which stimulation was applied. Baseline pressure is also reported, averaged over the deltaT seconds prior to the start of a trial. For intermittent drive, average and maximum $P_{ur}$ during individual burst cycles and intervening off-phases are determined. For the off-phases, processing begins after delay_int seconds to combat any $P_{ur}$ fluid tracking artifact.
$P_{\text{ves}}$ values are characterized without the need for taking a running average due to the lower frequency content of the detrusor smooth muscle response. The start and stop of a contraction are taken to be when $P_{\text{ves}}$ crosses a point $\text{numSD}$ SDs above baseline; the baseline is averaged over the $\text{deltaT}$ seconds before trial start, just as with $P_{\text{ur}}$. There is a separate absolute threshold, $P_{\text{ves\_thresh}}$, to eliminate low-amplitude transients. Candidate contractions shorter than $\text{con\_dur}$ are rejected as short-duration transients. If there is one more contraction stop event than start event, the code takes the end of the last trial in the file as the missing stop event; the assumption is that a premature end-of-file resulted in a “missed” stop event. Baseline pressure is also reported (averaged over $\text{deltaT}$ seconds prior to trial start). The contraction start and stop events are used as limits for determining average and maximum evoked $P_{\text{ves}}$ values.

The code detects whether or not surface stimulus was on during a trial and returns a binary flag. The code detects start and stop of surface stimulus—which is usually intermittent—using the same $\text{intermit\_sep}$ criterion as with detecting bladder drive. The code also calculates the average voided volume for $\text{deltaT}$ seconds before and after bladder stimulation, using these values to compute average volumetric flow (mL/s).

For output, the code plots the PSD for each trial’s raw data, computed from stimulus end to trial end (if continuous bladder drive used) or from bladder stimulus start to trial end (if intermittent bladder drive used). All computed results are saved to a text file with unique name.
MATLAB Code

% extract_data.m
% Tim Mariano
% Created 12/14/2006
% Last update 3/3/2009

% Descrip: Strips out data vector indices and timestamps of experimental trials. Note that using criterion of Peus (Pur) returning to within a certain number of SDs of pre-baseline pressure problematic as some of the resulting pressure differences are too small. Code also performs basic data analysis including spike detection and quantification. Processing of intermittent bladder drive (for dog experiments) implemented 8/27/2008.

clear all;
close all;

% Constants: may need to tweak for individual data files.
intermit_sep = 30;  % Minimum separation between adjacent trials in seconds (default: 30)
base_freq = 2;      % Lower bound of intermittent drive base frequency in Hz default: 2)
deltaT = 3;         % How many sec prior to stim start to grab as baseline (default: 3)
win = 3;            % Size of window for running average calculation in sec (default: 3)
refractory = 10;    % How many sec after stim end before seeking trial end (default: 10)
delay = 1;          % How many sec after stim end before seeking spikes (default: 1)
dly_int = 0.1;      % How many sec after burst end before analyzing off-phase (def: 0.1)
con_dur = 5;        % Minimum contraction duration to eliminate transients (default: 5)
frac_base = 0.1;    % Trial end when Peus within this fraction of baseline (default: 0.1)
numSD = 3;          % Sets max # SDs from pre-baseline Pves for contrac end (default: 3)
Pves_thresh = 10;   % Absolute Pves contrac threshold to eliminate transients (def: 10)
fc = 1;             % Cutoff frequency for HP filtering of Peus signal (default: 1)
mult = 10;          % Multiplier for thresholding of filtered Peus signal (default: 10)
fs = 100;           % Number of samples per second (default: 100)

% Startup text and user prompt for intermittent bladder drive (e.g. dog).
fprintf('
*** Automated Data Extraction and Reduction ***
(c) 2006-2009 Tim Mariano
Not to be used without permission.
');
intermit_user_input = [];
intermit_flag = -1;
while intermit_flag == -1
    % Keeps prompting until valid input.
    intermit_user_input = input('Parse intermittent bladder drive (e.g. dog)?  (Y/N) ','s');
    if intermit_user_input == 'Y' | intermit_user_input == 'y'
        intermit_flag = 1;
    elseif intermit_user_input == 'N' | intermit_user_input == 'n'
        intermit_flag = 0;
    end
    fprintf('
');
end

