Soft Silicone-based Neural Interface to Improve Bladder Function

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Lower urinary tract dysfunction is a serious ongoing medical complication that persists after spinal cord injury, multiple sclerosis, and diabetes mellitus. Losing the ability to empty the bladder voluntarily can significantly impact quality of life, and the current standard to treat this loss of control – intermittent catheterization – is associated with a high risk of urinary tract infections. Many neuromodulation interventions have been explored to empty the bladder but have not been translated to the clinic. Here, we designed a neural interface to electrically stimulate the bladder itself to evoke bladder emptying. Despite prior attempts in animals and people over the past decades, the success of direct bladder wall stimulation (DBWS) was limited by mechanical incompatibilities between the rigid electrodes and bladder tissue, especially during large volume changes, as well as by stimulation-induced co-activation of the urethra, legs, and other pelvic organs.

First, we designed a stretchable silicone electrode net that can be placed around the bladder, mapped the sensitivity of the bladder surface, and determined that the bladder base was the most sensitive location to stimulate in cats. We also minimized stimulation-induced co-activation of nearby muscles using different stimulation paradigms. Second, we created implantable versions of these electrode nets and tested them chronically in cats with and without anesthesia for 2-3 months. Direct bladder wall stimulation through various electrode configurations, temporal patterns, and stimulus intensities generated complete bladder emptying up to 15 weeks. In behaving cats, DBWS at different stimulus intensities elicited different voiding behaviors and could generate efficient

voiding at physiological bladder pressures. Third, we studied the neural mechanisms through which DBWS operates and found that robust contractions could be generated with or without peripheral innervation of the bladder, likely by activating post-ganglionic fibers in the bladder itself, but that activation of sensory pathways in the intact nervous system led to larger contractions at lower stimulus amplitudes.

Overall, experiments in cats demonstrated that these electrodes could be an effective neural interface to generate comfortable, complete bladder emptying and could be used after injury or disease where the bladder is underactive or atonic.

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Preface

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Introduction to Dissertation

This dissertation is aimed at developing a novel neural interface to improve bladder function. Normal bladder function is compromised by many pathological conditions such as spinal cord injury, multiple sclerosis, Parkinson's disease, and peripheral neuropathies. Here, we aimed at addressing those situations where the bladder becomes underactive or atonic, i.e., losing the ability to voluntarily void the bladder because of an inability to generate the muscular contractions and pressures required to empty the bladder. In this work, we attempted to revisit an old neuromodulation technique referred to as direct bladder wall stimulation (DBWS), which was an active area of research from the early 1960's through the 1990's. Scattered and infrequent reports have occurred since this time, but little progress has been made. Despite some successful pre-clinical and even clinical studies, these efforts were discontinued primarily due to technical challenges related to the electrode materials and design, and the corresponding physiological consequences.

In chapter 1, we provide a brief overview of the relevant anatomy and physiology of the lower urinary tract that is required to understand the major concepts of this thesis. This information was used for experimental design, ideation and data analysis.

In chapter 2, we review the historical context of DBWS and identify the challenges that lead to the discontinuation of this therapy in late 1990s. We found two major challenges. First, direct current spread, and stimulation-induced reflexes caused activation of urethral structures, activation of the legs, and co-recruitment of other pelvic organs. Second, the design and placement of electrodes on the detrusor muscle itself was a major challenge due to the unique structure and dynamic function of the bladder. This often led to displacement of electrodes and damage to the

bladder itself. We conclude that these challenges can all be attributed to the electrode design, size, placement on the bladder, and the high electrical stimulation currents that were required to overcome many of these electrode limitations.

In chapter 3, we present the design of a stretchable soft electrode net that can be placed on the bladder, and which conforms to the extreme volume changes that commonly occur in the bladder. This electrode was designed to mitigate many of the major challenges identified through the historical review and serves as a platform to explore the neurophysiology of DBWS more directly. We started by electrically stimulating different bladder areas and measuring bladder pressure. We found that the bladder base was the most sensitive to electrical stimulation, and electrodes confined to the base of the bladder were enough to generate robust bladder contractions that were not significantly different than employed a stimulation strategy that distributed electrodes across the entire surface. We further found that bipolar stimulation significantly reduced the co-activation of off-target tissues around the bladder including the external urethral sphincter, pelvic floor, and abdominal muscles. With these findings, we designed a stretchable soft silicone mesh to be placed around the bladder body to anchor the electrode array that interfaces directly with the base of the bladder. This design overcomes the technological limitations of DBWS, by testing this neural interface in acute experiments in cats.

In chapter 4, we implanted this neural interface in healthy, behaving cats to evaluate their function over months without the complicating effects of anesthesia. Even though the potential application of DBWS will be to restore bladder function in pathological conditions where the bladder is underactive or atonic, testing in healthy and awake animals offers the advantage of evaluating DBWS with intact bladder reflexes, the effect of DBWS on the bladder and pelvic sensation itself, while also studying the longevity of this neural interface. We found that DBWS

via soft silicone electrode nets could evoke complete voiding in both anesthetized and awake trials for the duration of the implant (3-4 months). Direct bladder wall stimulation also evoked comfortable voiding in behaving animals for the duration of the implant. Comfortable voiding in behaving animals supports the fact that co-activation of nearby tissue structures or sensory afferents conveying pain is limited with this interface. Data from the anesthetized and awake trials suggest the ability of electrode nets to generate motor contractions leading to bladder emptying at various stimulus intensities.

In chapter 5, we studied the neural mechanisms by which DBWS evokes bladder contractions. Using different anesthetics, we found that input from the spinal centers during DBWS under α-chloralose anesthesia ultimately facilitates larger bladder contraction, indicating the role of spinal reflexes in generating bladder contraction through DBWS. We transected the nerves responsible for these reflexes and found that robust bladder contractions could still be generated even with isoflurane at higher stimulation amplitudes and without spinal reflexes. We also used different pharmacological agents to examine the potential roles of direct activation of muscle tissue as well as activation of pre- and post-ganglionic neurons on the bladder surface. We found that even with ganglionic transmission blocked, DBWS still evoked functionally meaningful changes in bladder pressure.

Overall, using the novel electrode designed specifically for the bladder, the data presented in this thesis suggest that DBWS at the bladder base can generate extremely robust bladder contractions without co-activation of unwanted muscles in a way that is highly effective and has little direct sensory consequence. This is possible even in the absence of central reflexes and intact pre-ganglionic input. Ultimately, if the post-ganglionic neurons on the bladder surface are preserved – as is typically the case for spinal cord injury, peripheral neuropathies, and other

neurodegenerative diseases – DBWS using soft electrode arrays distributed around the base of the bladder could be used to overcome the inability to initiate voluntary voiding.

1.0 Introduction to The Lower Urinary Tract

1.1 A Brief Introduction to Bladder Neuroprostheses

Lower urinary tract (LUT) dysfunction is one of the most critical issues after spinal cord injury (SCI) and other pathological conditions such as multiple sclerosis and Parkinson's disease[1–3]. Historically, until the 1950's, the primary cause of mortality after SCI was renal failure due to the loss of voluntary and coordinated control of the bladder and urethra, leading to reflux of urine back to the kidney through the ureters[4]. In the following years, intermittent catheterization was introduced, reducing the cases of renal failure, but causing frequent and sometimes fatal urinary tract infections[5,6]. Since this time, significant progress has been made towards using pharmacological interventions to mitigate the effects of the loss of voluntary control and to reduce urinary tract infection rates due to catheterization[7]. Though these solutions helped minimize the most immediate and severe medical consequences of bladder dysfunction, the quality of life for many of these people remains affected. This is exemplified by surveys of people living with quadriplegia who prioritize restoring bladder, bowel, and sexual functions more than any other ability[8]. In people with tetraplegia, only improvements in hand function outrank improvements in bladder, bowel and sexual function. This priority is like driven by the fact that after a SCI, urinary tract infections and associated complications are the most common causes of hospitalization[9].

Since the 1950s, many research efforts have focused on the electrical stimulation of different tissue structures involved in the neural control of the LUT to improve function[10–12]. Still, they ultimately could not be translated into the clinic[10]. Peripheral nerve stimulation of the pelvic nerve (sensory and motor innervation of the bladder) and pudendal nerve (sensory innervation of

the urethra and genitalia and motor innervation of the external urethral and anal sphincters) has successfully restored bladder function in pre-clinical research[13–16] .Though these results were promising, these nerves are surgically intractable in humans and can lead to nerve damage[17,18]. Another promising solution is spinal cord stimulation (SCS) of the sacral spinal cord and nerves. Perhaps the most successful device for people with spinal cord injury is known as the Brindley-Finetech system, and works by activating the sacral ventral root leading to bladder emptying[19–21]. Though a promising technique, undesirable reflexes led to spastic bladder and EUS contractions, which are common after spinal cord injury. To address this issue, these implants were accompanied by a dorsal rhizotomy, which eliminates all sensory input to the sacral spinal cord. This device successfully emptied the bladder and eliminated the need for intermittent catheterization. However, there was a highly undesirable effect; people lost perineal sensation and sexual functions following the rhizotomy[22].

Though the primary functions of storage and voiding are simple in concept, the neural control of the LUT is complex and involves a variety of local, spinal, and supra-spinal reflexes, coordinating sympathetic, parasympathetic, and somatic pathways[23–25]. This complex control gives rise to challenges in developing neuroprosthetics to restore normal function, as illustrated in the prior description of the Brindley-Finetech system. In many cases, electrical stimulation approaches have had to be complemented by neurectomy procedures to reduce the system complexity[26,27].

Turning to the major focus of this thesis, direct bladder wall stimulation (DBWS) is another neuromodulation intervention that involves electrically stimulating the bladder – or more technically the detrusor muscle – directly to achieve micturition for people living with pathological conditions affecting the neural excitability of the bladder. Direct bladder wall stimulation has been

attempted for many decades to restore bladder function after spinal cord injuries and other voiding dysfunctions in both preclinical and clinical settings. This technique has theoretical advantages over other electrical stimulation interventions as the electrodes are placed directly in contact with the bladder and do not need to rely on intact reflex pathways like is required for pudendal nerve stimulation[15] or epidural spinal cord stimulation[28]. As a result, this approach could be used to empty the bladder emptying in various pathological conditions, including SCI. Although the conceptual simplicity of the technique was attractive, previous iterations contain several technological limitations and challenges that ultimately prevented any widespread translation of this approach.

Two factors mainly limited these efforts. Firstly, most systems used large electrodes distributed across the bladder[29,30], and through direct current spread as well as associated indirect reflex action caused by this current spread, urethral structures, off-target pelvic organs, and lower extremities were frequently activated, leading to urethral resistance and high residual urine volume in the bladder[31,32]. Secondly, the unique structure of the bladder made electrode placement difficult, and its dynamic contractions often resulted in the displacement of rigid electrodes and damage to the bladder[33]. With advances in technology, we aim to create a neural interface for DBWS which overcomes these challenges. To understand DBWS, it is important to understand the anatomy and physiology of the LUT. In the sections below, we summarize the relevant the anatomy and physiology of the LUT that is necessary to understand the rest of this thesis.

1.2 Anatomy and Neurophysiology of The Lower Urinary Tract

Many excellent reviews have been written about the anatomy and neurophysiology of the lower urinary tract (LUT)[23,25] , so only the most salient points, necessary for understanding the material presented in this thesis, are presented here. The LUT consists of the bladder, internal urethral sphincter (IUS), and external urethral sphincter (EUS). It primarily functions in two modes: voiding (micturition) and storage (continence) of urine. Micturition or voiding is the periodic emptying of urine from the bladder and continence is the storage of urine. In healthy individuals, coordinated activity of the bladder and the urethral sphincters is driven through complex control via descending pathways from the cortex and well as brainstem and spinal reflexes[34]. Coordinated activity between the urethral sphincters and bladder is necessary for normal LUT function. In the storage phase, the bladder relaxes and the urethral sphincters close, resulting in continence. In the voiding phase, the bladder contracts and the urethral sphincters relaxation such that urine is eliminated. This process can happen 3-10 times daily in a healthy individual. The following sections will discuss the anatomy and neurophysiology of the LUT.

1.2.1 Anatomy of The Bladder and Sphincters

A key goal of this thesis was to identify whether there were locations on the bladder surface that were particularly sensitive to electrical stimulation, such that robust bladder contractions could be evoked at low stimulus intensities. Prior clinical approaches often distributed electrodes broadly across the bladder without concern for the neuroanatomy of the bladder itself (REF). Further, more recent evidence suggests that the bladder has different densities of nerves in different areas (REF).

Therefore, it is important to understand the structure of the bladder in order to understand the particulars of the experimental design and analysis in future chapters.

The urinary bladder consists of three main regions: the bladder base, the bladder body, and the bladder dome[35]. The bladder base consists of the trigone and the bladder neck. The trigone is the triangular region at the bladder base identified by the triangle that connects the ureteral opening and the two urethral inlets[36]. The bladder dome is the top part of the bladder also called as apex of the bladder. The bladder body is located between below the bladder dome and above the opening of the ureters.

The bladder is composed of 4 layers: a mucousal layers called the urothelium, a submucousal layer, a muscular layer called the detrusor muscle, and finally serosal layer called the adventitia. The urothelium consists of epithelial cells that primarily prevent urine diffusion through the layer and prevent bacterial growth on the bladder's inner lining. The submucous layer consists of collagen and elastin fibers as well as myofibroblasts[37,38]. The arrangement of elastin and collagen fibers at this layer provides extensibility to accommodate large changes in bladder volume. This layer folds into rugae when the bladder is empty and stretches out as urine fills the bladder. The detrusor muscle consists of smooth muscle that contracts during micturition and relaxes during storage. It is this muscle that is the primary target of a directly bladder wall stimulation system. Finally, the adventitia consists of connective tissue that covers the outermost layer of the bladder.

The base of the bladder, where the bladder meets the tubular urethra, is called the bladder neck and consists of smooth muscle. At the junction between the bladder and urethra, there is a smooth muscle internal urethral sphincter (IUS). The urethra then continues as a smooth muscle

tubular structure for much of its length, but importantly is surrounded by a striated muscular ring called the external urethral sphincter (EUS).

1.2.2 Intramural Innervation of The Bladder Wall

Since muscle is far more easily activated by electrical stimulation of the axons innervating the muscle, than by direct stimulation of muscle, it is particularly important to consider the innervation of the bladder wall itself. As electrodes are placed on the bladder surface, electrical stimulation delivered through these electrodes could be activating different neurons in the bladder wall.

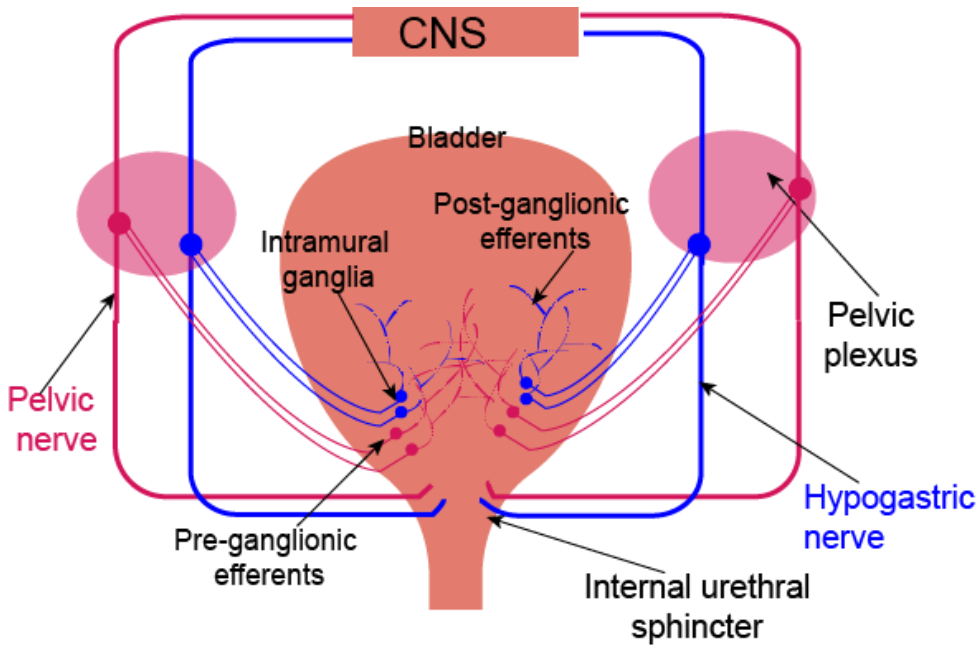

Figure 1.1 Intramural innervation of the bladder

The detrusor muscle is responsible for contracting the bladder during micturition and relaxing during the storage phase. These functions are under the control of the autonomous nervous system. More specifically, the excitatory drive to the bladder is under the control of the parasympathetic nervous system (pelvic nerve), while the inhibitory drive to the bladder is under the control of the sympathetic nervous system (hypogastric nerve)[39,40]. The innervation of the detrusor differs from striated muscles in important ways. In striated muscles, like the EUS, motor neurons project from the spinal cord directly to the muscles. However, in smooth muscle, like the detrusor, motor neurons in the spinal cord are preganglionic, and must first make synaptic contact with a postganglionic neuron in the periphery before these post-ganglionic neurons innervate the muscle. In the case of the excitatory drive to the detrusor, these autonomic ganglia are found in different places in different species, but in cats (the animal model used in this thesis), these ganglia are located on the bladder itself (figure 1.1).

Preganglionic efferents communicate to postganglionic efferents by releasing acetylcholine in the ganglia, which binds to nicotinic receptors on the postganglionic neuron. Next, the parasympathetic postganglionic efferents release acetylcholine at the neuromuscular junction, which binds to muscarinic receptors (M1) to generate muscle contractions (figure 1.2). Conversely, the sympathetic postganglionic efferents release norepinephrine at the neuromuscular junction, which binds to beta-adrenergic receptors to relax the detrusor muscle[41]. These mechanisms will become important to understand the pharmacological studies contained in the final research chapter of this thesis.

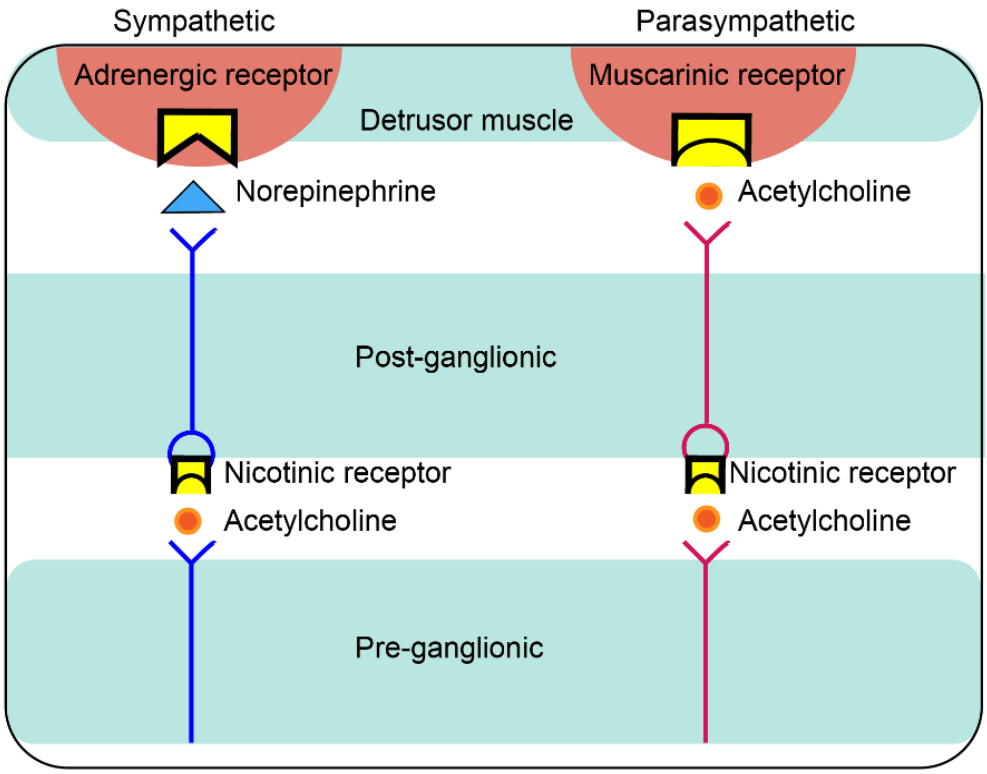

Figure 1.2 Sympathetic and parasympathetic innervation of the bladder wall

1.2.3 Peripheral Innervation of The Lower Urinary Tract

The bladder is connected to the central nervous system with the complex network of mixed sensory and motor nerves. Conceptually, we expect that DBWS activates neurons on the surface of the bladder that result in bladder contractions. In chapter 5, we seek to identify more specifically how different sensory and motor projections to the central nervous system affect the functional outcomes of DBWS. It is therefore important to understand the efferent and afferent innervation of the entire LUT.