% Call processing_basic.m to load data. Note that calibrations and zero offsets made in LabView DAQ are automatically applied to recorded data; no further adjustments are necessary in this program.
[filename, data] = processing_basic;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Find start and end of stimulus, recalling that stim is a TTL output that remains high for entire stim duration if continuous. If intermittent, separate handling required (below). Start is flagged 1, end is -1. FOR
% AB FILES, use "data(:,9)*10" (or "data(:,8)*10") to correct for aliasing.
stim_onoff = diff(data(:,9));  % BEFORE B022: "data(:,7)"; ELSE "data(:,8)"
stim_onoff = [0; stim_onoff];  % Correction for one fewer element

% If parsing intermittent stimulus, copy stim_onoff array for special
% processing and display minimum base frequency parameter to user.
if intermit_flag == 1
    burst_onoff = stim_onoff;
    burst_highlow = zeros(1,length(burst_onoff));
    fprintf(['Assumed minimum base frequency of intermittent bladder drive: ',
        num2str(base_freq), ' Hz\n']);
end

% Handles intermittent stim; don't want successive burst cycles to be
% considered separate stim start/stop events. For dog experiments, must
% also parse out individual burst cycles within stim period without
% counting the separate pulses that comprise them.
stim_high = find(stim_onoff > 2);
stim_low = find(stim_onoff < -2);
for v = 2:length(stim_high)
    % If parsing intermittent stim, find start of individual burst cycles
    % within a trial.
    if intermit_flag == 1 && stim_high(v) - stim_high(v-1) < fs/base_freq
        burst_onoff(stim_high(v)) = 0;
    end
    % Extracting true stim start events
    if stim_high(v) - stim_high(v-1) < intermit_sep*fs
        stim_onoff(stim_high(v)) = 0;
    end
end
for w = 2:length(stim_low)
    % For intermittent stim, find stop of individual burst cycles.
    % Extracting true stim stop events
    if stim_high(w) - stim_high(w-1) < intermit_sep*fs
        stim_onoff(stim_high(w-1)) = 0;
    end
end

% For intermittent bladder drive, also need an array that is ones for
% duration of each burst cycle plus "dly_int" secs (to combat Peus fluid
% tracking artifact) and zeroes otherwise. Used for spike counting
% during stim off-phases (below).
if intermit_flag == 1
    for aa = 1:length(stim_high)
        burst_highlow(stim_high(aa):stim_low(aa)+dly_int*fs) = 1;
    end
end

% High-pass filtered Peus trace (cutoff is "fc" Hz) with threshold for spike
% detection. Threshold is ultimately related to power of recorded signal.
filt_spikes = filtfilt(fir1(1000,fc/50,'high'),1,data(:,3));
filt_spikes = filt_spikes - mult*mean(abs(filt_spikes));
spike_on_index = zeros(1,length(stim_onoff));
spike_off_index = zeros(1,length(stim_onoff));

% Create arrays to store indices of stim start and stop and of burst cycle
% start and stop.
stim_on_index = zeros(1,length(stim_onoff));
stim_off_index = zeros(1,length(stim_onoff));
% Now strip out timestamps for start and end of stimulus and for intermittent burst cycles within stimulus period.

for m = 1:length(stim_onoff)
    % Find start and end of stimulus.
    if stim_onoff(m) > 2
        stim_onoff(m) = 1;
        stim_onoff(m-1) = 0;        % In case of false "double" start.
        stim_on_index(m) = m;
        stim_on_index(m-1) = 0;     % In case of false "double" start.
    elseif stim_onoff(m) < -2
        stim_onoff(m) = -1;
        stim_onoff(m-1) = 0;        % In case of false "double" start.
        stim_off_index(m) = m;
        stim_off_index(m-1) = 0;    % In case of false "double" start.
    else
        stim_onoff(m) = 0;
    end

    % Find start and end of intermittent burst cycles during stimulus.
    if intermit_flag == 1
        if burst_onoff(m) > 2
            burst_onoff(m) = 1;
            burst_onoff(m-1) = 0;
            burst_on_index(m) = m;
            burst_on_index(m-1) = 0;
        elseif burst_onoff(m) < -2
            burst_onoff(m) = -1;
            burst_onoff(m-1) = 0;
            burst_off_index(m) = m;
            burst_off_index(m-1) = 0;
        else
            burst_onoff(m) = 0;
        end
    end

    % Find start and stop indices for all candidate spikes.
    if m > 1 && filt_spikes(m) > 0 && filt_spikes(m-1) <= 0
        spike_on_index(m) = m;
    elseif m > 1 && filt_spikes(m) <= 0 && filt_spikes(m-1) > 0
        spike_off_index(m-1) = m - 1;
    end

% Get timestamps and indices for start and end of stimulus.
stim_on = stim_onoff.*data(:,1);
stim_on_stamp = stim_on(stim_onoff > 0)';
stim_on_index = stim_on_index(stim_on_index > 0);
stim_off_index = stim_off_index(stim_off_index > 0);

% Get timestamps and indices for start and end of burst cycles.
if intermit_flag == 1
    burst_on = burst_onoff.*data(:,1);
burst_on_stamp = burst_on(burst_onoff > 0)';
burst_on_index = burst_on_index(burst_on_index > 0);
burst_off_index = burst_off_index(burst_off_index > 0);
end

% Error checking
if length(stim_on_stamp) ~= length(stim_off_stamp)
    fprintf('ERROR: MISMATCH IN NUMBER OF STIM START AND STOP EVENTS!');
else
num_trials = length(stim_on_stamp);
end
if intermit_flag == 1 && length(burst_on_stamp) ~= length(burst_off_stamp)
    fprintf('ERROR: MISMATCH IN NUMBER OF BURST START AND STOP EVENTS!
');
end

% Now find start and end of surface stim (which is usually intermittent),
% using streamlined version of above methods with less error checking.
% The "2" is necessary to ignore the effects of noise.
surf_onoff = diff(data(:,end));
surf_onoff = [0; surf_onoff];
surf_high = find(surf_onoff > 2);
surf_low = find(surf_onoff < -2);
for y = 2:length(surf_high)
    if surf_high(y) - surf_high(y-1) < intermit_sep*fs
        surf_onoff(surf_high(y)) = 0;
    end
end
for z = 2:length(surf_low)
    if surf_low(z) - surf_low(z-1) < intermit_sep*fs
        surf_onoff(surf_low(z-1)) = 0;
    end
end
surf_on_index = find(surf_onoff > 2);
surf_off_index = find(surf_onoff < -2);
for k = 1:length(surf_on_index);
    surf_on_stamp = data(surf_on_index(k),1);
end
for l = 1:length(surf_off_index);
    surf_off_stamp = data(surf_off_index(l),1);
% For each trial, define the end as Peus running average falling within
% "frac_base" fraction of pre-stim baseline pressure and find corresponding
% timestamp and index. Find Pves stop as when Pves running ave falls
% within 2 SDs of baseline.
% Find running average of Peus using window of "win" seconds to remove
% effects of fluid tracking artifact.
running_ave_Peus = filter(ones(1,win*fs)/(win*fs),1,data(:,3));
Pves_adj = [];
for n = 1:num_trials
    % Find our baselines for Peus and Pves.
    pre_baseline_Peus(n) = mean(data(stim_on_index(n)-deltaT*fs:stim_on_index(n)-1,3));
    pre_baseline_Pves(n) = mean(data(stim_on_index(n)-deltaT*fs:stim_on_index(n)-1,2));
    deltaPves(n) = numSD*std(data((stim_on_index(n)-deltaT*fs):(stim_on_index(n)-1),2));
    % Find timestamp and index at which Peus running ave falls within
    % "frac_base" fraction of pre-baseline pressure, but only after
    % "refractory" seconds have passed.
    if n < num_trials
        for p = stim_off_index(n)+refractory*fs:stim_on_index(n+1);
            % Find trial end only after "refractory" seconds have passed.
            if running_ave_Peus(p) - pre_baseline_Peus(n) < 0.1*pre_baseline_Peus;
                trial_stop_stamp(n) = data(p,1);
                trial_stop_index(n) = p;
                break;
            end
        end
    else
        p = stim_on_index(n+1)
        trial_stop_stamp(n) = data(p,1);
    end
trial_stop_index(n) = p;
fprintf(['WARNING: TWO TRIALS WERE TOO CLOSELY SPACED
(','num2str(p),')\r']);
break;
ed
end