1.2.3.1 Efferent Innervation

The LUT is innervated by three nerves that arise from the thoracic, lumbar, and sacral spinal cord: the hypogastric nerve (sympathetic thoraco-lumbar), the pelvic nerve (sacral parasympathetic), and the pudendal nerve (sacral somatic). Sympathetic preganglionic axons in the hypogastric nerve descend from spinal segments T11- L2 to sympathetic chain ganglia and then to the superior hypogastric and pelvic plexus, and finally to the bladder. Hypogastric nerve postganglionic efferents release neurotransmitters that inhibit or relax the bladder and activate the internal urethral sphincter. Parasympathetic preganglionic axons from the pelvic nerve descend from the parasympathetic nucleus (SPN) from spinal segments S2-S4 to the pelvic plexus and finally to the bladder, where they synapse with postganglionic neurons in the bladder wall[41]. Pelvic nerve efferents release neurotransmitters that activate and contract the bladder and relax the internal urethral sphincter. The somatic efferent axons of the pudendal nerve descend from Onuf's nucleus in the S2-S4 spinal segments to synapse with the striated muscle of the EUS. Activity in these three nerves are coordinated by the central nervous system in a phasic on-off switch pattern to maintain coordinated activity between the bladder and the urethra. This leads to maintaining the bladder in either a micturition or continence phase[23].

1.2.3.2 Afferent Innervation

Afferent axons in the pelvic and pudendal nerves convey information from the LUT to the sacral spinal cord. There are two primary types of sensory fibers in the pelvic nerve that innervate the bladder and urethra. Small diameter fibers (A-delta mechanoreceptor afferents) in the detrusor muscle respond to stretch during bladder filling and convey a sense of fullness to the central nervous system. Activity in these axons in nearly absent when the bladder is empty, but gradually

increases as the bladder fills. The detrusor muscle is also innervated by c-fibers afferents that become active at high volumes in humans[42]. These nociceptive c-fibers also react to irritants such as cold stimuli and capsaicin. Afferents in the urethra also plays an essential role in augmenting or inhibiting reflex contractions of the bladder. The afferents are conveyed by the pudendal nerve to the spinal cord and carry the activity of flow-sensitive mechanoreceptors in the urethral wall. These afferents are involved in evoking or supporting ongoing detrusor contractions[43]. Electrical stimulation of pudendal nerve afferents that innervate the genitals can also inhibit or activate the bladder depending on the stimulation frequency[44,45].

1.2.4 Neural Control of The Lower Urinary Tract

We discussed the anatomy of bladder as well as the afferent and efferent innervation in the previous sections. However, continence (urine storage) and micturition (voiding) occur as the result of a complex series of spinal and supraspinal reflexes as well as descending control from the cortex[34]. Although a therapy like DBWS would be useful in pathological conditions such as spinal cord injury and diabetes, we plan to study DBWS is awake healthy behaving cats to study the longevity of the device, the effect on conscious sensation, as well as the interaction between the directly evoked effects of DBWS and this descending voluntary control. The following sections briefly summarize these two components of healthy LUT function.

1.2.4.1 Continence - Storage Phase

During bladder filling, pelvic nerve afferents communicate information about the state of the bladder to the spinal cord and brain by sensing stretch in the bladder wall itself. This leads to an increase in sympathetic outflow to the bladder wall and internal urethral sphincter via the

hypogastric nerve. This sympathetic activity has two actions. First, it directly relaxes the detrusor muscle by releasing norepinephrine in the bladder wall, which binds to beta-adrenergic receptors. Second, it norepinephrine contracts the internal urethral sphincter by binding to alpha-adrenergic receptors. The pudendal nerve also plays a significant role in continence, particularly at higher bladder volumes. Pudendal nerve efferents release acetylcholine at the neuromuscular junction of the striated muscle EUS and bind to nicotinic receptors that result in muscle contractions. These contractions of the EUS increase as the bladder gradually fills. At the brainstem, there is limited activity in the pontine micturition center during the storage phase[23,34].

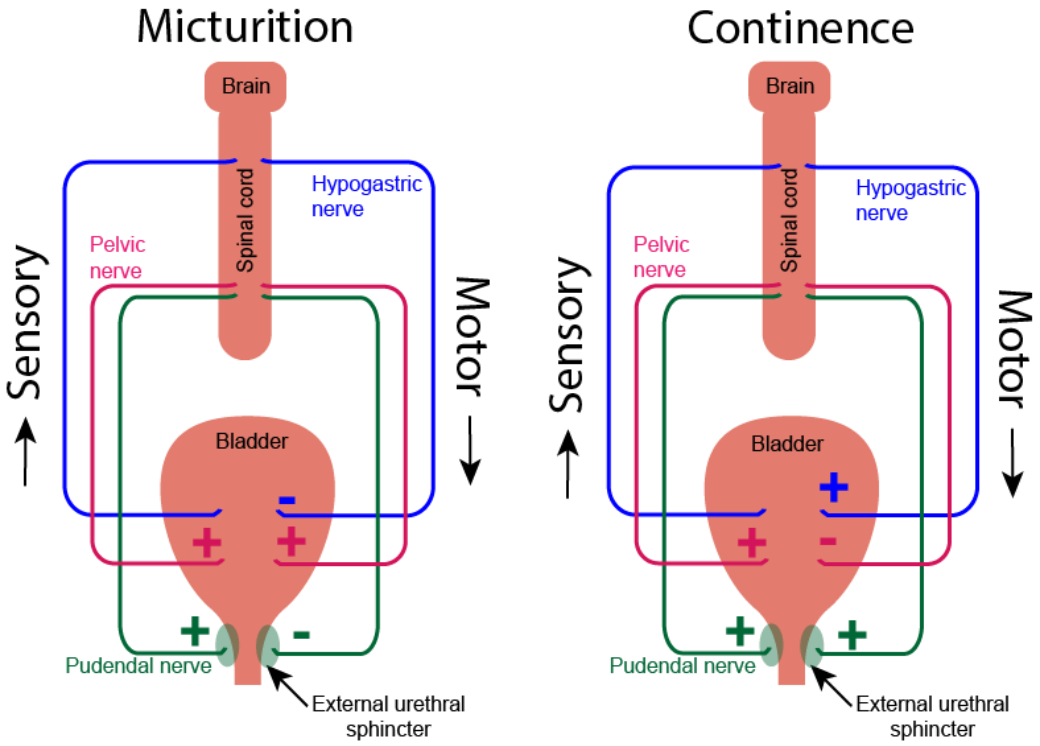

Figure 1.3 Neural control of the lower urinary tract

1.2.4.2 Micturition - Voiding Phase

The switching from the storage phase to micturition results from several convergent inputs to the pontine micturition center. A major contributor is from increased afferent activity in the

pelvic nerve that passes from the sacral spinal cord via the spinobulbospinal pathway, to the brainstem. This sense of bladder fullness, combined with higher-level input from the cortex, which ensures that voiding occurs at socially appropriate times, triggers a switch in the brainstem resulting in the micturition reflex. This state change leads to a coordinated increase in the activity of the sacral parasympathetic nucleus, causing bladder contractions, as well as inhibition of EUS motoneurons in Onuf's nucleus by increasing descending excitatory drive to a population of spinal interneurons that inhibit EUS motoneuron activity. This coordinated activity ultimately increases bladder pressure and decreases urethral resistance resulting in voiding[23,34]. Descending cortical and brainstem mechanisms are not the only factors involved during micturition. At the spinal level, flow-sensitive mechanoreceptors in the urethra project to the sacral spinal cord through the pudendal nerve and increase the synaptic drive to the sacral parasympathetic nucleus. This reflex is called the pudendo-vesical reflex and enhances bladder contractions during active voiding through this positive feedback loop (figure 1.3).

2.0 Direct Bladder Wall Stimulation: Success, Challenges, and Prospects

2.1 Overview

Direct bladder wall stimulation (DBWS) uses electrical stimulation through electrodes placed directly on the surface of the bladder to generate force in the detrusor muscle and empty the bladder. Direct bladder wall stimulation has been attempted for many decades to restore bladder function in people with spinal cord injury and other voiding dysfunctions where the bladder is underactive or atonic. In many preclinical and clinical studies, it was shown as a promising technique to improve bladder emptying; however, these efforts were discontinued due to several technological limitations and challenges. Two factors mainly limited these efforts. First, direct current spread as well as unwanted reflex activation led to contraction of urethral structures, activation of the legs, and co-recruitment of other pelvic organs. Current spread to urethral structures is a major concern as it increases urethral resistance, thereby preventing efficient bladder emptying. This current spread has been attributed to a number of factors including electrode design, the specific electrode placement on the bladder, and the high electrical stimulation amplitude required for DBWS. Second, placing electrodes on the detrusor itself is challenging due to its unique structure and extreme changes in volume. Rigid electrodes are simply not compatible with the tissue properties of the bladder. This review aims to identify these challenges and discuss technological advances that would be required to improve translational efforts.

2.2 Introduction

Lower urinary tract (LUT) dysfunction is one of the most critical issues after spinal cord injury (SCI) and other pathological conditions such as multiple sclerosis and Parkinson's disease[1–3]. The primary cause of mortality after SCI was renal failure up until the 1950s due to the loss of synchronized control of the bladder and urethral; dyssynergic contractions caused urine reflux back to the kidney through the ureters leading to kidney infections[4]. In the following years, intermittent catheterization was introduced, reducing the cases of renal failure, but causing frequent urinary tract infections due to the continued insertion or presence of the catheter[5,6]. Significant progress has been made since this time, including pharmacological interventions to relax the bladder and reduce the urinary tract infection rate due to catheterization[7]. Though these solutions helped minimize the severe medical consequences, they do not address the underlying issues and the quality of life of these people remains affected. People living with paraplegia prioritize restoring bladder, bowel, and sexual functions more than any other ability[8]. This is in large part do the fact that after a SCI, urinary tract infections and associated complications are the most common causes of hospitalization[9].

The LUT consists of the bladder, internal urethral sphincter (IUS), and external urethral sphincter (EUS) and primarily functions in two modes: voiding (micturition) and storage (continence). In healthy individuals, coordinated activity of the bladder and the urethral sphincter through spinal and supra-spinal reflexes is necessary to achieve these two modes. Since the 1950s, there has been significant effort directed towards using electrical stimulation of different tissue structures involved in the neural control of the LUT to improve function. However, at least for spinal cord injury, none have been translated into the clinic on a broad scale[10].

Peripheral nerve stimulation of the pelvic nerve (bladder) and pudendal nerve (external urethral sphincter) successfully improved bladder functions in preclinical research. Though the results were promising, these nerves are surgically intractable in humans and can lead to nerve damage[17,18]. Another promising solution is electrical stimulation of the sacral spinal cord, which is the location where the effect innervation to both the bladder and EUS originate[23]. In fact, stimulation of the sacral is the only device that has achieved any reliable improvements in bladder function for people living with SCI. This device is known as the Brindley-Finetech system, which works by activating the sacral ventral root, leading to bladder emptying[19–21]. Though a promising technique, undesirable reflexes led to spastic bladder and EUS contractions, and hence, a dorsal rhizotomy was necessary for all Brindley-Finetech implants. This device successfully emptied the bladder and eliminated the need for intermittent catheterization. However, there was a high risk-to-benefit ratio, as people lost perineal sensation and sexual functions following the rhizotomy[22].

Though the primary functions of storage and voiding are simple in concept, the neural control of the LUT is complex and involves a variety of local, spinal, and supra-spinal reflexes coordinating sympathetic, parasympathetic, and somatic pathways[23–25]. This gives rise to challenges in designing electrical stimulation systems that can successfully control these various functions; these implants often needs to be complemented by neurectomy procedures to reduce complexity or improvement that cannot be successfully restored by stimulation[26,27].

Direct bladder wall stimulation (DBWS) is a neurostimulation intervention that involves electrically stimulating the detrusor muscle directly to achieve generate bladder contractions for people living with pathological conditions affecting the neural excitability of the bladder. This technique has several potential advantages over other electrical stimulation interventions. The primary advances are that it is not necessary to identify and stimulation the pelvic nerve, which is

a difficult anatomical target, and as electrodes are directly in contact with the bladder it is not necessary to rely on activating unreliable reflex pathways. Although DBWS was a promising technique to improve bladder emptying, previous iterations contain several technological limitations and challenges. Two factors mainly limited these efforts. First, large stimulation currents were typically used leading to direct current spread and indirect reflex action, which caused unwanted activation of urethral structures, pelvic organs, and lower extremities. These off-target effects and stimulation-induced activation of the pelvic floor and EUS prevented low-pressure voiding and led to high residual urine volumes in the bladder. Secondly, the unique structure of the bladder made electrode placement difficult, and its dynamic contractions often resulted in the displacement of rigid electrodes. This review aims to summarize the success and challenges of DBWS as well as suggest pathways to improve the clinical potential of this approach.

2.3 Experimental Successes and Failures

2.3.1 Animals

The first instance of DBWS was reported in 1962 when acute and chronic experiments with paraplegic dogs produced bladder emptying. Two to six stainless-steel discs, 5 mm in diameter, were sutured to the external surface of the bladder wall. Electrodes were placed on the anterior and posterior surfaces of the bladder body. Bladder emptying was achieved by stimulating these electrodes using biphasic pulses at 15 volts and a pulse width of 1-5 ms. This stimulation resulted in complete emptying of the bladder[46].

Following this, many other groups attempted DBWS in dogs in both acute and chronic experiments in intact and spinalized animals[27,33,47–53]. Across these experiments, different groups varied the stimulation voltage, electrode locations, electrode material, and electrode size in an attempt to optimize the functional outcomes[52]. Walter et al. also attempted DBWS in intact and spinalized cats where they used different types of electrodes to accommodate changes in the bladder and found that they could also generate substantial bladder pressures and efficient voiding with a variety of different electrodes placements and stimulus intensities. This was in spite of a number of challenges that included mechanical incompatibility between electrodes and the bladder wall, as well as some co-activation of nearby tissue structures. These challenges will be be discussed in details in following sections[54–57].

2.3.2 Humans

Given these successes in experimental animals, several groups began to move rapidly towards human trials. The first clinical trial of DBWS was reported by Bradley et al., who reported studied seven human patients with a variety of spinal cord and brain injuries[58]. In this study, 2 out of the 7 implants were considered successful as stimulation led to emptying of the bladder. However, in 5 of the patients, stimulation generated significant bladder pressures but did not empty the bladder. These failures were the first suggestion that DBWS in humans might not mirror the success that were seen in animals. Failure in these 5 patients were attributed to several factors including the co-activation of nearby tissue structures, pain, and electrode lead failure.

Within the next few years, multiple reports of DBWS in humans were published. Scott et al. implanted a 51-year-old male with a neurogenic bladder in which DBWS led to changes in bladder pressure, but without successful voiding. Stimulation caused spasms in the urethral

sphincter due to detrusor sphincter dyssynergia that is common after upper motor neuron lesions[33]. Stenberg et al. reported 4 cases of DBWS in people with supra sacral SCI where prolonged bladder wall stimulation led to the reflex recovery of bladder function in 3 patients, as well as stimulation evoked voiding in 1 patient[59]. Halverstadt et al. completed the most comprehensive clinical study of DBWS; 10 patients with neurogenic bladders due to iatrogenic factors and procedures such as radical hysterectomy and pelvic lymphadenectomy were implanted. In these 10 cases, seven were considered successful. The longest follow-up was eight years for one patient with hypotonic bladder who remained catheter free with DBWS. Three failed cases were attributed to technical challenges, abdominal pain, co-activation of nearby tissue structures, and mechanical failure of the bladder wall at the electrode-tissue interface[26,60,61]. Merill et al. reported 5 cases of patients with upper motor neuron lesions using a novel electrode device intended to overcome some of the limitation of large metal disks, which were common in prior implants. Helically wound wire electrodes were implanted in the bladder wall and 2 out of the 5 cases were considered successful over a period of 3 to 18 months. However, these cases required secondary procedures such as phenol block or pontocaine spinal anesthetic to suppress stimulation induced pelvic floor contractions that were common in people with upper motoneuron lesions. Similar to prior studies, electrode displacement, co-activation of nearby structures due to high stimulus currents, and reflex spasms of the pelvic floor led to failure[62]. In the last clinical trials, Magasi et al. reported 32 clinical cases (21 peripheral nerve injuries and 11 central injuries) and 29 of the 32 cases were considered successful in the primary goal of emptying the bladder. The three unsuccessful cases occurred in subjects with central nervous system lesions that led to rigidity and spasticity in the external urethral sphincter[63,64].

Across all these studies, there were varying degrees of success and failure that were attributed to different electrode designs, electrode placement, stimulus intensity, therapeutic targets, or pathological conditions. In both animal and human studies there were many successful cases; however, serious attempts at DBWS were discontinued because of the inability to successfully and reliably address the various challenges. These challenges consisted of both technical issues as well as physiological issues resulting from the various clinical indications in which DBWS had been attempted.

Citation	Cases (Successful out of total)	Injury	Secondary procedure	Challenges	Electrode and placement	Stimulation parameters
Bradley, 1963	2 out of 7	7 UMN, 1 LMN	None	Current spread to pelvic musculature, hind limb, Pain	2-6 disc or tape electrodes on the bladder dome	Biphasic pulse, 5-15 V, 1-8 ms, 20-25 Hz
Kantrowitz,1965,	1 out of 4	3 UMN, 1 LMN	None	Pelvic floor muscle spasms due to reflexic activity and current spread	2-4 silastic insulated leads in loop-like fashion in the bladder wall	10v, 20-25pps, 0.5-2 ms pulse width
Scott, 1965	0 out of 1	1 UMN	None	Current spread to perineal musculature	10 perforated platinum disc electrodes, 4 between UVJ and 6 all over the bladder	2-10 V, 20 Hz, 2-4 ms
Susset, 1966	1 out of 1	1 UMN	Intrathecal alcoholization	Initial pelvic floor spasticity, current spread, pain	8 platinum disc electrodes on the bladder body	Biphasic pulses: 10-20V, 20Hz,1ms
Stenberg, 1967	4 out of 4	4 UMN	None	Closing of the bladder neck due to stimulation	stainless steel wires embedded into anterior bladder wall	2.5-18 V, 4ms, 20 Hz
Halverstadt,1968, 1971, 1975	7 out of 10	Hypotonic bladder due to peripheral surgical hysterectomy, pelvic resection, sacral rhizotomy for pelvic pai, post pregnancy urinary retention	None	Current spread to the pelvic and perineal musculature, ejaculation, erection, and desire to defecate. Pelvic pain if electrodes place near bladder the neck.	Stainless steel wires embedded in the bladder wall to cylindrical flexible wire imbricating serosal tissue, cephalad placement in dome of the bladder, farther from UVJ preferred.	2.5 to 30 V, 1-4 ms, and 20 pps
Magasi 1969-1986	37 out of 45	Paralyzed bladder with lower motor neuron lesion (32), UMN lesion (13)	Transurethral bladder resection for UMN lesion	Current spread, urethral spasm for UMN lesion, successful for LMN lesion	8 platinum disc electrodes, 2 near UVJ and 6 all over the bladder	1 ms pulse width mentioned, not other parameters
Merill 1974	3 out of 5	UMN lesion	Phenol block, pontocaine spinal anesthesia	Current spread to perineal and pelvic musculature and pelvic foor spasms due to spinal reflexes preserved after in UMN lesion	Helical electrodes embedded in the bladder wall at the base of the bladder	Unclear
Jonas, 1978	8 out of 11	2 UMN, 9 LMN lesions (5 central, 4 peripheral)	Transurethral resection of sphincter, bladder neck incision	Pain and co-activation	8 platinum disc electrodes on the bladder body	Unclear

Figure 2.1 Summary of clinical trials in literature

2.4 Challenges

In this section we identify and discuss three major challenges that have been associated with DBWS (fig 2.1). These challenges are current spread, mechanical compatibility at the electrode-tissue interface, and the specific physiological issues that arise after damage to the nervous system. Current spread frequently caused unwanted activation of nearby tissue because of the large voltages and currents that were typically used. Mechanical incompatibilities between the electrodes and the bladder, which undergoes extreme volume changes, frequently resulted in damage to the electrodes, or worse, major damage to the bladder itself. Finally, after neurological injury, the neural control of the lower urinary tract is changed in ways that create additional challenges for DBWS. Each of these issues will be addressed in turn.

2.4.1 Current Spread

One of the most persistent challenges reported that prevented complete emptying of the bladder using DBWS was the spread of current that resulted in pain and co-activation of nearby tissue structures such as muscles of legs, pelvic floor, and perineum. Most functionally problematic, this current spread caused stimulation-induced occlusion of the urethra, resulting in high urethral resistance. Pelvic and abdominal pain in pre-clinical[49,58,65] and clinical studies[26,60,61,66–68] have also been attributed to this current spread. If current spread was a recognized problem, then a question that arises is what factors contributed to this. These various issues are discussed below and include electrode location, electrode insulation and tissue contact, electrode size, and stimulation paradigms.

2.4.1.1 Electrode Location

The location of the electrode on the bladder surface plays a vital role in the stimulation amplitude that is required to successfully emptying the bladder with DBWS. The stimulus amplitude that is required to generate a contraction can be different throughout the bladder surface. Since overall stimulus intensity is one of the most obvious factors that contributions to current spread and unwanted tissue co-activation, placing electrodes on the bladder in locations that minimize the required stimulus intensity can is essential. There are two basic ways in which electrical stimulation can cause contractions of the bladder wall: myogenic contractions, or stimulation of the detrusor muscle cells themselves; neurogenic contractions, or stimulation of axons that innervate detrusor muscle cells.

Direct stimulation of the detrusor muscle has been reported in many studies and has shown success in cases with complete lower motor neuron lesions[61,64]. It can generate bladder contractions of large magnitudes, even in denervated bladders[69]. However, the stimulus intensities needed to generate a robust contraction is very high (40 V-100 V). Because of the high stimulus intensities needed to evoke a direct myogenic contraction, the stimulus current can easily spread to nearby tissue structures leading to the excitation of other muscles directly, or more commonly by activating nearby sensory or motor axons. Moreover, generating large bladder pressures through myogenic contractions is challenging as the smooth muscle contractions are limited to the location around the stimulating electrode. Therefore, the smooth muscle contractions generated by single electrode stimulation do not generate large bladder contractions. Stimulation through many electrodes across the bladder surface is then needed to generate a strong bladder contraction and it commonly requires high stimulus intensities distributed across many locations to generate a strong contraction capable of emptying the bladder. While this type of stimulation is useful in cases where

the detrusor itself may be denervated, this is not the case usually in many cases of underactive bladder. Specifically, even if preganglionic projections to the bladder are damaged, postganglionic efferents are typically preserved, even after lower motor lesions[70,71].

Unlike myogenic contractions, neurogenic contractions require less stimulation current as the target of the electrical stimulus is the innervation of the muscle itself. As described earlier, in the bladder and other smooth muscles, excitatory output from the central nervous system must first make synaptic contact in a ganglia before postganglionic neurons innervate the detrusor itself. This postganglionic efferent population is preserved on the bladder surface after sacral spinal cord injury and in incomplete spinal lesions[71,72]. Stimulating these postganglionic efferents led to more powerful bladder contractions and lower stimulus intensities could evoke a coordinated bladder contraction through the intramural network[73]. These intramural ganglia and nerves can high a high density near the ureterovesical junction both in animals and humans as the parasympathetic nerves of the bladder typically make contact with the bladder in this area[70]. For example, Stenberg et al. identified pelvic nerve efferents contacting the detrusor near the ureterovesical junction in human cadaver[59]. However, no systematic identification of ideal stimulation locations has ever been reported, and different studies have used many different electrode placements. Even in cases where stimulation locations near the ureterovesical junction were reported as being highly effective, electrodes were still distributed across the bladder surface[53,67]. Scott et al. reported that in dogs, stimulation through a single electrode placed near densely innervated regions of the bladder generated much higher pressure changes than simultaneous stimulation of many electrodes distributed across the bladder surface[33]. Similarly, Bradley et al. placed electrodes on the dome of the bladder in dogs, and while they were able to empty the bladder, stimulation at 120 V was required and also led to pain[46]. In the end, electrical stimulation near the bladder neck in the

vicinity of ureterovesical junction can generate large bladder contraction in dogs[27,33,48,53,74,75] cats[55,76] and humans[32,63,64]. However, Halverstadt et al. point out that electrodes near the bladder neck increase the chance of evoked simultaneous contractions of the bladder neck and external urethral sphincter and may be more likely to cause pain[26].

2.4.1.2 Electrode Design, Electrode Insulation and Electrode-tissue Contact

Since there are many other excitable tissues around the bladder, poor insulation of the side of the electrode that does not face the bladder, or poor contact between the of the electrode and bladder surface can lead to current spread to nearby tissue structures. Ellis et al. reported that proper insulation led to a significant reduction in current spread in dogs[50]. It was also reported that embedding the electrodes within detrusor muscle itself also limited current spread to nearby structures[27,50]. Halverstadt et al. reported the spread of current even with silastic insulation of electrodes in people, which was then reduced by shielding electrodes with polyethylene[60,61]. Talibi et al. reported the influence of electrode insulation on the stimulus intensity required to evoke bladder contractions[77]. Suppose the current is not only leaking under or in the vicinity of the electrode to the bladder. If not insulated, it will draw current to other structures, and high stimulus intensities will be needed. Merill et al. reported both silastic insulation and imbricating the bladder wall over the platinum electrodes in the bladder in dogs and humans[62,78]. Another significant factor that contributes to current spread is the design of the electrode itself. Electrode design is related charge density and can affect the stimulus intensity needed to generate robust bladder contractions, but can also lead to activation of nearby structures[46]. If an electrode with a smaller surface area is positioned near an excitable location of the bladder, like in the vicinity of UVJ, a higher charge density can be achieved for the same stimulus current, which helps to activate neurons. Conversely, electrodes with large surface areas potentially cover more neuronal targets, but significantly higher

stimulus intensities are required to achieve the same current density, which could lead to co-activation of nearby tissue structures[79].

Researchers have used different electrode designs, to place electrodes in the bladder wall and on the surface of the bladder such as suture, and disc electrodes. Stimulus intensity is relevant to the vicinity of neural targets from electrodes. For example, the stimulus intensity needed to generate a bladder contraction near the ureterovesical junction will be lower than the stimulus intensity required to generate a contraction from the electrode placed towards the dome of the bladder[74].

Similarly, a surface electrode on the bladder surface will need higher stimulus intensity than an electrode embedded in the bladder wall. Although suturing the electrodes to the bladder wall provides better electrode-tissue contact, which leads to less stimulus intensity requirements, however; it causes trauma to the bladder wall and poses a risk of electrode migration due to the dynamic nature of the bladder. Electrode design is again related to the stimulus intensity, and the bigger the area of the electrode, the lower the charge density, and high stimulation charge will be needed to generate bladder contractions. Electrode design also complicates the electro-tissue contact integrity as well as the bladder health. Habib et al. noticed that disc electrodes are better than needle electrodes due to trauma caused by needle electrodes to the neural targets in the bladder wall[48]. Bradley et al. demonstrated a reduction in current spread using small contact point electrodes[46].

2.4.1.3 Type of Stimulation and Stimulus Intensity

Through these sections we have discussed how different stimulus intensities were required to evoke bladder contractions and that the stimulus intensity varied with electrode location, size, electrode-tissue contact, and insulation. Wear et al. reviewed many of the stimulation parameters

that successfully evoked bladder emptying and found that the optimal stimulation parameters were typically biphasic pulses at 10-25 V, with a stimulus frequency of 15-30 Hz and pulse duration of 4-7 ms[52]. Here, we discuss the various effects of different stimulation patterns such as monopolar stimulation, bipolar stimulation, and simultaneous stimulation. Peterson et al. reported that monopolar stimulation generated stronger bladder contractions than bipolar stimulation when stimulation was delivered to the bladder neck, but that this effect was very dependent on the exact location of the electrode. For example, if an electrode pair was placed directly over a highly excitable tissue region – presumably one with a high density of postganglionic fibers under the electrode – then bipolar stimulation could evoke higher pressures than monopolar stimulation. Bipolar stimulation also reduced current spread to nearby tissue. However, monopolar stimulation had a more broadly distributed electric field that increased the chance of successfully stimulated the desired tissue[48,56,75]. This makes sense as bipolar stimulation limits the electric field to the near vicinity of the electrode poles. In a different study Walter et al. found that bipolar stimulation generated similar bladder pressures to monopolar stimulation when the return electrode was placed on the abdomen[76].

Increasing the number of electrodes on the bladder can also have a positive effect. Bilateral stimulation was able to generates more powerful bladder contractions that unilateral stimulation[76]. Further, Pagano et al. reported that simultaneous stimulation of 4 electrodes improved bladder emptying[53]. However, these effects of multi-electrode stimulation were not universally found. In some cases, multi electrode stimulation caused no more robust contraction than a single well-placed pair of electrodes on the bladder surface[52].

2.4.2 Mechanical Challenges at the Electrode-tissue Interface

Co-activation of nearby tissue structures has been a major challenge for DBWS. However, the mechanical challenges have been at least as difficult to overcome. The bladder experiences very large volume changes many times a day. In humans, average voiding volumes can be approximately 400 ml, and in more extreme cases can reach more than 1000 ml. Maintaining electrode-tissue contact on the bladder surface in a system that experiences such extreme volume changes is challenging. Perhaps unsurprisingly, failures due to the mechanical displacement of electrodes have been reported in both pre-clinical[53,80] and clinical studies[32,60] and many different electrode placement techniques have been used in an attempt to minimize these mechanical challenges.

Halverstadt et al. used stainless steel wires embedded them into the bladder wall and positioned towards the bladder dome in people[61]. This was ineffective and the electrodes migrated. Later, cylindrically shaped flexible electrodes were embedded into the bladder wall, which improved the mechanical integrity of the electrode but still led to the displacement of electrodes in the bladder wall[26]. Magasi et al. placed rigid metal disks around the bladder in people, which led to significant damage to the bladder in some people[32]. Kantrowitz et al. implanted electrodes on the bladder surface in a loop-like fashion to accommodate changes in the bladder volume which increased the electrode stability but still resulted in displacement of electrodes[81].

Mechanical complications increase with the number of electrodes and lead wires as the pelvic area is dense and covered with fatty tissue[53]. Researchers have used different electrodes and suturing techniques to overcome this issue. Peterson et al. sutured carbon fiber electrodes like a nerve in a zig-zag manner in the bladder wall near the ureterovesical junction with the proline suture to accommodate changes in bladder volume[75]. This resulted in efficient bladder emptying

over a longer time span but reported mechanical impediment to bladder contraction leading do displacement of electrodes. Timm et al. used coils of platinum-iridium wires insulated with silastic tubing in a zig-zag pattern over the bladder surface to accommodate changes in bladder volume during micturition which increased the positional stability over time[82]. Walter et al. used placed suture electrodes on in the outer serosal layer of the bladder wall at the base of the bladder to prevent eroding of electrodes into the bladder wall, which can cause trauma, and to accommodate changes in bladder volume while still maintaining contact with the neurovascular bundle that innervating the bladder leading to efficient bladder emptying[76]. Later, Walter et al. used Permaloc helical stainless electrodes in a swine model for better flexibility of electrodes with respect to the bladder surface. These electrodes would stretch with the bladder and were more stable for longer durations of the implant and generated complete bladder amptying[80].

In many of these cases, the ultimate success of a particular electrode approach was unclear. Mixed successes were common. However, it can be stated with confidence that none of these devices were ever tested in more than small numbers of people, and no device exists today, suggesting that none of these approaches overcame the fundamental mechanical challenges.

2.4.3 Neural Control of The Lower Urinary Tract After Injury

One of the most difficult challenges for DBWS is not related to the design or use of the device itself, and rather, results from the altered neural control the lower urinary tract that often occurs in injury or disease. The primary issue that often arises is reflexive urethral and pelvic floor contractions, where spasticity of these structures, either electrically induced, or evoked by voiding itself, leads to urethral obstruction and high residual volumes. This was a significant problem identified in many studies of DBWS.

In dogs, DBWS was evaluated in cases of both upper motor neuron (UMN) lesions (supra sacral spinal cord injury) and lower motor neuron (LMN) lesions (cauda equina injury). Complete emptying of the bladder was routinely demonstrated with lower motor neuron lesions[29,46,48,49,51–53,75] and also in chronic studies of upper motor neuron lesions[33,46,48,51,83]. Similarly, Kantrowitz et al. reported successful emptying of the bladder with lower motor neuron lesions but found that urethral spasticity in animals with upper motor neuron lesions prevented efficient voiding. In these cases, a pudendal neurectomy was required to achieve bladder emptying in these cases[29,47]. Pagano et al. also achieved bladder emptying only with LMN lesions[53]. More generally, urethral contractions and spasticity occurred often with UMN lesions[29,33,47]. However, Bradley et al. reported success in UMN lesions (upper thoracic) and LMN lesions (below L7) in achieving bladder emptying with DBWS[58]. They also reported that the stimulus intensities did not change before and after spinal cord injury. This was in contrast to other studies in which stimulation intensities had to be significant increase after SCI[47,50,52].

Cats are a common model of the neural control of bladder function before and after SCI and Walter et al. reported successful bladder emptying with both UMN and LMN lesions in cats. Similar to the studies in dogs, cats with UMN lesions had higher urethral resistance, but stimulation could nevertheless evoke bladder emptying, although this was not complete[55,57]. Again, similar to dogs, LMN lesions in cats led to low urethral tone and inactive pelvic floor reflexes, and complete bladder emptying could be achieved[84]. The authors also emphasize that cats are a better model for LUT studies than dogs, as cats have presentations of bladder and urethral activity as humans with UMN lesions.

In humans, most of the successful bladder emptying cases occurred in people with LMN lesions of both peripheral and central origin. In the longest clinical trials, reported by Halverstadt

et al., people were followed for up to nine years and bladder emptying was achieved in 5 out of 10 patients with hypotonic bladder due to peripheral surgical hysterectomy, pelvic resection surgery, and sacral rhizotomy for pelvic and post-pregnancy urinary retention[26,60,61]. Unfortunately, there have been few successes in people with UMN lesions like are common after SCI. In these cases, secondary surgical procedures like sphincterometry, pudendal neurectomy, and transurethral resection, were required to reduce outlet pressure during bladder emptying due to pelvic floor and urethral spasticity[68]. In one of the most comprehensive studies Magasi et al. reported 32 clinical cases (21 peripheral, 11 central type bladder paralysis), and 29 of 32 cases were considered successful. However, only three of these were in people with UMN lesions, and urethral spasticity and rigidity made it harder to achieve bladder emptying; transurethral resection of the bladder neck was required to facilitate voiding[64]. Merill et al. again attempted DBWS in people with UMN lesions, but these implants were unsuccessful due to outlet obstruction from detrusor sphincter dyssynergia[62].

Across these animal and human studies, several high-level conclusions can be made. First, successful outcomes were often more common in animals than in humans. Second, and perhaps most critically, preserved innervation of the urethra and pelvic floor was likely to prevent DBWS from successfully emptying the bladder. This was due to either direct or reflexively evoked contractions of the EUS and pelvic floor which caused high outlet resistance. Therefore, a major challenge for DBWS is to minimize these contractions. This may be particularly challenging after SCI where dyssynergic contractions of the EUS are common.

2.5 Discussion And Future

During the 1950s through 1990s DBWS was widely studied in both animal and human studies. However, for a number of reasons, including current spread from the electrodes, mechanical incompatibilities between the bladder and electrode, and physiological constraints exacerbated by the injuries and diseases in which it was tested, efforts to continue clinical translatability of DBWS were discontinued. This is somewhat surprising as there were numerous examples of successful outcomes in people, and many of the challenges were likely driven in large part by technological limitations at the time. With recent advancement in implantable technologies, such as flexible electronics, better stimulators, and biomaterials, this technology hold potential to help people with underactive bladder.

Several specific design criteria can be identified based on the successes and failures described above. First, a DBWS device should be flexible to accommodate changes in the bladder volume without electrode displacement or causing trauma to the bladder wall. Second, the device should prioritize minimizing the number of electrodes on the bladder and target these electrodes towards locations around the base of the bladder so that low stimulus intensities can be used to generate large, coordinated bladder contractions. This targeting is especially important as it will minimize the number of the electrodes required, as well as minimize the stimulation currents required to evoke large contractions. Electrodes distributed across the entire bladder may never be an effective approach because of the large currents required to above these contractions, which lead to co-activation of nearby muscles that often prevent voiding. Finally, in addition to minimizing the stimulus currents, every effort should be made to limit the fields to the immediate vicinity of the electrodes. This will further limit the chances of activating nearby tissue structures.

After decades of near silence on DBWS, several recent studies have either developed technologies that could apply to DBWS or have directly revisited the core idea using newer technologies. In one example, an epicardial mesh was created using a flexible nanowire mesh that allowed recording and stimulation from the epicardial surface to treat arrythmias[85]. While the heart undergoes much smaller volume changes, this concept could potentially be adapted to bladder wall stimulation, providing mechanical flexibility, biocompatibility, and consistent electrode-tissue contact. In a direct test of DBWS, an ultra-compliant carbon nanotube-based interface was created that could both sense changes in bladder volume using a strain gauge and also deliver stimulation current[86]. This device was tested in short-term cat experiments, and although changes in bladder pressure were reported, there were only limited data about the functional outcomes. More uncommon approaches have also been attempted in small animals. In one instance a closed-loop soft sensor and actuator system was developed that mechanically compresses the rat bladder to evoke voiding[87]. In another, an expandable mesh was placed around the bladders of mice that contained light emitting diodes which activated light sensitive ion channels (channelrhodopsin) targeted to detrusor muscles cells using an adeno-associated virus[88]. This interface was tested in was able to expand up to 300% of the initial bladder volume and successfully generated bladder pressures.

With the rapid advances in neurotechnologies, including novel thin and flexible electrodes, it is possible that DBWS could be developed into a device that could help people with injuries or diseases where the bladder is underactive or atonic. Perhaps the most significant unaddressed challenge is a reliable, non-surgical method to reduce outlet pressure in scenarios where these EUS or pelvic floor contractions are driven by reflex mechanisms in the sacral spinal cord.