else    % Handle the case when we are on last trial.
    for p = stim_off_index(n)+refractory*fs:length(running_ave_Peus)
        if running_ave_Peus(p) - pre_baseline_Peus(n) < 0.1*pre_baseline_Peus;
            trial_stop_stamp(n) = data(p,1);
            trial_stop_index(n) = p;
            break;
        % Handles case in which next trial starts before Peus drops to
        % right level.
        elseif p == length(running_ave_Peus)
            trial_stop_stamp(n) = data(p,1);
            trial_stop_index(n) = p;
            fprintf(['WARNING: TWO TRIALS WERE TOO CLOSELY SPACED
(','num2str(p),')\r']);
            break;
        end
    end
end

% Subtract "pre_baseline_Pves" and "deltaPves" from corresponding
% trial's Pves data to apply threshold for contraction. Finds
% CANDIDATE contractions.
Pves_adj(stim_on_index(n):trial_stop_index(n)) =
data(stim_on_index(n):trial_stop_index(n),2) - pre_baseline_Pves(n) - deltaPves(n);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% ANALYZE DATA %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Loop to find start and stop points of candidate contractions.
mark = 0;
for t = stim_on_index(1)+1:length(Pves_adj)
    if Pves_adj(t) > 0 && Pves_adj(t-1) <= 0
        poss_con_start(t) = t;
    elseif Pves_adj(t) <= 0 && Pves_adj(t-1) > 0
        poss_con_stop(t) = t;
        mark = t;
    end
end
poss_con_start = poss_con_start(poss_con_start > 0);
poss_con_stop = poss_con_stop(poss_con_stop > 0);

% CONTRACTION ERROR CHECKING
%
% If one more contrac start than stop event, assume end of last trial to be
% contrac end.
if length(poss_con_start) == length(poss_con_stop) + 1
    poss_con_stop(end+1) = trial_stop_index(end);
% If first contrac stop event is time stamped before first contrac start
% event, ignore the first contrac stop event.
elseif poss_con_stop(1) < poss_con_start(1) && length(poss_con_stop) ==
    length(poss_con_start)+1
    poss_con_stop(1) = [];
% Otherwise, report error message for mismatch.
elseif length(poss_con_start) == length(poss_con_stop)
    fprintf('ERROR: MISMATCH IN NUMBER OF CANDIDATE CONTRACTIONS!\r');
end

% Now check each candidate contraction and keep only if peak is above
"Pves_thresh."  If kept, record average and peak Pves values.
tot_con = 0;
ave_Pves = [];
max_Pves_stamp = [];
max_Pves_amp = [];
contrac_start_index = [];
contrac_stop_index = [];
contrac_start_stamp = [];
contrac_stop_stamp = [];
for u = 1:length(poss_con_start)
  [temp_Pamp temp_Pind] = max(data(poss_con_start(u):poss_con_stop(u),2));
  % Correction because "max" command only returns relative index.
  temp_Pind = temp_Pind + poss_con_start(u) - 1;
  if temp_Pamp > Pves_thresh && poss_con_stop(u) - poss_con_start(u) > con_dur*fs
    tot_con = tot_con + 1;
    ave_Pves(tot_con) = mean(data(poss_con_start(u):poss_con_stop(u),2));
    max_Pves_stamp(tot_con) = data(temp_Pind,1);
    max_Pves_amp(tot_con) = temp_Pamp;
    contrac_start_index(tot_con) = poss_con_start(u);
    contrac_stop_index(tot_con) = poss_con_stop(u);
    contrac_start_stamp(tot_con) = data(poss_con_start(u),1);
    contrac_stop_stamp(tot_con) = data(poss_con_stop(u),1);
  end
end