3.0 Design of a Soft Silicone Based Neural Interface on Basis of Functional Mapping of The Bladder Surface

3.1 Overview

Direct bladder wall stimulation (DBWS) has been attempted for many decades to restore bladder function in people with spinal cord injuries and other voiding dysfunctions. However, these efforts were limited by mechanical incompatibilities between the rigid electrodes and bladder tissue – especially during large volume changes – as well as stimulation-induced co-activation of the urethra, legs, and other pelvic organs. The co-activation and mechanical challenges have been attributed to the electrode design, electrode placement on the bladder, and the high electrical stimulation amplitudes typically required for DBWS. We aim to design a neural interface to address these challenges. We stimulated the bladder surface at many locations and recorded bladder pressure to identify locations which led to large pressure changes at minimal stimulation amplitudes. We also recorded the electromyographic activity from nearby muscles in response to stimulation to evaluate if co-activation can be minimized.

With these results, we designed a stretchable soft silicone mesh that could be placed around the bladder to provide a sutureless method to anchor the electrode array to the base of the bladder, which was found to be the most sensitive region for electrical stimulation. We also found that bipolar stimulation significantly reduced, or even eliminated, co-activation of nearby muscles at stimulation amplitudes that evoked large bladder pressures. This device was tested in both acute and chronic implants where stimualtion reliably emptied the bladder. This device design overcomes many of the historically significant challenges of DBWS.

3.2 Introduction

Spinal cord injury and neurodegenerative diseases such as multiple sclerosis and Parkinson's disease can lead to major problems with lower urinary tract (LUT) control[2,3]. This is highlighted by the fact that restoring bladder and bowel function is one of the highest priorities for people living with spinal cord injury[8]. In attempts to restore bladder function, neurostimulation has been attempted at many points in the LUT neural circuit[10]. Direct bladder wall stimulation (DBWS) is one such stimulation technique, which involves electrical stimulation of the detrusor muscle itself or axons innervating the detrusor. With electrodes implanted on the bladder wall, stimulation can cause complete emptying of the bladder[33,46,52,76]. As discussed in previous chapter, DBWS has been performed in animals and human clinical studies to treat various pathological conditions and has successfully produced micturition. Moreover, DBWS directly activates the bladder rather than relying on reflex mechanisms, as is the case with sacral neuromodulation. This can have the effect of increasing voiding efficiency, and minimizing unwanted off-target effects of sacral nerve stimulation, such as leg movement[55]. DBWS also avoids some of the challenges of pelvic nerve stimulation, which is challenging in humans because of difficulties that are experienced in locating and instrumenting these fine nerves[89].

In previous studies, DBWS was mainly limited by the current spread that caused co-activation of nearby tissue structures and challenges in maintaining the mechanical stability of the electrodes on the bladder surface. Current spread arises from excitation of nearby tissue structures directly, or by reflex activation, and can lead to pain as well as activation of urethral, hind limb, and pelvic musculature[89,90]. More specifically, a major challenge of previous studies of DBWS was activation of the external urethral sphincter due to direct current spread to the pudendal nerve resulting in mechanical occlusion of the urethra leading and a dyssynergia-like behavior[29,47,49,58].

Poor electrode insulation[27,50,60,66,91], large electrodes[52,79], suboptimal stimulation parameters[52], poor placement of electrodes on the bladder wall[26,55,63,64], and mechanical and positional instability of electrodes have been the main factors leading to this current spread[26,75,80,82].

Poor electrode insulation was addressed relatively easily by simply insulating the non-bladder-contact side of electrodes or by embedding the electrodes in the serosal layer of the bladder wall[6, 23,29]. On the other hand, optimal electrode sizes and locations have yet to be established even through these two factors clearly have effects on current spread[8, 28]. Various stimulation waveform pulses were also evaluated, and similar to many other studies of electrical stimulation, square pulses have been proven to be the most effective[65]. However, other stimulation parameters that maximize bladder contractions have varied widely; pulse amplitudes range from 5-80 V, stimulus frequencies from 10-40 Hz, and pulse widths from 1-5 ms[52].

One factor the likely drives the large parameter range is the specific location of the electrodes. Bladder contractions can be achieved either by direct muscle stimulation with electrodes placed on the bladder dome[46] or by stimulating motor axons in the bladder wall[56]. High stimulation stimulus amplitudes (20-80V) have been required for stimulation the bladder dome to achieve significant bladder contractions. In contrast, bladder contractions can be more easily evoked by stimulating the nerve network innervating the bladder wall at lower amplitudes (5-50V), especially in the vicinity of the ureterovesical junction (UVJ)[76]. However, it has been reported by some researchers that electrodes closer to the bladder base cause more current spread to urethral structures[61] although bipolar stimulation may reduce this problem[56].

Because the bladder can increase in size by ~10 times during filling, there can be substantial mechanical tension placed on electrodes attached to its surface. This has led to the detachment of electrodes and attachment sutures, leading to electrode displacement and trauma to the serosal

layer[26,32,53,60,80]. To address this issue electrodes and suturing techniques were developed to accommodate the conformal changes in the bladder. Examples include suture electrodes and woven eye electrodes, but this issue has remained a challengingproblem[56,57].

To address these various challenges, it is necessary to develop electrodes and stimulation techniques that optimize electrode design, location, and stimulation parameters to maximize bladder contractions at the lowest optimal stimulation amplitude while also constraining the electric field around the electrodes. Further, electrodes should be positionally and mechanically stable to accommodate the conformal changes in the bladder during natural filling and emptying. In this paper, we develop and evaluate a novel neural interface for DBWS in cats. We mapped the bladder surface to find the optimal locations and stimulation parameters using customized 3D-printed electrode arrays. Further, the effects of different configurations (monopolar, bipolar stimulation) and co-activation of nearby tissue structures were evaluated.

3.3 Methods

All experiments were performed under the approval of the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC). Terminal acute experiments were performed using 10 anesthetized cats (7 males and 3 females) weighing 2.6-5.5 Kg.

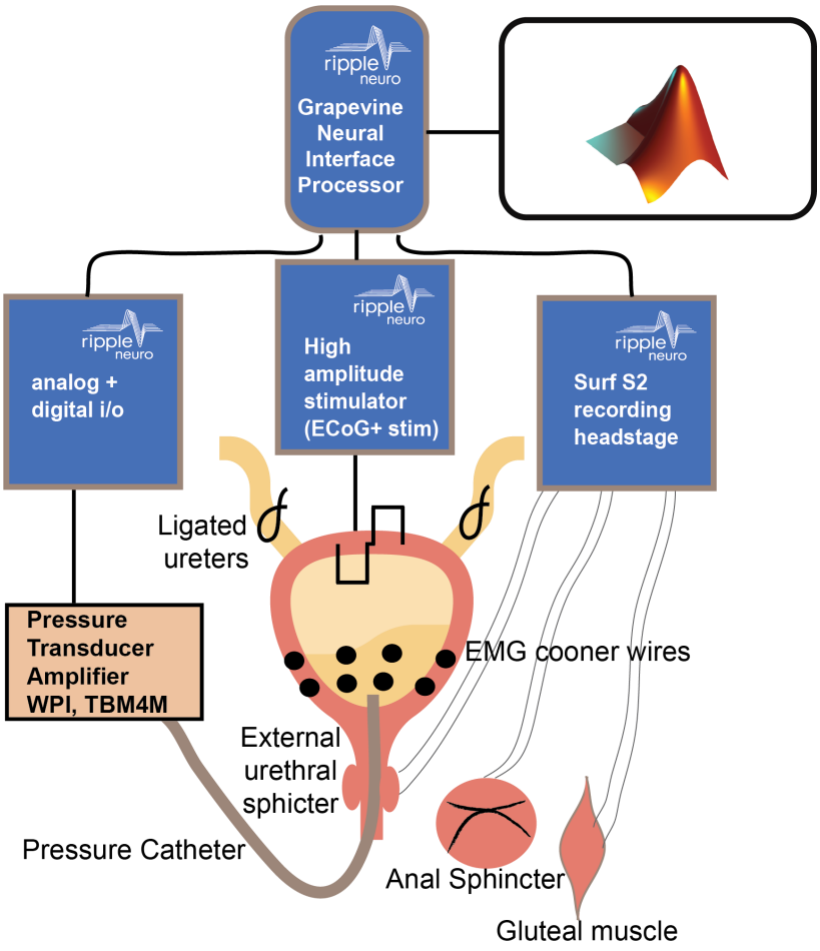

Figure 3.1 Experimental setup A transurethral catheter was inserted for fluid infusion and pressure monitoring. Three different electrode arrays were placed on the bladder. Stimulation and data recording were performed using Grapevine neural interface processor (Ripple Inc). Ureters were ligated and drained externally with a suture on the urethra to maintain the bladder under isovolumetric conditions.

3.3.1 Surgical Preparation

Anesthesia was induced with ketamine (10 mg/kg) and was maintained via inhaled isoflurane (1%-2%). Isoflurane suppresses the reflex activity of the LUT[92]. A tracheostomy was performed to connect the trachea to an artificial respiration system. Animals were artificially ventilated at 12-14 breaths per minute throughout the procedure. Animals were monitored

continuously for heart rate, SpO2, blood pressure, and body temperature on a vital monitoring system (SurgiVet). A pressure-sensing catheter (AD Instruments) was inserted in the carotid artery for blood pressure monitoring. Warm air heating pads were used to maintain temperature, and IV fluids (Nacl-Dextrose) were administered continuously.

A midline abdominal incision was made to expose the bladder. The proximal urethra and the ureters were ligated to maintain the bladder at an isovolumetric state. The right ureter from the kidney was sutured to drain urine from the kidney externally. An electrode array was placed on the bladder for stimulation. The proximal urethra was ligated to the pressure catheter to keep the bladder at isovolumetric condition. A single-lumen polypropylene pressure catheter was inserted via the urethra to record bladder pressure and infuse saline into the bladder. Bladder pressure was recorded from this catheter with a pressure transducer connected to a Trans-Bridge amplifier (WPI Inc.). For experiments including EMG recording, fine wire (Conner wire inc.) bipolar EMG electrodes were inserted in the external urethral sphincter, external anal sphincter, and the gluteal muscle of the left hindlimb through needles (figure 3.1).

3.3.2 Electrode Design and Placement

For the first two experiments (n=2, subjects A and B), 3D-printed, straight strips of platinum electrodes embedded in soft silicone with a diameter of 1 mm and spaced 1 cm apart were placed on the bladder wall. These straight polymer strips with platinum embedded circular electrodes (16 electrodes, diameter 1mm, interelectrode distance: 1cm) were developed (figure 3.2a), which were placed on the dorsal, ventral, and both lateral aspects of the bladder, and stimulation was performed on each electrode for functional mapping of bladder surface. For the following six experiments (Subjects C-H), an X-strip electrode array with electrodes of a diameter

of 1.5 mm (figure 3.3a) was developed based on the observations of the first set of experiments to study optimal electrode locations and parameters further. The electrodes were arranged on the X strip array such that the bladder dome and the bladder base had the highest density of electrodes (8 on the dome with 4mm inter-electrode distance, 4 on the body with interelectrode distance of 16 mm from the dome and base electrodes and 20 on bladder base covering dorsal, ventral and both the lateral aspects with 5 electrodes with interelectrode distance of 4 mm). For the remaining two experiments (subjects I and J), based on the observations from experiments with previous arrays, 12 electrodes were placed on the bladder base in two concentric circles of 6 electrodes, each separated by 2.0 mm vertically, as shown in figure 3.5a. These electrodes were anchored with a soft silicone mesh with a convoluted structural design over the bladder (figure 3.5a), allowing accommodation of changes in the bladder while maintaining electrode stability.

3.3.3 Determination of Isovolumetric Bladder Volume

Prior to ligation of the urethra, the bladder was manually filled with saline through a transurethral catheter until saline began to leak past the catheter. The volume at which the bladder started leaking was considered the maximum bladder capacity, and the volume was maintained between 70-90% of maximum bladder capacity for optimally placing the electrodes.

3.3.4 Data Collection and Instrumentation

The analog output from the pressure amplifier was recorded using an analog to digital headstage connected to the Grapevine Neural Interface Processor (Ripple Inc.). In the first two experiments the electrode array on the bladder surface was connected to an AM Systems Model

2100 stimulator. Stimulation was performed manually and stimulation event triggers from the stimulator were recorded through the same analog to digital headstage connected to the Grapevine. For later experiments, we used a custom programable stimulator (Ripple) that had a compliance voltage of 30V and a maximum current amplitude of 15 mA. This stimulator was interfaced with a Grapevine Neural Interface Processor through MATLAB (MathWorks Inc.). EMG signals were sampled at 30 kHz with Surf S2 headstage connected to the Grapevine Neural Interface Processor.

3.3.5 Stimulation Protocol

For functional mapping, we individually stimulated each electrode in the net using biphasic pulses (cathodic first) with an interpulse interval of 66 μs. For the first two experiments, stimulation amplitude was varied from 2-6 mA while the pulse width of 1 ms and frequency of 10 Hz were kept constant. For functional surveys, we stimulated at 2, 4, and 6 mA and stimulation-evoked bladder pressures were monitored and considered significant if the stimulation-evoked bladder pressures were greater than 10 cmH_2O. In some experiments, the same stimulation protocol was performed at two additional frequencies: a low (3 Hz) and a high (30Hz) frequency. For the last two experiments, the amplitude was varied from 1-4 mA with a constant frequency of 30 Hz and pulse width of 1 ms. Other stimulation parameters were occasionally tested.

3.3.6 Data Analysis and Statistics

All data analysis was performed in MATLAB (Mathworks Inc). The pressure change was calculated as a difference between the average pressure in a 100 ms window prior to stimulation onset and the highest value reached during the stimulation time window. Pressure changes greater

than 10 cmH_2O were considered to indicate a contraction and was used for further statistical analyses. Stimulation artifact was removed from EMG signals by blanking the signal for either 6 ms (animals G, I, J) and 10 ms (animal H) from stimulation onset and the signal was interpolated in this window. Following blanking, the EMG signals were rectified, band pass filtered (100-1000 Hz). The mean absolute value (MAV) in a 10 s pre-stimulation window was subtracted from the MAV during stimulation as a measure of EMG activity during stimulation.

Ten animals were used in this overall study with smaller numbers in each group depending on the specific details of the experiments. Pressure change data were typically non-normally distributed ($p< 0.05$, Shapiro-Wilk normality test), so we used the Kruskal-Wallis test to comparison across unpaired groups with the Wilcoxon rank sum test as a post hoc test to compare across two groups. For paired data across multiple groups, we used Freidman test followed by post hoc testing with the Wilcoxon sign rank test. A p value < 0.05 was considered as a significant effect. We also performed linear mixed model analyses, details of which are reported in Appendix-A.

3.4 Results

3.4.1 Effects of Electrode Location

We stimulated multiple locations covering the surface of the bladder to determine whether specific areas differentially evoked bladder contractions. We compared different monopolar stimulation parameters and the evoked bladder pressure changes while the bladder was maintained

at a constant volume. These tests were performed with three customized electrode arrays and are reported according to the array type.

The first type of array (n=2, subject A, B) was a straight strip electrode array with 16 contacts out of which 6 electrodes were placed in contact with the bladder on 4 aspects of the bladder (24 sites on the bladder), each with a 1 mm diameter (Figure 3.2a). These strips were placed on four different aspects of the bladder, oriented along the axis from the bladder dome to the bladder base. We varied the stimulation amplitude while holding the frequency and pulse width constant at 10 Hz and 1 ms, respectively. The threshold amplitude (A1) was determined to be the value at which maximum electrodes generated a change in the bladder pressure higher than 10 cmH_2O. The threshold amplitude (A1) varied for two subjects (A1=2 mA, A2 = 4 mA for subject A, A1=4 mA, A2 = 6 mA for subject B). An example of stimulation evoked bladder pressure changes from one subject at stimulation amplitudes A1, A2, and at different locations on the bladder surface is shown in figure 3.2b. Stimulating through an electrode at the bladder base generated higher bladder pressures than an electrode at the bladder dome at both these amplitudes. Figure 3.2c shows the evoked bladder pressure responses upon stimulating different locations on the bladder surface (subject A, A2=4 mA). The maximum changes in bladder pressure were observed at the bladder base. The evoked pressure responses to stimulation at stimulation amplitudes A1 and A2 and locations on the bladder surface for both the subjects (A, B) are shown in figure 3.2d.

There was a no statistically significant difference between the pressures evoked by stimulation with respect to amplitude or location ($p=0.1547$, Kruskal-Wallis test). This could be dure to the small sample size. However, at both A1 and A2 at the bladder dome, stimulation evoked pressures were higher for the bladder base (A1, median: 16 cmH_2O, IQR: 20 cmH_2O) followed by

bladder dome (A1, median: 13 cmH_2O, IQR: 7.5 cmH_2O) and then the bladder body (A1, median: 10 cmH_2O, IQR: 5.5 cmH_2O). Similarly, at amplitude A2, evoked pressures were higher for the bladder base (A2, median: 17.5 cmH_2O, IQR: 35 cmH_2O) followed by bladder dome (A2, median: 16.8 cmH_2O, IQR: 4.5 cmH_2O) and then the bladder body (A2, median: 12 cmH_2O, IQR: 8 cmH_2O).

At the maximum stimulation amplitude (A2), 9 out of 16 electrodes on the bladder dome generated bladder contractions leading to a change in pressure threshold of 10 cmH_2O, whereas 3 out of 8 electrodes responded on the bladder body. 9 out of 24 electrodes responded at the bladder base, but these pressure changes were higher than the bladder dome and the bladder body. At maximum stimulation amplitude (A2), stimulating electrodes at the bladder base yielded maximum pressure changes of up to 52 cmH_2O, while stimulating electrodes at the bladder dome generated bladder contractions not more than 25 cmH_2O. This preliminary data indicated higher neural excitability of the bladder dome and bladder base region.

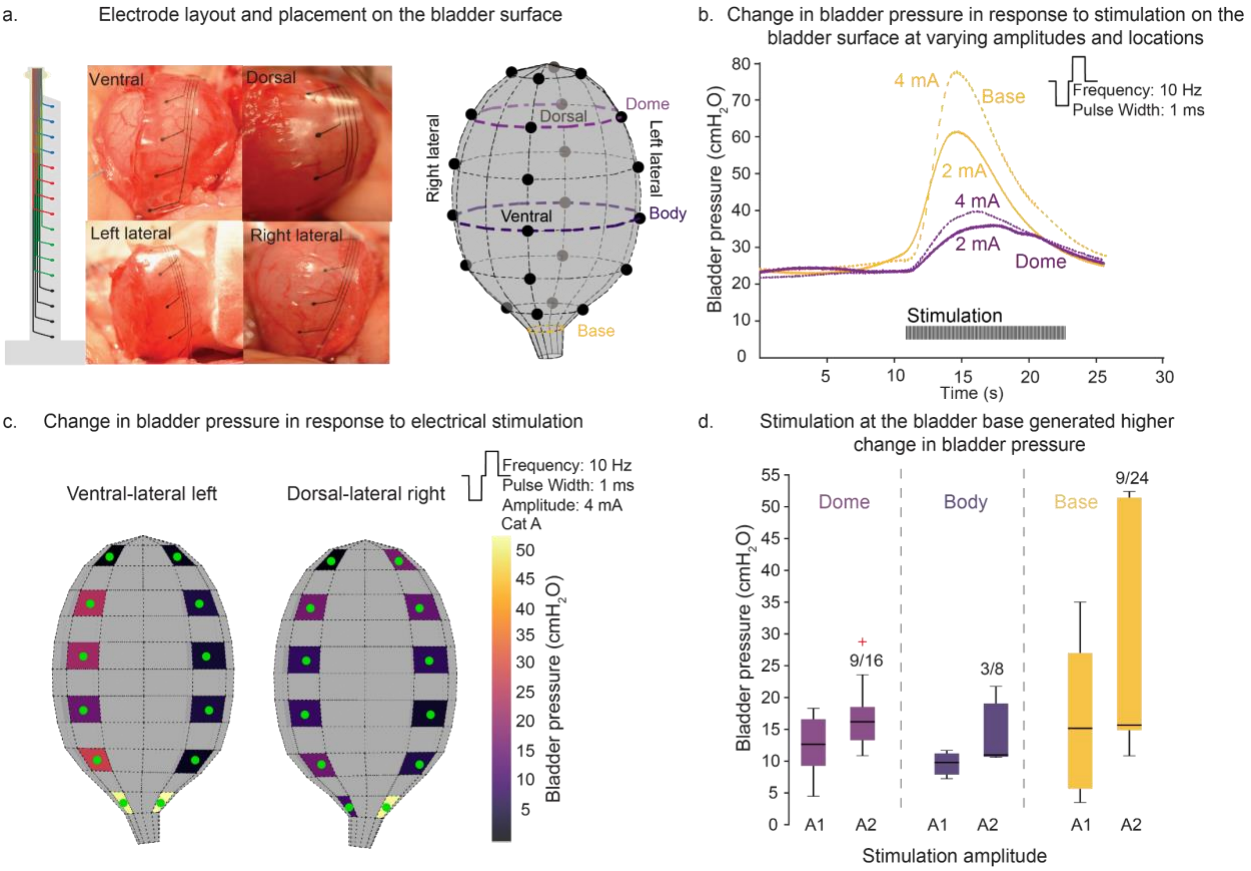

Figure 3.2 Effect of electrode location and stimulation amplitude with the first type of array a) Straight strip electrode array (first type of array) and straight strip electrode array placed on different aspects of the bladder. b) Raw bladder pressure signal upon stimulating electrode at bladder dome and bladder baser at A1=2 mA and A2=4 mA for subject A. c) Evoked responses while stimulating electrodes on bladder surface shown with different aspects of the bladder at stimulation parameters (Amplitude: A2=4mA, Frequency: 10 Hz, Pulse Width: 1 ms). d) Evoked pressure responses w.r.t change in amplitude (A1, A2) and location on the bladder surface (dome, body, base) for subject A and subject B.

Based on these results, we designed an electrode array with a higher density of electrodes on the bladder dome and the bladder base. ***This second type of array looked like 'X' with 32 electrodes*** which were placed on the bladder surface such that 20 electrodes were on the bladder base (5 each on each dorsal, ventral, and both lateral aspects of the bladder), as well as 8 electrodes

on the bladder dome and 4 on the bladder body as shown in figure 3.3a. This array was used in five experiments to further map the regions on the bladder with higher neural excitability (n=5, Subject C, D, E, F, G). For each animal, two stimulation amplitudes (A1, A2) were used (A1 = 2 mA, A2= 4 mA for subject C, D, E, G and A1= 4mA, A2= 6 mA for subject F)**.** The pulse width of 1 ms and frequency of 30 Hz was kept constant to compare the pressure changes evoked by stimulation at different locations. Figure 3.3b shows the evoked bladder pressure responses upon stimulating different locations on the bladder surface (subject C, A2= 4 mA). Stimulation of electrodes at the bladder base generated higher bladder pressure than electrodes at the bladder dome and the bladder body. The evoked pressure responses to stimulation at both stimulation amplitudes and different locations on the bladder surface for 5 subjects (C, D, E, F, and G) are shown in figure 3.3c. Stimulation evoked pressure were found to be significantly different with respect to amplitude and the location (p=3.3e-13, Kruskal-Wallis test). So, further group-wise testing was nonparametric tests as post-hoc with Bonferroni correction.

At stimulation amplitude A1, there was no significant difference between the bladder pressure changes across the dome, body, and base (dome-body: adjusted p=0.15, body-base: adjusted p =0.23, dome-base: adjusted p =0.1, Wilcoxon rank-sum test). At stimulation amplitude A2, the pressures generated at the bladder base were significantly higher than the bladder dome (p adjusted: 1.71e-21, Wilcoxon rank sum test) and the bladder body (p adjusted: 6.03e-20, Wilcoxon rank sum test). Pressure evoked at amplitude A2 were significantly higher than pressure evoked at A1 for the bladder body (adjusted p=3.5e-5) and the bladder base (adjusted p=1.1e-19). However, this difference was not statistically significant for the bladder body(adjusted p=0.54, n=3) although the pressures evoked at A2 (median: 21 cmH_2O, IQR: 12 cmH_2O) were higher than at A1(median: 4 cmH_2O, IQR: 7 cmH_2O)

At the maximum stimulation amplitude (A2), the bladder dome and bladder base showed minimal activation compared to electrodes on the bladder base. 9 out of 40 electrodes on the bladder dome generated bladder pressure changes greater than 10 cmH_2O, 3 out of 20 electrodes responded on the bladder body, and 50 out of 100 electrodes at the bladder base. Further, pressures evoked by stimulating electrodes at the bladder base on different aspects of the bladder (ventral, left lateral, dorsal, and right lateral) were compared at maximum amplitude (A2). An example of pressure responses evoked by stimulating electrodes on different aspects of the bladder base (subject C) is shown in figure 3.3d. Figure 3.3e compares stimulation evoked bladder pressure changes across these four aspects of the bladder. We found no significant difference between the pressures evoked by stimulating electrodes on different aspects of the bladder at the bladder base ($p=0.279$, Kruskal Wallis test).

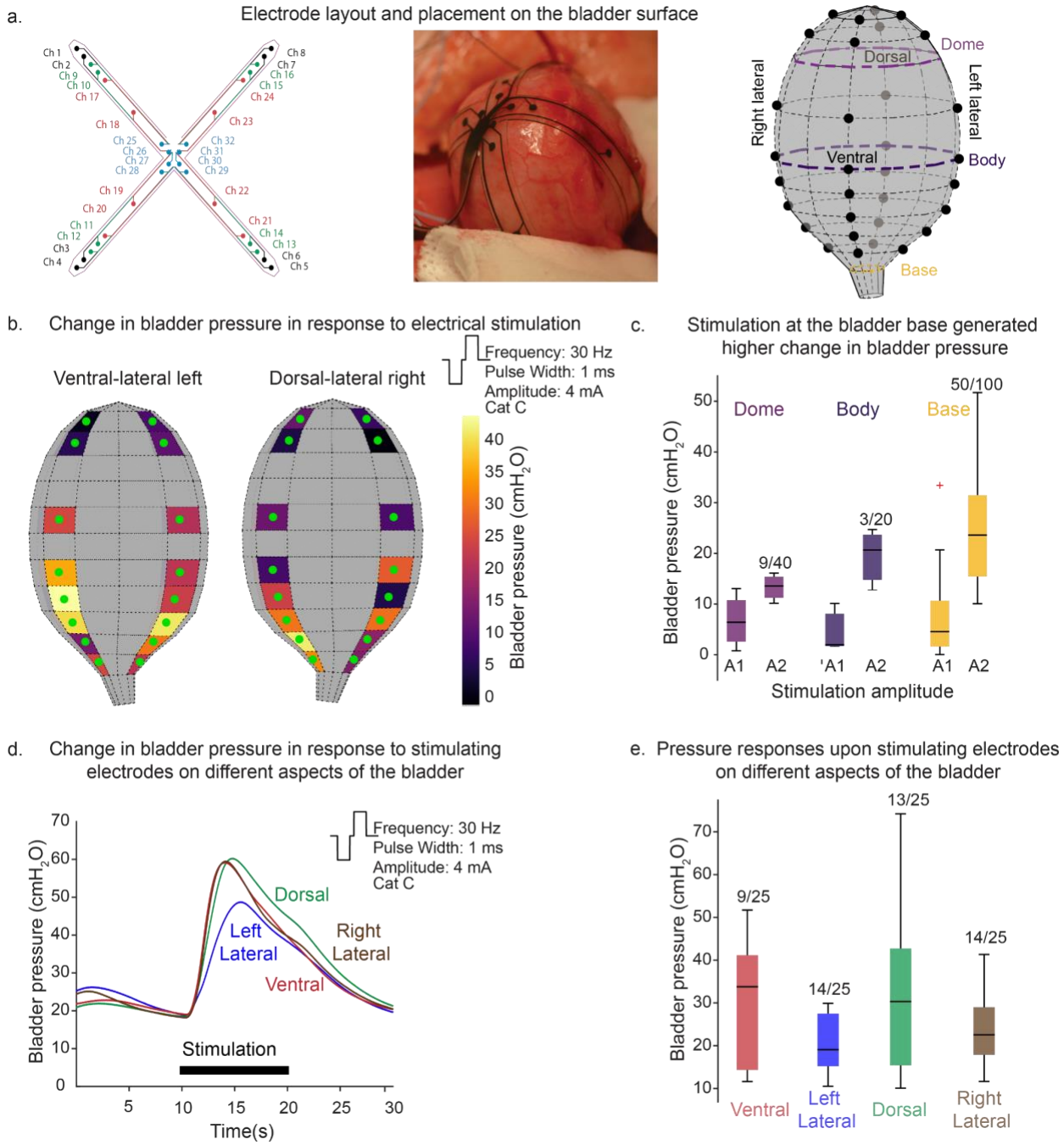

Figure 3.3 Effect of electrode location and stimulation amplitude with the second type of array a) X-strip electrode array and X strip electrode array placed on the bladder covering different locations. b) Evoked responses while stimulating electrodes on the bladder surface are shown with different aspects of the bladder at stimulation parameters (Amplitude: A2=4mA, Frequency: 30 Hz, Pulse Width: 1 ms). c) Comparison of evoked pressure changes w.r.t location on bladder and stimulation amplitudes (A1, A2).). d) Raw stimulation evoked pressure responses illustrated according to different aspects of the bladder while

stimulating single electrodes on bladder base at stimulation amplitude A2 in subject C. e) Evoked responses at stimulation amplitude=A2, frequency: 30Hz, Pulse width: 1 ms while stimulating electrodes on different aspects of the bladder at the bladder base in 5 subjects.

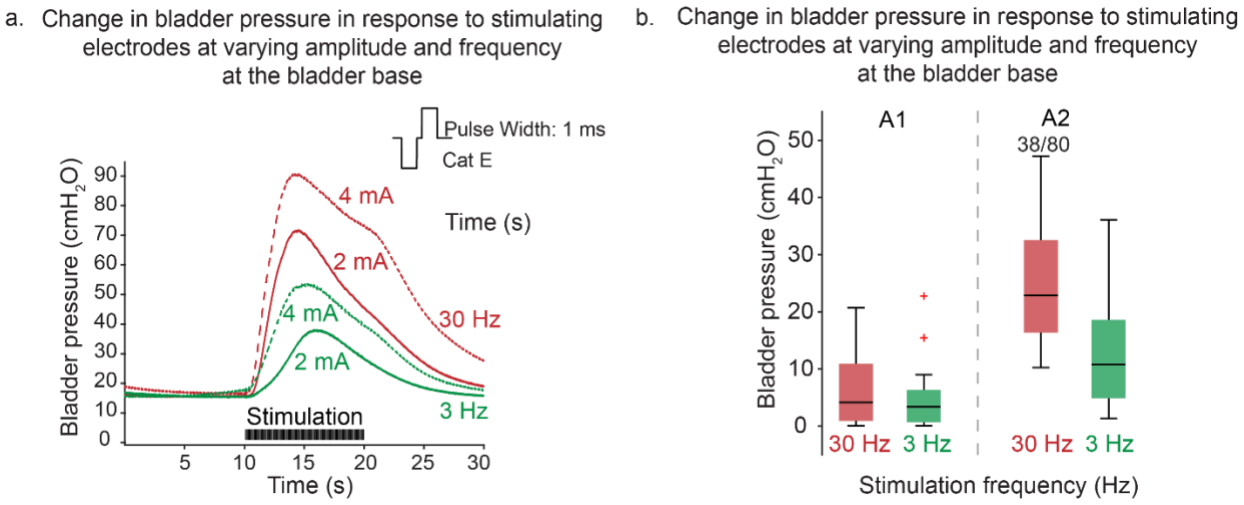

Figure 3.4 Effect of stimulation frequency on evoked bladder pressure upon stimulating the bladder base electrodes a) Evoked bladder pressure responses at stimulation amplitude A1 and A2 at 3 and 30 Hz on a single electrode on the bladder base in a single subject. b) Evoked responses at stimulation amplitude A1 and A2 for two frequencies (3 and 30 Hz at pulse width 1 ms across subjects (n=4) at the bladder base.

Further, we also stimulated electrodes on the bladder surface at a lower frequency (3 Hz) to test the effects of electrical stimulation at both stimulation amplitudes (A1 and A2) in 4 subjects (C, D, E, and F). A typical example illustrating the change in bladder pressure upon stimulating the electrodes on the bladder base at these two frequencies (3 and 30 Hz) and stimulation amplitude (A1 and A2) is shown in figure 3.4a. We compared stimulation evoked bladder pressure changes at the electrode base at these two amplitudes and frequencies (3 and 30 Hz), as shown in figure 3.4b. There was a significant difference in bladder pressure with respect to amplitude and the frequency ($p=0.001$, Friedman test). We found that the pressure changes evoked at 30 Hz for both

stimulation amplitudes (A1 and A2) were significantly higher than 3 Hz (p-adjusted *p<0.0125, Wilcoxon sign-rank test*). Moreover, the pressures generated at amplitude A2 were significantly higher than pressure generated at amplitude A1 for both frequencies (p-adjusted *p<0.0125, Wilcoxon sign-rank test*). This graded response of stimulation parameters further strengthens the robust effects of electrical stimulation at the bladder base in evoking robust bladder contractions.

*Based on these results, we created the **third type of array** used in 2 subjects (n=2, subject I, J),* shown in figure 3.5a. In this design, the electrode array had 12 electrodes, arranged in two concentric circles of 6 electrodes at the bladder base with equal interelectrode distance and attached to a backbone style soft silicone mesh around the bladder to anchor the electrode array. This soft silicone mesh keeps the electrode in contact with the bladder base, and the silicone mesh conforms during fill and empty cycles. All 12 electrodes were stimulated individually at increasing amplitudes (1, 2, 3, 4 mA) at a frequency of 30 Hz and pulse width of 1 ms, and evoked bladder pressures were measured. Figure 3.5b shows the evoked bladder pressure responses upon stimulating different locations on the bladder base (subject I, 3 mA). Figure 3.5c shows the stimulation produced changes in bladder pressure at different amplitudes. Bladder pressures were significantly different across the amplitudes (p=0.001, Friedman test). Bladder pressure changes significantly increased with stimulation amplitude up to 3 mA (1-2 mA, p-adjusted = 0.0067, 2-3 mA, p-adjusted = 0.02, Wilcoxon paired test with Bonferroni post hoc). No significant difference in pressure changes was observed between 3 mA and 4 mA (p-adjusted=0.6874, Wilcoxon paired). At 4 mA, 19 out of 24 electrodes generated pressure changes above the threshold of 10 cmH_2O. Overall, these data suggested that the bladder base is the most sensitive to electrical stimulation, and electrodes confined to that region are enough to generate robust bladder contractions.

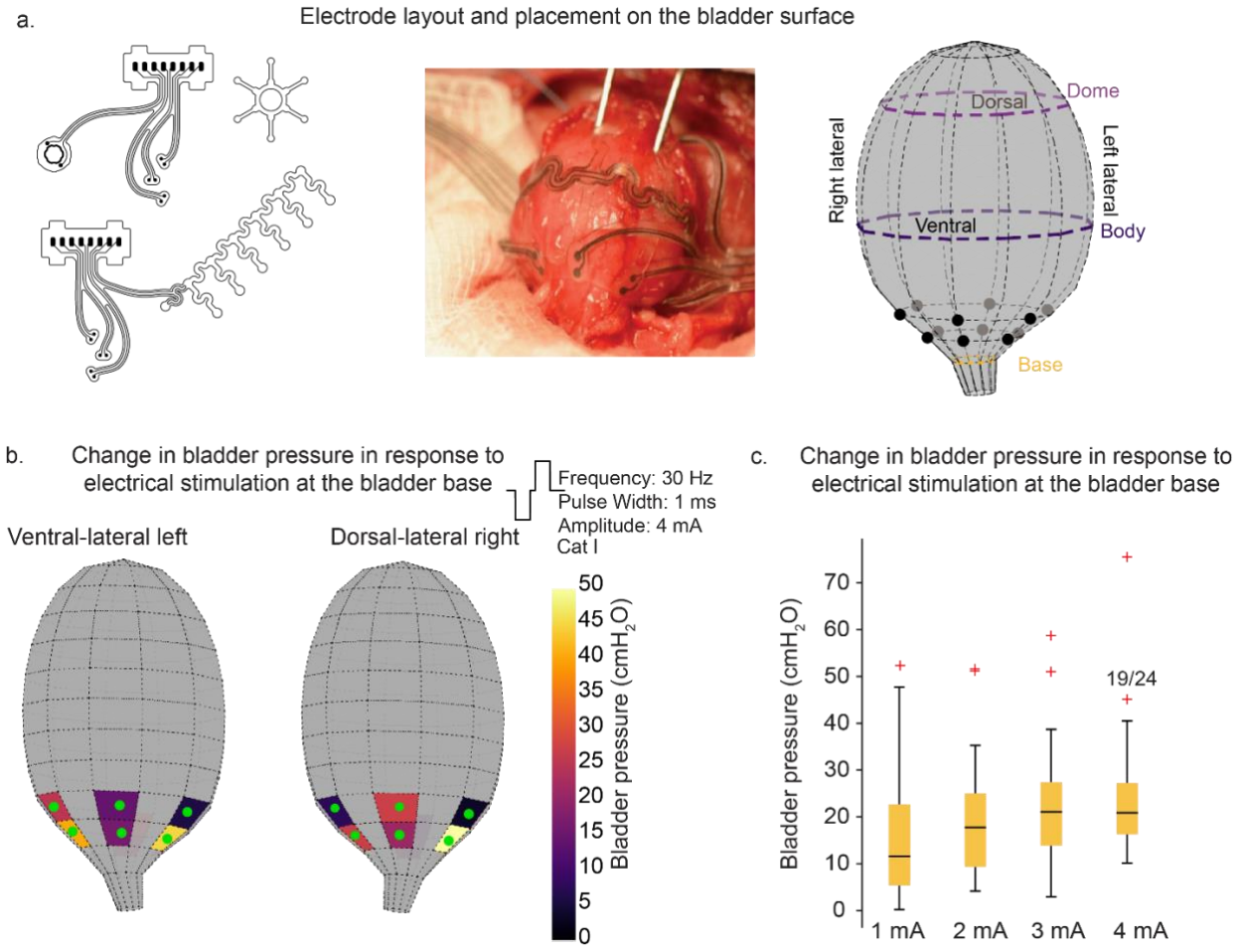

Figure 3.5 Effect of electrode location and stimulation amplitude with the final array design a) (left) Instrumented electrode array and (middle) Instrumented electrode array placed on bladder (n=2), (right) Arrangement of electrodes on bladder. b) Evoked pressure responses at stimulation amplitude: 3 mA, frequency: 30Hz, pulse width: 1ms for subject I at different electrode locations on bladder base. c) Comparison of evoked pressure responses with increase in amplitude (1-4mA) at 30 Hz and 1 ms.