% Do some basic analysis on Peus values: sum, cumulative sum, power
% spectrum with plots.  Note that product or cumulative product grow too
% quickly to be useful.  Also find Pves maximum pressure for each trial and
% volume values at start and end of trial.
for q = 1:num_trials
  if intermit_flag == 0
    chunk = data(stim_off_index(q):trial_stop_index(q),3);
  elseif intermit_flag == 1
    chunk = data(stim_on_index(q):trial_stop_index(q),3);
  end
  mean_peus(q) = mean(chunk);
  % Sum and cumulative sum.
  % summed(q) = sum(chunk);
  % cum_sum(Chancellor et al.) = cumsum(chunk);
  % Determine whether or not surface dermatome stim was on during a given
  % trial by seeing if logic 1 anywhere in stim portions of block channel.
  % if max(data(stim_on_index(q):stim_off_index(q),end)) > 2 &&
  % max(data(stim_on_index(q):stim_off_index(q),end)) < 10
  surf_applied(q) = 1;
  else
    surf_applied(q) = 0;
  end
  % PSD using Welch's method; need to use cell arrays.
  % Zero-pad if chunk is shorter than largest NFFT value.
  if length(chunk) < 4096
    chunk_padded = [chunk;zeros(4096-length(chunk),1)];
  else
    chunk_padded = chunk;
  end
[p1(Chancellor et al.), f1(Chancellor et al.)] = pwelch(chunk_padded, [], 2048, 4096, fs);
[p2(Chancellor et al.), f2(Chancellor et al.)] = pwelch(chunk_padded, [], 1024, 2048, fs);
[p3(Chancellor et al.), f3(Chancellor et al.)] = pwelch(chunk_padded, [], 512, 1024, fs);
[p4(Chancellor et al.), f4(Chancellor et al.)] = pwelch(chunk_padded, [], 256, 512, fs);
pwr1_temp = p1();
pwr2_temp = p2();
pwr3_temp = p3();
pwr4_temp = p4();
max_pwr = max([max(pwr1_temp) max(pwr2_temp) max(pwr3_temp) max(pwr4_temp)]);

% Make plots.
% Start time for plot will depend on whether or not intermittent bladder drive used (e.g. a dog experiment).
if intermit_flag == 0
    plot_start_index(q) = stim_off_index(q);
elseif intermit_flag == 1
    plot_start_index(q) = stim_on_index(q);
end
figure;
subplot(211), plot(data(plot_start_index(q):trial_stop_index(q), 1), chunk);
xlabel('Time (sec)');
ylabel('P_E_U_S (cm H_2O)');
title(['Trial ', num2str(q), ': Chunk Analyzed']);
subplot(312), plot(data(plot_start_index(q):trial_stop_index(q), 1), cum_sum(Chancellor et al.));
xlabel('Time (sec)');
ylabel('Cumulative Sum');
title(['Trial ', num2str(q), ': Cumulative Sum']);
subplot(212), plot(f1(Chancellor et al.), pwr1_temp, 'b-');
hold on;
plot(f2(Chancellor et al.), pwr2_temp, 'r-');
plot(f3(Chancellor et al.), pwr3_temp, 'm-');
plot(f4(Chancellor et al.), pwr4_temp, 'g-');
axis([0 1 0 max_pwr]);
xlabel('Freq (Hz)');
ylabel('Power');
title(['Trial ', num2str(q), ': Power Spectrum']);
hold off;
legend('4096, 2048', '2048, 1024', '512, 1024', '256, 512', 'Location', 'Northeast');

% Calculate additional parameters.
% End-of-stim pressures
end_stim_Pves(q) = data(stim_off_index(q), 2);
end_stim_Peus(q) = data(stim_off_index(q), 3);
end_stim_diff(q) = end_stim_Peus(q) - end_stim_Pves(q);
% Average and max Peus (1) for whole trial if not intermittent bladder drive or (2) for each burst cycle and off-phase during intermittent bladder drive.
if intermit_flag == 0
    during_stim_ave_Peus(q) = mean(data(stim_on_index(q):stim_off_index(q), 3));
    [during_stim_max_Peus(q), junk_ind] = max(data(stim_on_index(q):stim_off_index(q), 3));
elseif intermit_flag == 1
    % For each trial, how many burst cycles?
    burst_cycle_count(q) = length(find(burst_onoff(stim_on_index(q):stim_off_index(q)) > 0));
    for bb = 1:burst_cycle_count(q)
        % Adjust cycle count indexing as we advance through the trials.
        if q > 1
            cc = sum(burst_cycle_count(1:q-1));
        end
    end
    end_stim_ave_Peus(q) = mean(data(burst_cycle_count(q)(bb):stim_off_index(q), 3));
    [end_stim_max_Peus(q), junk_ind] = max(data(burst_cycle_count(q)(bb):stim_off_index(q), 3));
end
else
    cc = 0;
end
during_stim_on_ave_Peus_per_cycle(bb,q) =
mean(data(burst_on_index(bb+cc):burst_off_index(bb+cc),3));
[during_stim_on_max_Peus_per_cycle(bb,q), junk_ind] =
max(data(burst_on_index(bb+cc):burst_off_index(bb+cc),3));
% Now want to calculate values for off-phases between burst
% cycles, knowing that we have one fewer off-phase.  Correct
% for possible Peus fluid tracking artifact.
if bb < burst_cycle_count(q)
during_stim_off_ave_Peus_per_cycle(bb,q) =
mean(data(burst_off_index(bb+cc)+dly_int*fs:burst_on_index(bb+cc+1),3));
[during_stim_off_max_Peus_per_cycle(bb,q), junk_ind] =
max(data(burst_off_index(bb+cc)+dly_int*fs:burst_on_index(bb+cc+1),3));
end
end
% Take average of averages over all on or off phases within
% a single trial's stim
during_stim_on_ave_Peus(q) =
mean(during_stim_on_ave_Peus_per_cycle(1:burst_cycle_count(q),q));
during_stim_on_max_Peus(q) =
mean(during_stim_on_max_Peus_per_cycle(1:burst_cycle_count(q),q));
during_stim_off_ave_Peus(q) =
mean(during_stim_off_ave_Peus_per_cycle(1:burst_cycle_count(q)-1,q));
during_stim_off_max_Peus(q) =
mean(during_stim_off_max_Peus_per_cycle(1:burst_cycle_count(q)-1,q));
end
% Find average volume for deltaT seconds before start of bladder stim
% and for deltaT seconds after end of stim.  Calculate average flow
% (mL/sec).  CHANGE TO DATA COLUMN 6 if B019 (or earlier) data.
vol_temp = mean(data((stim_on_index(q)-deltaT*fs):stim_on_index(q),7));
volume_start(q) = vol_temp;
vol_temp = mean(data(stim_off_index(q):(stim_off_index(q)+deltaT*fs),7));
volume_end(q) = vol_temp;
Q_ave(q) = (volume_end(q)-volume_start(q))/(stim_off_stamp(q)-
    stim_on_stamp(q)+deltaT);
end
% Peus spike analysis: start by removing zeros from start/stop vectors.
% spike_on_index = spike_on_index(spike_on_index > 0);
% spike_off_index = spike_off_index(spike_off_index > 0);
% Error checking.
if length(spike_on_index) ~= length(spike_off_index)
    fprintf('ERROR: MISMATCH IN NUMBER OF SPIKE START AND STOP EVENTS!\r');
end
% Loop to find max value for each spike and ensure it occurs between end of
% stim and end of trial for the relevant trial.
spike_count = zeros(1,num_trials);
ave_spike_amp = zeros(1,num_trials);
tot_spk = 0;
for r = 1:length(spike_on_index)
    [temp_amp, temp_ind] = max(data(spike_on_index(r):spike_off_index(r),3));
    % Correction because of way "max" command works (returns relative index).
    temp_ind = temp_ind + spike_on_index(r) - 1;
    % Is detected spike within appropriate period for correct trial?
    for s = 1:num_trials
% If parsing intermittent bladder drive, include spikes during off-phases between burst cycles. ADDITION 2/26/09: "temp_amp > 0" check.
if intermit_flag == 1 && temp_ind > stim_on_index(s) && temp_ind < stim_off_index(s) && temp_amp > 0 && burst_highlow(temp_ind) == 0
    spike_count(s) = spike_count(s) + 1;
tot_spk = tot_spk + 1;
spike_max_index(tot_spk) = temp_ind;
spike_max_stamp(tot_spk) = data(temp_ind,1);
spike_max_amp(tot_spk) = temp_amp;
spike_max_by_trial(r,s) = temp_amp;
elseif intermit_flag == 1
    spike_max_by_trial(r,s) = 0;
end
% Now count post-stim spikes for both intermittent and continuous bladder drive. Wait "delay" seconds after end of bladder drive % before seeking post-stim spikes to avoid Pves fluid tracking % artifact at stim end. ADDITION 2/26/09: "temp_amp > 0" check.
if temp_ind > stim_off_index(s)+delay*fs && temp_ind < trial_stop_index(s) && temp_amp > 0
    spike_count(s) = spike_count(s) + 1;
tot_spk = tot_spk + 1;
spike_max_index(tot_spk) = temp_ind;
spike_max_stamp(tot_spk) = data(temp_ind,1);
spike_max_amp(tot_spk) = temp_amp;
spike_max_by_trial(r,s) = temp_amp;
elseif intermit_flag == 0
    spike_max_by_trial(r,s) = 0;
end
end
end
% Final computations to get actual average spike amplitude per trial.
for a = 1:num_trials
    if mean(spike_max_by_trial(:,a)) > 0
        trial_spikes = nonzeros(spike_max_by_trial(:,a));
        ave_spike_amp(a) = mean(trial_spikes);
    end
end
% % Values to output and save to file. Order and format last changed 5/16/08.
% format compact;
% format short g;
stim_on_stamp
stim_off_stamp
contrac_start_stamp
% max_Pves_stamp;
max_Pves_amp
contrac_stop_stamp
ave_Pves
trial_stop_stamp
end_stim_Pves
end_stim_Pves
end_stim_diff
spike_count
ave_spike_amp
surf_applied
if sum(surf_applied) > 0
    surf_on_stamp = surf_on_stamp';
    surf_off_stamp = surf_off_stamp';
end
if intermit_flag == 0
    during_stim_ave_Peus
during_stim_max_Peus
end
if intermit_flag == 1
    during_stim_on_ave_Peus
during_stim_on_max_Peus
during_stim_off_ave_Peus
during_stim_off_max_Peus
end
volume_start
volume_end
Q_ave
pre_baseline_Pves
pre_baseline_Peus
% summed;