3.4.2 Effects of Bipolar Stimulation

In many experiments, we observed that stimulation evoked contractions of abdominal and even leg muscles at sufficiently high current amplitudes. Current spread to nearby tissue structures in the perineal and pelvic region lead to high urethral resistance due to mechanical occlusion of the urethra and muscle spasms of the leg, leading to residual urine in the bladder. This has been one of the major problems reported in the literature. To address that, we stimulated bipolar electrode pairs to orient the electric field between them, thereby reducing the activation of nearby tissue. We tested two different electrode spacing, as shown in figure 3.7a, using electrodes on the bladder base, and measured evoked EMG in surrounding muscles to determine the extent of charge spread. In subject H, we stimulated electrodes on the bladder base at high stimulation currents (8, 10 mA) at 30Hz frequency and 1 ms pulse width. An example of typical stimulation-evoked pressures is shown in figure 3.6a. All three EMGs (EUS, EAS, and Gluteal muscle), as well as the hind limb movement data from an accelerometer, shows a substantial decrease in MAV from recorded EMGs during bipolar stimulation compared with monopolar stimulation ($p<0.005$, Kruskal-Wallis test, post-hoc with Bonferroni correction, figure 3.6b) while evoking similar changes in bladder pressure ($p=0.9$, Kruskal-Wallis test, figure 3.6a). Two cases of EMG activity from the external urethral sphincter are shown in figure 3.6c. In the first case, where bipolar stimulation eliminated the current spread currently. Second, where bipolar stimulation led to a decrease in co-activation of EUS compared to monopolar stimulation. The stimulation evoked hind limb movement was eliminated with bipolar stimulation at both stimulation amplitudes (figure 3.6b).

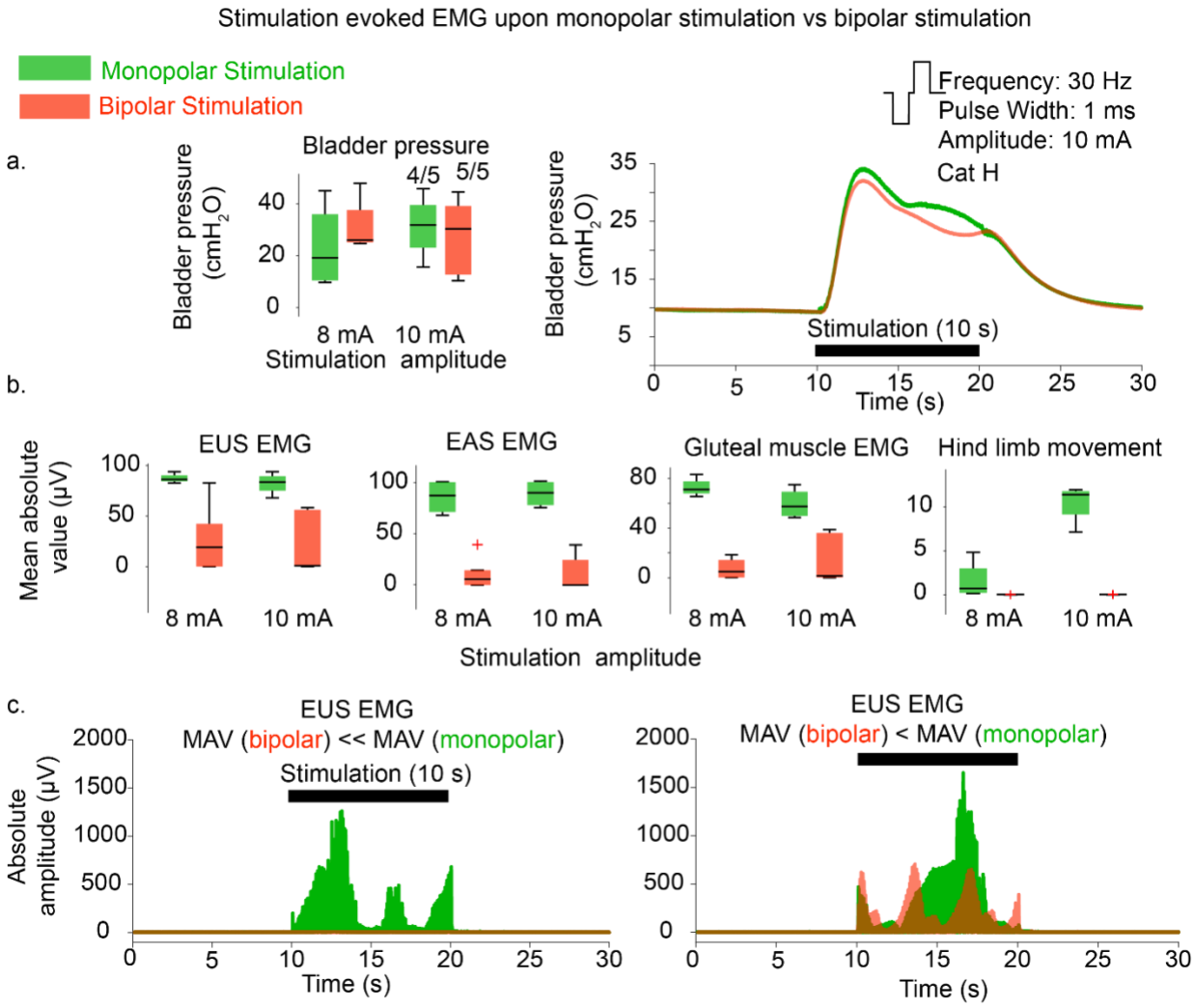

Figure 3.6 Stimulation evoked EMG upon Monopolar stimulation and bipolar stimulation at higher amplitudes a) Left: Comparison of evoked bladder pressure at two high amplitudes (8, 10 mA) comparing monopolar stimulation vs. bipolar stimulation. Right: Bladder pressure traces during monopolar and bipolar stimulation on stimulating one electrode at amplitude: 10mA, frequency: 30Hz, pulse width: 1ms. Right: Comparison of Pressures upon monopolar and bipolar stimulation at 8, 10 mA. b) Left-right: the mean absolute value of EMGs recorded from EUS, EAS, and Gluteal EMG and the hind limb movement from the 3-axis accelerometer. c) Two cases showing the EMG from EUS where bipolar stimulation eliminated the co-activation of EUS (left) and a case where co-activation was reduced with bipolar stimulation in comparison to monopolar stimulation (right).

After testing the current spread at higher amplitudes, we tested for the current spread at functionally relevant and reasonable stimulation parameters. In subjects G, I, and J, EMGs were recorded during monopolar and bipolar stimulation at 2 and 4 mA while a frequency of 30 Hz and pulse width of 1 mA was kept constant. The mean absolute value (MAV) as a measure of current spread was observed to be increasing with amplitude for both monopolar and bipolar stimulation with stimulation amplitude. In figure 3.7b, it is shown that there is not a significant difference between the bladder pressure evoked by monopolar and bipolar stimulation ($p=0.3$, Kruskal-Wallis test) at 2 mA and 4 mA. However, the mean absolute values of all three EMGs were significantly different with respect to the type of stimulation ($p<0.005$, Kruskal-Wallis test)

At lower stimulation amplitude (2 mA), there is a significant reduction in the mean absolute value of all 3 EMGs (EAS, EUS, and Gluteal muscle) with bipolar stimulation (p-adjusted<0.005, Wilcoxon rank sum test). At higher amplitude (4 mA), there is a significant reduction in the mean absolute value of all EAS and gluteal muscle EMGs with bipolar stimulation (p-adjusted<0.005, Wilcoxon rank sum test). For EUS EMG, there was a reduction in mean absolute value with bipolar stimulation, but no statistically significant difference was found (p-adjusted=0.5, Wilcoxon unpaired*).* Even with the monopolar stimulation, the mean absolute values of EUS EMGs have a median value of 7.6 μV, which might be insufficient to generate force in the EUS muscle. These data indicate that the co-activation of nearby tissue structures can be reduced upon stimulating the bladder surface with bipolar stimulation.

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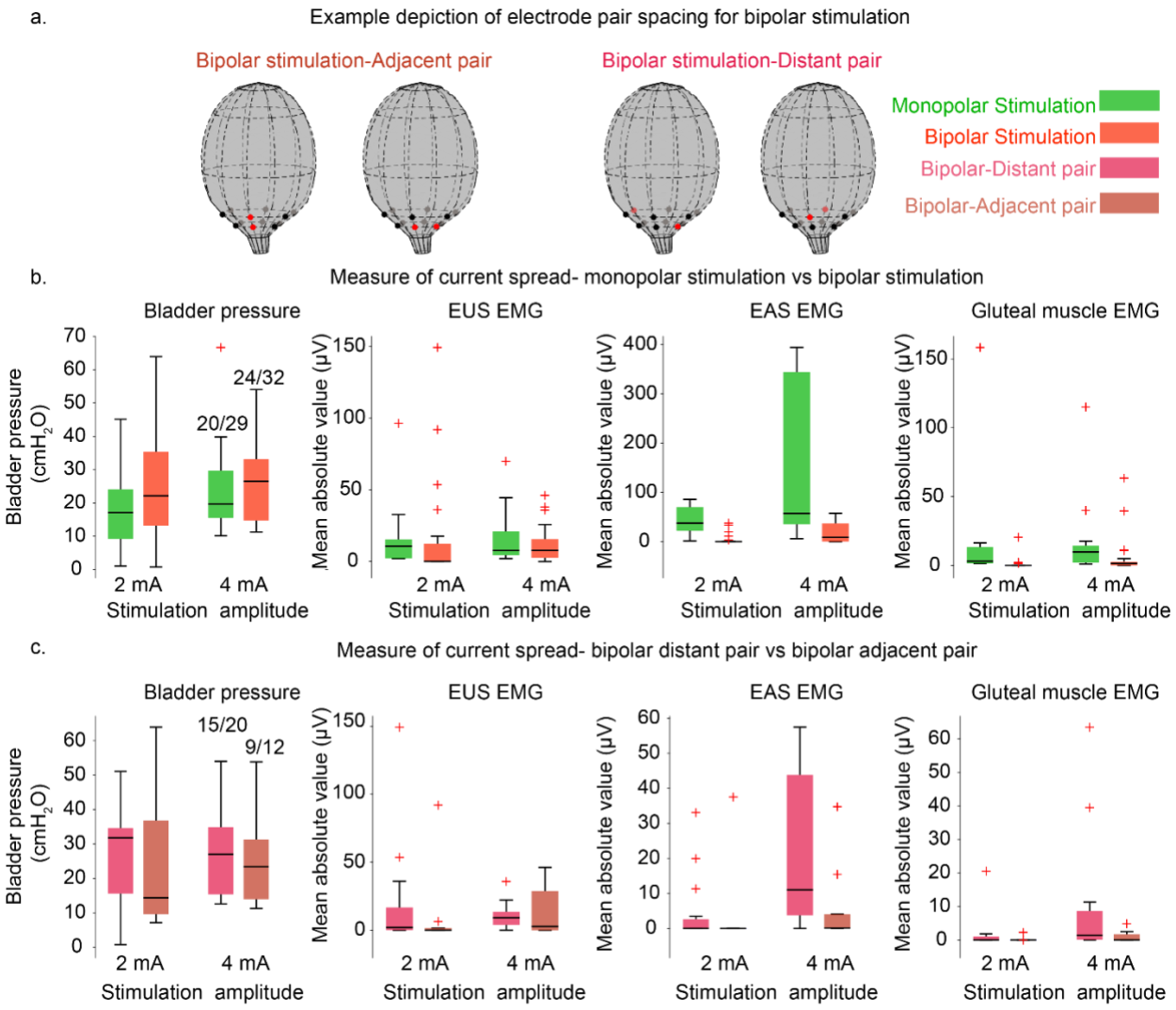

Figure 3.7 Stimulation evoked EMG upon Monopolar stimulation and bipolar stimulation at functionally relevant amplitudes a) Example depiction of bipolar electrode pair spacing for adjacent electrodes (left) and distant electrodes (right). b) Measure of current spread (Mean absolute value of EMG signal) at two amplitudes (2, 4 mA) in three subjects (n=3). From left to right: Evoked pressure changes are compared at two amplitudes (2, 4 mA) with monopolar and bipolar stimulation followed by the MAV of EMG signal for external urethral sphincter (EUS), external anal sphincter (EAS), and Gluteal muscle EMG. c) Measure of current spread (MAV of EMG signal) at different amplitudes in three subjects (n=3). From left to right: Evoked pressure changes are compared at different amplitudes for adjacent and distant pairs during bipolar stimulation, followed by the MAV of EMG signal for EUS, EAS, and Gluteal muscle EMG.

Further, we investigated the differences in current spread with bipolar pairs adjacent and distant, as shown in figure 3.7a. Although distant pairs generated higher changes in bladder pressure in some trials, it was observed that the evoked pressure from a bipolar stimulation pair with adjacent electrodes was not significantly different (p-adjusted>0.05, Wilcoxon rank sum test) than the bipolar stimulation pair with distant electrodes. Mean absolute values of EMGs recorded from the external anal sphincter were significantly reduced with adjacent bipolar stimulation at 4 mA(p-adjusted = 0.02, Wilcoxon unpaired). The mean absolute values recorded from the external urethral sphincter and the gluteal muscle followed a similar trend where the current spread was reduced with adjacent pair simulation but were not statistically significant (p-adjusted>0.05, Wilcoxon rank sum test). This can be attributed to the fact that the bipolar stimulation, in general, reduced the co-activation of nearby muscles, evident from the low median of mean absolute values of recorded EMGs, which are not enough to generate the force in muscles to co-activate these muscles which might cause urethral resistance. For distant bipolar pair at maximum stimulation amplitude (4 mA), the median for the mean absolute values was low (EUS = 9.1462 µV, EAS = 11.01 µV, Gluteal muscle= 1.4402). For the adjacent bipolar pair at maximum stimulation amplitude (4 mA), the median for the mean absolute values was lower than the bipolar distant pair (EUS = 2.85 µV, EAS = 0.1 µV, Gluteal muscle= 0 µV).

3.5 Discussion

Optimal placement: We found that the electrodes placed at the bladder base generated higher stimulation-evoked bladder contractions compared to the bladder body and the bladder base at similar stimulus intensities. Detrusor muscle contractions can be evoked either by myogenic

stimulation of the bladder wall or by nerve-evoked contractions mediated by synaptic transmission[27,73] through the intramural network in the bladder wall. While the former is easier as it doesn't require finding the optimal location, it requires high amplitudes of stimulation to generate a robust bladder contraction which is likely to result in pain and co-activation of the pelvic floor, perineal and hind limb muscles, which additionally create bladder outlet obstruction and uncomfortable voiding. Instead, nerve-evoked responses requiring lower stimulation thresholds are ideal for the long-term feasibility of direct bladder stimulation and mitigate the co-activation and pain problems. Significant pressure changes upon stimulating a single electrode on the bladder base might indicate nerve-evoked contractions through the intramural nerve network, resulting in whole bladder contractions[76]. Stimulating single electrodes on other locations such as the bladder body and bladder dome, both of which produced smaller pressure changes in this study, are indicative of local direct contraction of the detrusor muscle, which is confined to the near vicinity of the stimulating electrodes and therefore results in only small changes in net bladder pressure[27]. Intramural innervation on the bladder wall is denser at the bladder base in the vicinity of the ureterovesical junction (UVJ) than on the bladder body[48]. Innervation from the pelvic plexus enters into the bladder wall near this junction. Conversely, innervation on the bladder body is spread out, and electrodes in this region are less likely to be near a nerve in this area. It is therefore a viable option to place electrodes at bladder base, which facilitates whole bladder contractions at smaller amplitudes.

Stimulation parameters: Stimulation-evoked bladder pressures could be generated by stimulating electrodes at the bladder base at stimulus amplitudes ranging from 1- 6 mA. Stimulation parameters vary a lot depending on the electrode-tissue contact and the location of electrodes on the bladder [65]. Previous literature has reported different stimulation amplitudes

generating significant bladder contractions. However, the pulse width(1-4 ms) and frequencies (10-40 Hz) are majorly the same in previous studies[91]. That is why the pulse width was kept constant, and frequency comparisons were made using 3 and 30 Hz to distinguish stimulation evoked changes in bladder pressure clearly. Concerning the placement of electrodes on the bladder, substantially higher stimulation amplitudes have been reported previously when electrodes were placed on the bladder dome[46,74] compared to when electrodes were placed on the bladder base[56,64], which is consistent with our findings. The electrode contact was maintained in the first two types of arrays used for functional mapping because the polymer material insulating electrodes could adhere to the bladder wall and was visually checked periodically. Significant contractions were seen at 4-6 mA across 7 subjects in these cases. However, in the instrumented mesh, electrodes at the bladder base were anchored through pressure on the polymer mesh providing a robust electrode-tissue contact. This improved contact could be the reason for significant bladder contractions with current amplitudes as low as 1 mA. However, we did not quantify the electrode-tissue contact in this study.

Current spread: One of the major challenges in the history of DBWS is the current spread to the nearby pelvic floor, perineal and hind limb muscles. Current spread to nearby structures can lead to reflex or direct contraction of EUS, as well as the perineal and pelvic floor muscles, which on contraction, can lead to mechanical occlusion of the urethra during stimulation. Various factors result in the current spread, such as electrode size, electrode contact, electrode insulation, stimulation amplitude, and the location on the bladder. Here, we show minimal current spread during monopolar and bipolar stimulation at low amplitudes and a substantial reduction of current spread at very high amplitudes during bipolar stimulation. Even with the monopolar stimulation, the mean absolute values of EUS EMGs have a median value less than 10 μV, which might be

insufficient to generate force in the EUS muscle[93]. The median MAV values for EAS and gluteal muscles were less than 15 µV and 10 µV for monopolar stimulation, which might not be enough to generate a functional contraction of these muscles. No visual leg movement was reported during these trials up to 6 mA. Leg movements were observed at very high amplitudes (8, 10 mA) visually and are evident in the accelerometer data, and these movements were eliminated by bipolar stimulation at these amplitudes. While generating similar bladder pressure changes, the current spread to EUS, EAS, and gluteal muscles is substantially reduced during bipolar stimulation. The fact that monopolar and bipolar stimulation generated similar bladder pressures also supports the idea that electrodes on the bladder base produce pressure changes by accessing the dense innervation in that region.

Moreover, during bipolar stimulation, the distance between the cathode and anode affects its ultimate effectiveness. This is evident in figure 3.7c, wherein closer pairs have less current spread but comparatively lower changes in bladder pressure compared to farther bipolar pairs, supporting the observations by Walter et al. as farther pairs here were mostly bilateral[76]. The EMG activities produced upon monopolar stimulation at 2 and 4 mA might not indicate a significant force generation to cause outlet obstruction. Still, a substantial reduction in these values at very high amplitudes shows the capability of limiting the current to the vicinity of electrodes. In humans, DBWS has been shown to be highly successful after a pudendal neurectomy procedure, as that minimizes the impact of current spread on the urethral sphincter [26,27] . Because the human abdomen and urethral sphincter are closer to the bladder than in cats, bipolar stimulation could be a necessity[57]. However, this study was done in anesthetized cats in which we only looked at the stimulation-evoked bladder pressure changes and the EMG activity of nearby muscles. Based on the stimulation-evoked pressures and the limited co-activation of nearby muscles, we believe it

would lead to complete bladder emptying, however, we did not do any test to confirm if these stimulaton-evoked bladder pressure changes would lead to bladder emptying.

3.6 Conclusion

In the literature, DBWS has been demonstrated successful in humans and animals with certain limitations and challenges. Here, we show the development of a neural interface for DBWS, which could overcome the co-activation of nearby muscles, one of the major challenges in DBWS literature. We performed functional mapping for the bladder, determined the optimal locations for DBWS at the bladder base with 2 different types of arrays, and then developed an electrode mesh array with 12 electrodes on the bladder base anchored with a soft silicone convoluted mesh structure such that minimal stimulus intensity could evoke large bladder contractions. We showed that current spread could be constrained to the vicinity of electrodes and the ability of this electrode mesh to conform according to the volume changes in a dynamic organ such as the bladder.