fid = fopen(['extracted_',filename],'wt');
fprintf(fid,'stim_on_stamp	');
fprintf(fid,'%f	',stim_on_stamp);
fprintf(fid,'
stim_off_stamp	');
fprintf(fid,'%f	',stim_off_stamp);
fprintf(fid,'
contrac_start_stamp	');
fprintf(fid,'%f	',contrac_start_stamp);
fprintf(fid,'
max_Pves_amp	');
fprintf(fid,'%f	',max_Pves_amp);
fprintf(fid,'
contrac_stop_stamp	');
fprintf(fid,'%f	',contrac_stop_stamp);
fprintf(fid,'
ave_Pves	');
fprintf(fid,'%f	',ave_Pves);
fprintf(fid,'
trial_stop_stamp	');
fprintf(fid,'%f	',trial_stop_stamp);
fprintf(fid,'
end_stim_Pur	');
fprintf(fid,'%f	',end_stim_Peus);
fprintf(fid,'
end_stim_Pves	');
fprintf(fid,'%f	',end_stim_Pves);
fprintf(fid,'
end_stim_diff	');
fprintf(fid,'%f	',end_stim_diff);
fprintf(fid,'
spike_count	');
fprintf(fid,'%f	',spike_count);
fprintf(fid,'
ave_spike_amp	');
fprintf(fid,'%f	',ave_spike_amp);
fprintf(fid,'
surf_applied	');
fprintf(fid,'%f	',surf_applied);
if sum(surf_applied) > 0
    fprintf(fid,'
surf_on_stamp	');
    fprintf(fid,'%f	',surf_on_stamp);
    fprintf(fid,'
surf_off_stamp	');
    fprintf(fid,'%f	',surf_off_stamp);
end
if intermit_flag == 0
    fprintf(fid,'
during_stim_ave_Pur	');
    fprintf(fid,'%f	',during_stim_ave_Peus);
    fprintf(fid,'
during_stim_max_Pur	');
    fprintf(fid,'%f	',during_stim_max_Peus);
end
if intermit_flag == 1
    fprintf(fid,'
during_stim_on_ave_Pur	');
    fprintf(fid,'%f	',during_stim_on_ave_Peus);
    fprintf(fid,'
during_stim_on_max_Pur	');
    fprintf(fid,'%f	',during_stim_on_max_Peus);
fprintf(fid, '\nduring_stim_off_ave_Pur\t');
fprintf(fid, '%f\t', during_stim_off_ave_Peus);
fprintf(fid, '\nduring_stim_off_max_Pur\t');
fprintf(fid, '%f\t', during_stim_off_max_Peus);
end
fprintf(fid, '\nvolume_start\t');
fprintf(fid, '%f\t', volume_start);
fprintf(fid, '\nvolume_end\t');
fprintf(fid, '%f\t', volume_end);
fprintf(fid, '\nQ_ave\t');
fprintf(fid, '%f\t', Q_ave);
fprintf(fid, '\npre_baseline_Pves\t');
fprintf(fid, '%f\t', pre_baseline_Pves);
fprintf(fid, '\npre_baseline_Pur\t');
fprintf(fid, '%f\t', pre_baseline_Peus);
fprintf(fid, '\n');
fclose(fid);