This design can be customized for other organs for direct organ stimulation. This neural interface motivates more efforts into DBWS as a feasible neuromodulation intervention for overcoming many pathological conditions related to the LUT in which the bladder can't generate enough pressure to release the urine.

4.0 Direct Bladder Wall Stimulation in Behaving Cats Through a Soft Silicone-based Flexible Neural Interface

4.1 Overview

Direct bladder wall stimulation has been attempted for decades to restore bladder function in people with spinal cord injuries and other voiding dysfunctions. However, these efforts were limited by the co-activation of the urethra, legs, and other pelvic organs at stimulus intensities that evoked bladder contractions. Neural interfaces for the detrusor muscle face several challenges due to its structure and the volume changes it undergoes in normal function. We designed a stretchable silicone net that can be placed around the bladder body to anchor a soft electrode array that interfaces directly with the base of the bladder to generate bladder contractions. We created implantable versions of the electrode nets and tested them in chronic experiments. We implanted 5 healthy cats (4 females and 3 males) and tested them with and without anesthesia for 2-3 months.

Bladder wall stimulation through various electrode configurations (monopolar, bipolar), temporal patterns (single electrode, sequential stimulation of multiple electrodes), and stimulus intensities were able to generate complete bladder emptying. In behaving cats, bladder wall stimulation at many different stimulus intensities elicited efficient voiding at physiological bladder pressures. We found a weak linear relationship between stimulation amplitude and bladder pressure itself; however, there was a longer time between stimulation onset and voiding at low stimulation amplitudes than at higher amplitudes, which evoked more rapid voiding. This contrasts with anesthetized tests, where stimulation at similar stimulus intensities evoked increased bladder pressure. These results suggest that stimulation of the bladder wall recruits sensory pathways

leading to an urge to void, but the animals can suppress that. For most of the duration of the implants, stimulation evoked functional voiding with consistent electrical stimulation thresholds. However, after several months there was a failure of an implanted bond that limited the overall duration of the implants. Chronic experiments demonstrate that these electrode nets can be used as a neural interface to generate or initiate comfortable, complete bladder emptying in awake, behaving cats.

4.2 Introduction

Spinal cord injury and neurodegenerative diseases such as multiple sclerosis and Parkinson's disease affect the regulation of lower urinary tract (LUT) functions[2,3]. Restoring bladder and bowel function is one of the highest priorities for people with spinal cord injury[8]. To restore bladder function, neuromodulation can intervene at many points in the LUT neural circuit[10]. Direct bladder wall stimulation (DBWS) is one such neuromodulation technique, which involves stimulating the detrusor muscle of the bladder wall directly. With electrodes implanted on the bladder wall, contractions to achieve complete emptying of the bladder can result from either direct electrical stimulation of the detrusor muscle or by stimulation of neurons in the bladder wall leading to contraction of the detrusor muscle through intramural innervation[33,46,52,76]. DBWS has been performed in animal[29,56,74,83,94] and human clinical studies[26,63,64] to treat various pathological conditions and has successfully produced micturition with certain limitations. Moreover, DBWS has been shown to be better than neuromodulation interventions such as sacral stimulation mainly due to the involvement of dorsal rhizotomy, lower voiding efficiency, and co-activation of other adjacent tissues such as hind limbs involved in the sacral neural pathways[55]. DBWS has also been

shown to be a better neuromodulation intervention than direct stimulation of the pelvic nerve due to the trauma caused to the nerve by implanted nerve electrodes[89].

In previous studies, DBWS was mainly limited by the current spread that caused the co-activation of nearby tissue structures and challenges maintaining the mechanical stability of the electrodes on the bladder. The current spread is the excitation of tissue structures directly or by reflex activation other than the bladder wall, leading to pain and activation of the urethral, hind limb, and pelvic musculature[89,90]. One of the major problems was the direct current spread to urethral muscles and the urethral spasm caused due to direct spread of current to perineal and pelvic structures, causing a reflex or direct contraction of these muscles through the pudendal nerve resulting in mechanical occlusion of the urethra leading to a dyssynergia like behavior due to co-activation of the urethra and detrusor muscle[29,47,49,58]. The poor insulation of electrodes[27,50,60,66,91], size of electrodes[52,79], optimal stimulation parameters[52], placement of these electrodes on bladder wall[26,55,63,64], and the mechanical and positional stability of electrodes have been the main factors leading to current spread[26,75,80,82].

DBWS has been tested in longitudinal animal studies to regulate voiding function in animal SCI models. Limited experiments have been performed in an awake, behaving spinally intact model[75]. Even though the potential application of DBWS will be to restore bladder function in pathological conditions where the bladder is underactive or atonic, testing in healthy and awake animals offers the advantage of studying intact sensory reflexes and their role in voiding during DBWS. Unlike in an isoflurane/dexdomitor anesthetized model where LUT neural reflexes are diminished, in conscious animals, enhanced sensory inputs from social and brainstem centers related to micturition are known to have a profound effect on micturition behavior[95]. We evaluated the effect of DBWS in free-behaving subjects in their natural environment where voluntary control

of micturition is active. It was anticipated that these isovolumetric changes in bladder pressure reported in previous chapter will lead to functional voiding in awake behaving and anesthetized cats. In previous chapter, we designed a stretchable soft silicone electrode mesh that can be placed around the bladder body to anchor a soft electrode array that interfaces directly with the base of bladder to generate bladder contractions. We implanted this electrode mesh for longitudinal studies in healthy cats.

4.3 Materials and Methods

4.3.1 Experiment Overview

Seven adult cats (N= 7, 3 males, 4 females) were implanted with the electrode nets and were tested for voiding function for 2-3 months. A bladder dome catheter was implanted to infuse saline and measure bladder pressure. A pelvic nerve cuff was implanted unilaterally to compare if bladder wall stimulation and pelvic nerve stimulation evoked similar functions. Following one week of the implant as the recovery phase, DBWS or pelvic nerve stimulation was tested every week. On average, 2 behavioral testing sessions were performed every week, and one anesthetized testing session was conducted to search the responsive electrodes every two to three weeks for the first 3 cats (A-C). The goal of anesthetized trials was to troubleshoot the technical issues and find the responsive electrodes to be tested in awake behaving cats. However, for the following 4 cats (D-G), we compared the results of anesthetized trials and awake behaving trials to quantify the effects of stimulation. A summary of experiments conducted in each cat is shown in table 4.1. To do so, one anesthetized session was performed every week with 2 awake behaving sessions for 2-

3 months. These experiments involved determining the bladder capacity every week, followed by electrical stimulation trials to evoke voiding function at 60-80 % of the bladder capacity. All experiments were performed under the approval of the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC).

Table 4.1: Summary of experiments conducted in each cat X indicates that the study was conducted. Cat F shown in red, did not voluntarily void; the analysis for this cat is reported separately.

Cat	Comparison between anesthetized and awake behaving trials	Effects of stimulus intensity in awake behaving trials	Pelvic nerve stimulation	Stimulation Types
A		X		Bipolar, Sequential
B		X	X	Monopolar, Bipolar, Sequential
C		X		Monopolar, Bipolar, Sequential
D	X	X		Monopola, Bipolar, Sequential
E	X	X	X	Monopolar, Bipolar, Sequential
F	X	X	X	Bipolar, Sequential
G	X	X		Monopolar, Bipolar, Sequential

4.3.2 Surgical Preparation

Anesthesia was induced with inhaled isoflurane in a closed chamber and was maintained via inhaled isoflurane (1-2 %). Cats were monitored for vital signs, including heart rate, core temperature, SpO_2, and $ETCO_2$. Each animal's abdominal region and back were shaved and cleansed with isopropyl alcohol and iodine. A midline abdominal incision was made to expose the bladder and the pelvic nerve. Electrode nets (custom printed by Ripple inc.) were placed on the bladder, and the positions of electrodes were noted. Electrode nets were made such that 12 electrodes were placed on the bladder base in two concentric circles of 6 electrodes, each separated by 2.0 mm vertically, as shown in figure 4.1. A single-lumen bladder dome catheter (Custom-made silicone tubing, diameter-1 mm) was placed on the dome through the electrode nets to infuse saline and measure bladder pressure. A bipolar pelvic nerve cuff (Microleads Inc, 800-1000 μm) was implanted on the left pelvic nerve. Electrode nets and pelvic nerve were tested for stimulation, and monitoring stimulation evoked bladder pressure before closing the abdomen. A large surface area ground electrode patch (custom made using cooner wire and silicone substrate) was placed in the abdominal muscle. The catheter and the cables from electrode nets, pelvic nerve cuff, and ground patch electrode were then routed out of the peritoneum through the lateral side onto the back of the cat. The abdomen was closed using a suture. The wires were then passed through a custom-made backpack and a circuit board mounted on the titanium base plate. This backpack assembly housed a custom-made circuit board with electrode nets connected by an omnetics connector and the pelvic nerve cuff leads connected with a Samtec connector. The backpack also had ports to fit in the Luer fitting connector to which the pressure catheter was connected. A header circuit board was connected on the top side of the board to establish a connection between the external recording and stimulation equipment during anesthetized and behavioral experiments.

4.3.3 Data Collection and Instrumentation

Grapevine neural interface processor (Ripple Inc.) was used for recording and stimulation. For electrical stimulation of the bladder wall through electrode nets, either the high current stimulator (Ripple Inc., compliance: 30 V, max current: 15 mA) was used with grapevine neural interface processor, which was programmed using MATLAB (Mathworks Inc.), or the AM systems stimulator (compliance: 100 V, max current: 10 mA) for which stimulation event triggers were recorded using headstage connected through the grapevine neural interface processor. For pelvic nerve stimulation, either AM systems stimulator or Ripple Nano 2+ stim headstage interfaced with a Grapevine (compliance: 8 V, max current: 1.5 mA) was used. The bladder pressure catheter was connected to a pressure transducer, which was connected to a Trans-bridge amplifier (WPI Inc.). A removable plate mounted on a custom-made load sensor was used to measure the weight of the urine and as a metric for initiation of voiding. The load sensor was connected to the amplifier. The analog output from the trans-bridge amplifier and the load sensor amplifier was then recorded using an Analog-Digital I/O head-stage connected through the Grapevine neural interface processor.

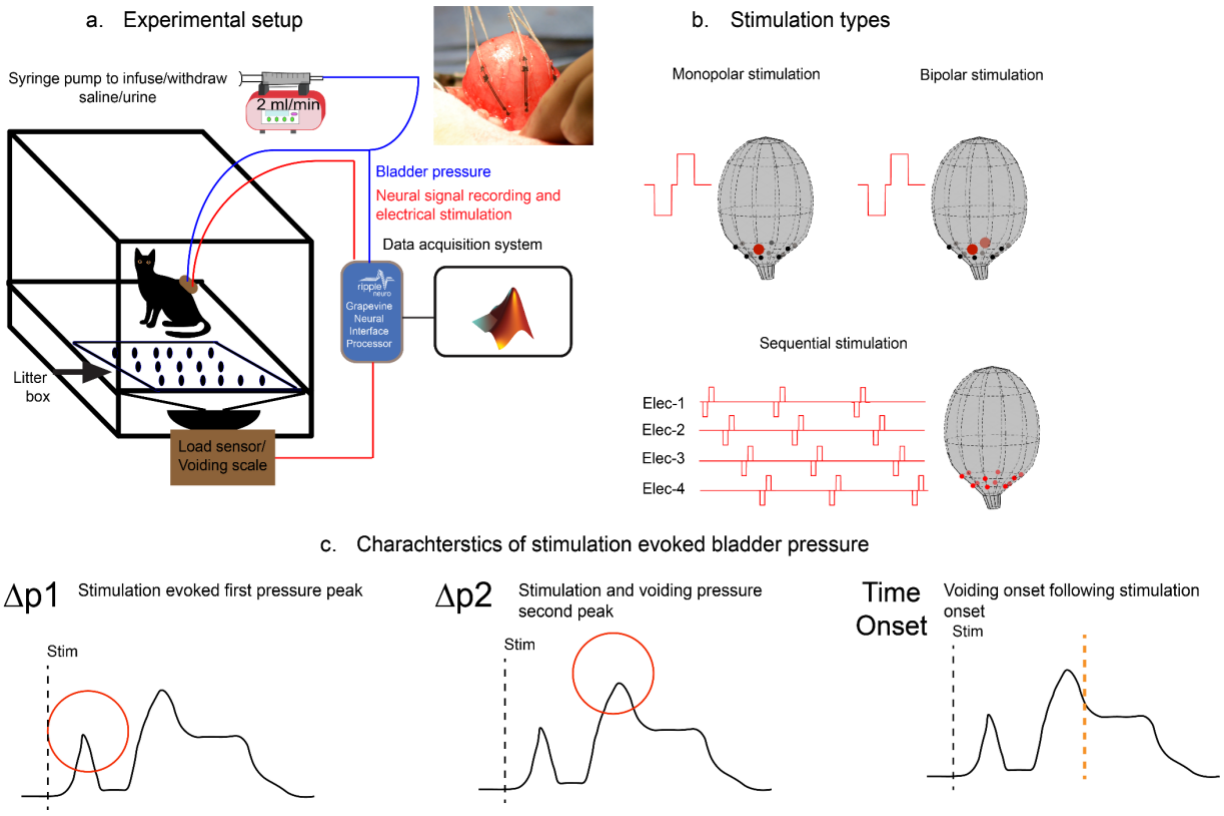

Figure 4.1 Experimental setup. a) Experimental design for awake behaving trials. b) Stimulation types: Monopolar stimulation, bipolar stimulation, and sequential stimulation. Monopolar stimulation involves one electrode on the bladder as a stimulating electrode and a large return electrode on the abdomen. Bipolar stimulation involves both the stimulating and ground electrodes on the bladder. Sequential stimulation is the stimulation of all 12 electrodes on the bladder sequentially and is a more powerful stimulation than Monopolar or bipolar stimulation. c) Characteristics of stimulation evoked pressure illustrating Δp1, Δp2, and time onset in awake behaving trials. These curves are ideal representations of bladder pressures and were not always seen. Stimulation-evoked first pressure peak is the pressure change immediately after (7s) stimulation onset. Stimulation-evoked voiding peak pressure is the bladder pressure reached during the voiding. Voiding onset following stimulation onset is the time from the stimulation onset to the second-voiding peak bladder pressure.

4.3.4 Anesthetized Experiments

Dexdomitor (0.04 mg/kg) was injected for anesthetized experiments. For cats A-C, anesthetized trials were performed only to find responsive electrodes. The anesthetized experiments aimed to find electrodes that generated bladder pressure upon stimulation. Usually, monopolar stimulation on electrodes at 3 mA, 1ms, and 30 Hz was performed, and bladder pressure responses were recorded. Electrodes that generated bladder pressures greater than 10 CmH_2O pressure or any voiding in these trials were chosen to be used to awake behaving trials that week.. For cats D-G, anesthetized trials were conducted every week to compare the effects of stimulation between anesthetized and awake behaving trials. Similar bladder wall stimulation paradigms were performed. For anesthetized trials, a quiet time of 10-30 s were kept at the 60-80 % capacity before stimulation, and then bladder wall stimulation was applied for 20-30 s and a post-stimulation time of 10-30s. A similar approach was used for finding pelvic nerve stimulation thresholds to generate bladder pressure and voiding in anesthetized trials and was then repeated with the animals awake. These anesthetized sessions lasted 1- 2 hours, depending on the cat's response to the dexdomitor anesthesia. In the case of hardware or apparatus troubleshooting, additional dexdomitor sessions were performed.

4.3.5 Awake Behaving Experiments

Awake behaving experiments were performed two times a week. A 30*30*30 inches cage placed on 80-20 frames was used for these experiments (figure 4.1 a). A load sensor was placed on the bottom of frame where urine output was measured. An external sterile pressure tubing was connected to infuse and withdraw saline into the bladder and to measure the bladder pressure.

Cystometry at an infusion rate of 2 ml/min was performed each week to measure the bladder capacity. For the first 1-3 sessions, cats were trained to get accustomed to peeing in the litter box placed in the cage. Since these were intact awake, behaving cats, they had voluntary control of the urethra and could suppress bladder pressure if they could control the urge to urinate. After the bladder capacity was determined every week, saline was infused up to 60- 80% of the capacity, and stimulation trials were performed at that bladder volume. The urine output was measured using the load cell and checking the residual volume by withdrawing from the external tube in cases where bladder volume output was low. A quiet time of 10-60 s was kept at the 60-80 % capacity before stimulation, bladder wall stimulation was applied for 20-60 s and a post stimulation time of 30-120s was kept depending on the relative time the cat was taking to void. These sessions lasted up to 45-90 mins.

4.3.6 Stimulation Protocol and Data Collection

A typical stimulation trial lasted for about 150 s (30-60 s quiescent recording followed by 10-30 s of stimulation followed by 30-60 s quiescent recording) for awake behaving trials and about 70 s in anesthetized trials (10-30 s quiescent recording followed by 10-60 s of stimulation followed by 10-30 s quiescent recording). Biphasic, charge-balanced symmetric, cathodic first stimulation pulses were applied with an inter-pulse interval of 66 μs. The pulse width was kept constant at 1 ms, and frequency was kept constant at 30 Hz for all the trials, which was determined to be most effective in previous studies. The stimulation amplitude was varied between 1 mA- 6 mA. Different types of stimulation configurations involving monopolar stimulation, bipolar stimulation, and sequential stimulation were performed, as shown in figure 4.1b. Monopolar stimulation involves one electrode on the bladder as a stimulating electrode and a large return

electrode on the abdomen. Bipolar stimulation involves both, the stimulating electrode and the ground electrode on the bladder. Sequential stimulation is stimulation of all 12 electrodes on the bladder sequentially and is powerful stimulation than Monopolar or bipolar stimulation. In previous experiments, we explored this type of stimulation but did not collect the data for it as the goals were different. Anecdotally, we noticed higher pressure changes with sequential stimulation, it is stimulating all the electrodes on the bladder surface.

4.3.7 Data Processing and Statistics

All data were recorded using MATLAB (Mathworks Inc.). The pressure change was calculated as a difference between the pressure value 100 ms prior to the stimulation onset, and the highest value reached in the first 10 seconds of stimulation (first peak, $\Delta p1$,), and the highest pressure values reached during voiding (second peak, $\Delta p2$). Stimulation trials that led to voiding were involved in the analysis for awake, behaving cats. The quantification metrics are shown in figure 4.1c. Voiding efficiency was calculated as a percentage of volume output from the infused volume.

The voiding event was noted from the load cell, and the time difference from the onset of stimulation to the voiding onset was calculated. For anesthetized trials, a similar approach was used; however, there was only stimulation evoked pressure peak in these trials as voluntary control and reflexes are absent in anesthetized trials. The anesthetized and awake behaving data was compared for cats D, E, F, and G. Cat F couldn't be trained for voluntary voiding, so the results from this cat are reported separately. Data from cats A, B, C, D, E, and G were considered to see the effects of stimulus intensity in awake trials. Data were non-normal and were confirmed by the Shapiro-Wilk normality test. Spearman correlation was used for non-parametric data to see the

effect of stimulus intensity. For comparison between Anesthetized and awake behaving trials, the Wilcoxon rank sum test was used. To compare the effects of stimulus intensity in awake behaving animals, data were binned according to the day in terms of lowest and highest stimulus intensity, which led to a void, and a Wilcoxon-paired sign-rank test was used. A p-value less than 0.05 was considered significant. In addition, median values are reported for effects with no statistical significance.