fprintf('\r***DONE***\r');
function [filename, data] = processing_basic

%%% Extract data, create timestamp from the hour, min, sec, ms fields. %%%

% 1. Find all the times that are recorded. In the majority of cases they
%    are 10 samples apart.
% 2. Find the times that are recorded 11 samples apart.
% 3. Strip out sample before 11th in those cases so that all recorded times
%    are 10 samples apart.
% 4. Create a time stamp from hour, min, sec, centisecond.
% 5. Shift origin so first time is zero.
% 6. Linearly interpolate to get a unique time for each sample.
% 7. Plot

% Note: Calibrations and zero offsets made in LabView DAQ are
% automatically applied to recorded data; no further adjustments are
% necessary in this program.

% Set path, open dialog to load tab-delimited text file, import text file.
% Remember to add paths to MATLAB for all new data directories!
cd 'D:\My Documents\CWR\GustafsonData\';
[filename, pathname] = uigetfile({'*.txt','Tab-Delimited Text Files';'*.*','All Files'},
   'Load Data File');
readin = dlmread(filename,'\t');       % For reading ASCII files.

% Get date information from file name.
year = 2000 + str2num(filename(1:2));
month = str2num(filename(3:4));
day = str2num(filename(5:6));

% There is only a timestamp every 10th-11th sample with all zeros
% otherwise. Find these timestamps and strip out sample before 11th if
% such a case arises.
ind = find(~(((readin(:,1) == 0 & readin(:,2)==0) & readin(:,3)==0) & readin(:,4)==0));
dind = diff(ind);
null = (ind(find(dind==11) + 1) - 1);
readin(null,:)=[];

% Create new standardized timestamp with origin shifted to zero.
t_abs = datenum(year,month,day,readin(:,1),readin(:,2),readin(:,3)+readin(:,4)/10);
tmidnight=diff(t_abs(t_abs==datenum(year,month,day,0,0,0)));
tmidnight_time = (midnight(midnight<0));
t_abs(midnight_time:end) = t_abs(midnight_time:end) + datenum(year,month,day+1,0,0,0);
t_start = t_abs(1);
t_stamp = rem(t_abs,1)*24*60*60;
t_stamp = max(t_stamp - t_stamp(1),0);

% Interpolation filter to upsample for time only, since timestamps taken
% only every 10th sample. This does not affect the recorded data y-values.
interpolation_filter = [.1 .2 .3 .4 .5 .6 .7 .8 .9 1 .9 .8 .7 .6 .5 .4 .3 .2 .1];
t_stamp = filter(interpolation_filter,1,t_stamp);

% Extracting 5th through 11th or 12th columns of original data file as vector
% "data." These columns contain all relevant data, in the following order:
% Pves / Pabd / Pdet / Peus1 / Peus2 (if present) / Volume / Stim / Block.
% Omits final five columns of data file, which are just flags. Note that
% Peus2 added between B021 and B022.
data = [t_stamp,readin(:,5:end-5)];
% UNCOMMENT IF Stim AND Block CHANNELS REVERSED (as in D04 and D10)
%temp_data = data(:,end);
ndata(:,end) = data(:,end-1);
ndata(:,end-1) = temp_data;

% UNCOMMENT IF Pabd SUBSTITUTED FOR Pves (as in B040)
%ndata(:,2) = data(:,3);

% UNCOMMENT IF USING MICROTRANSUDER
%ndata(:,3) = data(:,5);

% First column is standardized time; rest are the data extracted above.
figure;
plot(data(:,1),data(:,2:end));
title(['Raw Extracted Data: ',filename(filename ~= '_')]);
xlabel('Time (seconds)');
ylabel('Pressure (cm H_2O) / Volume (mL)');
References