4.4 Results

4.4.1 Longevity

We implanted 7 cats (4 females and 3 males) and tested DBWS for up to 2-3 months. For the duration of the implant, DBWS through multiple stimulation amplitudes (1-6 mA) and configurations (monopolar, bipolar, sequential) could evoke voiding in both anesthetized and awake behaving trials. Fig 4.2a shows the voiding pressures achieved at maximum stimulus amplitude per week for 7 cats within the implant duration. Cat B, D, E, F, and G were tested for three months, and bladder wall stimulation could evoke voiding. Cat A had to be terminated due to surgical complications, and cat C was terminated in week 10 due to electrode lead failure. Cat A took a long time before it voluntarily voided in the cage. Cat F did not voluntarily void, so the data shows involuntary voiding evoked by bladder wall stimulation. Different stimulation evoked bladder pressure responses were noted in these cats. Figure 4.2b depicts three distinct cases from cat E at 3 stimulus amplitudes of bladder pressures where complete emptying of the bladder was achieved. At stimulus amplitude 2 mA, stimulation evoked first peak pressure ($\Delta p1$) is very low,

and cat voids 30 seconds later than stimulation onset. At stimulus amplitude 4 mA, Δp1 is larger, and the cat voids within 15 seconds of stimulation onset. At stimulus amplitude 6 mA, Δp1 is even higher, and the cat voids within 10 seconds of stimulation onset.

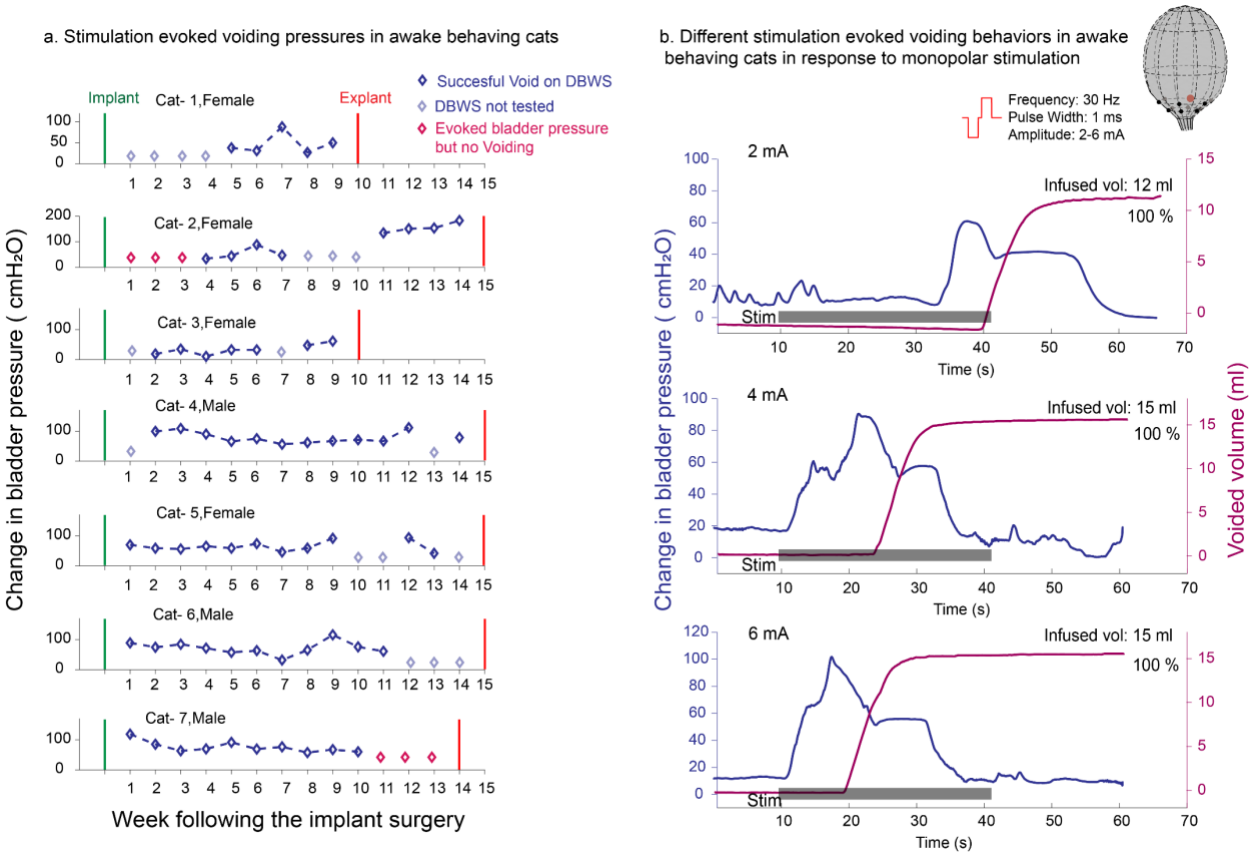

Figure 4.2: Longevity of electrode nets in evoking functional voiding. a) Stimulation evoked pressure at maximum stimulus intensity in awake behaving experiments per week. b) Different stimulation evoked voiding behaviors in response to monopolar stimulation in cat E, at 2 mA [top], 4 mA [middle], 6 mA [bottom]. As the stimulation amplitude increases, the stimulation evoked pressure (Δp1) increases, and the time onset decreases.

4.4.2 Anesthetized vs. Awake Animals

We conducted experiments at similar stimulation configurations and settings to compare the effects of DBWS between anesthetized and awake, behaving animals. Since cat F did not

voluntarily void, the comparison between anesthetized and awake trials is shown in the next section. DBWS through multiple stimulation amplitudes (1-6 mA) and configurations (monopolar, bipolar, sequential) could evoke voiding in both anesthetized and awake behaving trials.

Monopolar stimulation

Fig 4.3 compares anesthetized and awake behaving trials for cats D, E, and G upon monopolar stimulation at increasing stimulus intensities. Figure 4.3a-b shows an example of bladder pressure responses for anesthetized and awake trials. In anesthetized trials, bladder pressure and voiding efficiency increased with stimulus intensity right after the stimulation onset as reflexes and voluntary control were absent. However, in awake behaving trials, at 1 mA, there is no change in first peak pressure (Δp1) but increases with stimulation amplitude with minimal change in the second peak or voiding pressure (Δp2) and cat voids after 30 s of the stimulation onset and this time decreased as the stimulation intensity increased from 2-4 mA.

Stimulation evoked first peak pressure (Δp1): Δp1 increases with stimulus intensity for both anesthetized ($\rho = 0.36$, $N=82$, $p<0.05$) and awake behaving trials ($\rho=0.23$, $N=127$, $p<0.05$). Median values of Δp1 for anesthetized trials are higher than that of awake behaving trials at all the stimulation amplitudes but statistically significant only for 3mA (figure 4.3c, $p<0.05$).

Stimulation evoked voiding pressure (Δp2): We also compared the bladder pressure change in anesthetized trials with Δp2 in awake behaving trials at increasing stimulus intensities (figure 4.3d). There was a minimal increase in Δp2 with stimulus intensity ($\rho=0.0128$, $N=127$, $p>0.05$); however, Δp2 was significantly higher than bladder pressure changes in anesthetized trials ($P<0.05$).

The onset of voiding following stimulation onset: The time onset of voiding following stimulation is significantly higher for awake behaving trials compared to anesthetized trials ($p<0.05$) at all the

stimulus intensities (figure 4.3e). In awake behaving trials, this time onset decreases as the stimulation amplitude increases (ρ= -0.27, N=127, $p<0.05$).

Voiding efficiency: The voiding efficiency increases with stimulation amplitude increases in anesthetized trials (figure 4.3f, ρ=0.59, N=82, $p<0.05$), however, voiding was always complete in awake, behaving trials, given the voiding is a result of stimulation and cats' voluntary relaxation of the urethra which is then accompanied by the pudendo-vesical reflex, unlike anesthetized trials where reflexes are suppressed. Similar relations were found with sequential and bipolar stimulation.

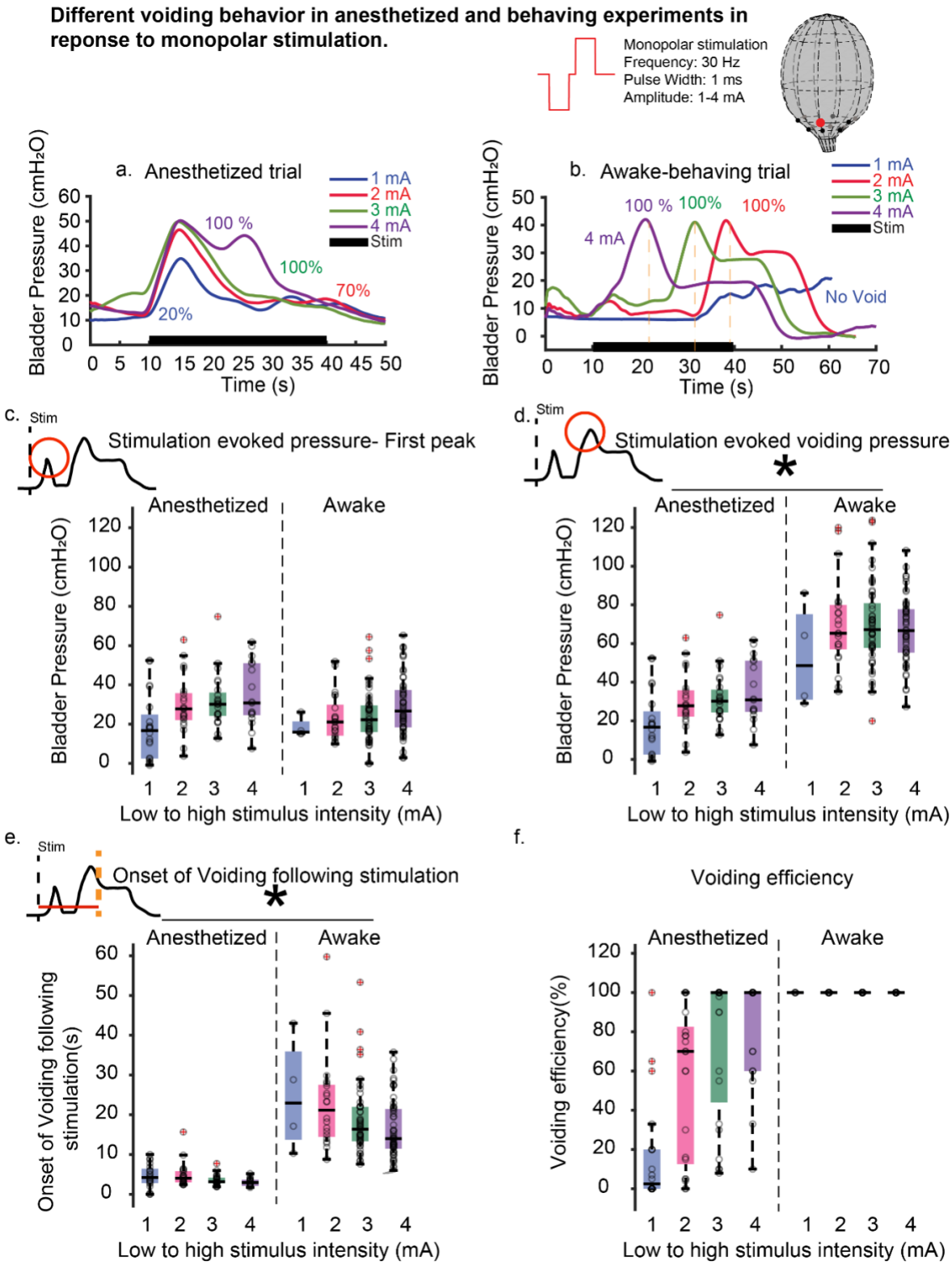

Figure 4.3 Different voiding behavior in anesthetized and behaving experiments in response to monopolar stimulation a) Stimulation evoked pressure response at different stimulus intensities in an anesthetized trial.

b) Stimulation evoked pressure response at different stimulus intensities in a behaving trial. c) Cumulative comparison of Δp1 between anesthetized and behaving trials. d) Cumulative comparison of Δp2 between anesthetized and behaving trials. e) Cumulative comparison of time onset between anesthetized and behaving trials. f) Cumulative comparison of voiding efficiency between anesthetized and behaving trials.

Sequential stimulation

Sequential stimulation involved sequentially stimulating each electrode on the bladder surface one after the other, as represented in figure 4.4. The frequency of this sequential train of pulses was kept at 30 Hz, and the pulse width of each pulse was kept at 1 ms. We often used sequential stimulation in experiments as a first check as it doesn't rely on a single electrode (Monopolar) or an electrode pair (Bipolar) to generate bladder contractions as stimulation is delivered sequentially through all the electrodes.

Stimulation evoked first peak pressure (Δp1): Δp1 increases with stimulus intensity for both anesthetized (ρ= 0.5, N=92, $p<0.05$) and awake behaving trials (figure 4.4a, ρ=0.53, N=111, $p<0.05$). Median values of Δp1 for anesthetized trials are higher than that of awake, behaving trials the stimulation amplitudes (1-3 mA) but statistically significant only for 2mA ($p<0.05$). At 4 mA, Δp1 is higher for awake behaving trial ($p<0.05$).

Stimulation evoked voiding pressure (Δp2): There was an increase in Δp2 with stimulus intensity (ρ=0.47, N=111, $p<0.05$), however, Δp2 was significantly higher than bladder pressure changes in anesthetized trials (figure 4.4b, $p<0.05$) for 2-4 mA.

The onset of voiding following stimulation onset: The time onset of voiding following stimulation is significantly higher for awake behaving trials compared to anesthetized trials (figure 4.4c, $p<0.05$) at all the stimulus intensities. In awake behaving trials, this time onset decreases as the stimulation amplitude increases (ρ= -0.25, N=111, $p<0.05$).

Voiding efficiency: The voiding efficiency increases with stimulation amplitude increases in anesthetized trials ($\rho=0.72$, $N=92$, $p<0.05$), however, voiding was always complete in awake behaving trials (figure 4.4d).

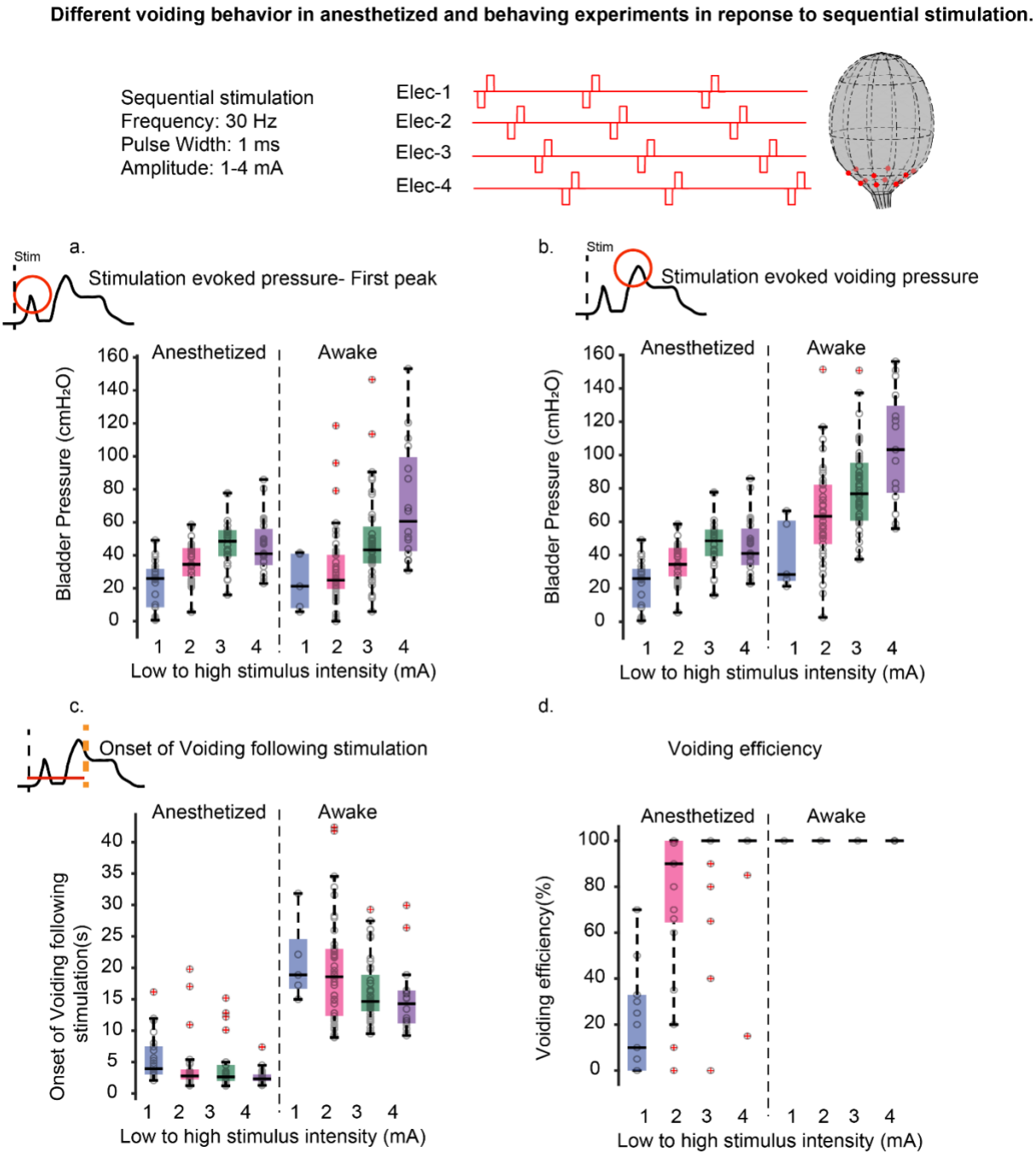

Figure 4.4 Different voiding behavior in anesthetized and behaving experiments in response to sequential stimulation a) Stimulation evoked pressure response at different stimulus intensities in a behaving trial. b) Cumulative comparison of Δp1 between anesthetized and behaving trials. c) Cumulative comparison of Δp2 between anesthetized and behaving trials. e) Cumulative comparison of time onset between anesthetized and behaving trials. d) Cumulative comparison of voiding efficiency between anesthetized and behaving trials.

Bipolar stimulation

Stimulation evoked first peak (Δp1): Δp1 increases with stimulus intensity for both anesthetized (figure 4.5a, $\rho = 0.4$, N=30, $p<0.05$) and in awake behaving trials ($\rho=0.07$, N=36, $p>0.05$). Median values of Δp1 for anesthetized trials are higher than that of awake behaving trials, the stimulation amplitudes (2-4 mA), and statistically significant ($p<0.05$). At 1 mA, median Δp1 is higher for awake, behaving trials, but with only two samples in behaving trials.

Stimulation evoked voiding pressure (Δp2): There was an increase in Δp2 with stimulus intensity (figure 4.5b, $\rho=0.25$, N=36, $p>0.05$); however, Δp2 was significantly higher than bladder pressure changes in anesthetized trials ($p<0.05$) for 2-4 mA.

The onset of voiding following stimulation onset: The time onset of voiding following stimulation is significantly higher for awake behaving trials compared to anesthetized trials ($p<0.05$) at all the stimulus intensities. In awake trials, this time onset decreases as the stimulation amplitude increases (figure 4.5c, $\rho = -0.3$, N=36, $p<0.05$).

Voiding efficiency: The voiding efficiency increases with stimulation amplitude increases in anesthetized trials ($r=0.46$, N=30, $p<0.05$), however, voiding was always complete in awake behaving trials (figure 4.5 d).

Overall, DBWS through multiple stimulation amplitudes ranging (1-6 mA) and configurations (monopolar, bipolar, sequential) could evoke voiding in both anesthetized and awake behaving trials.

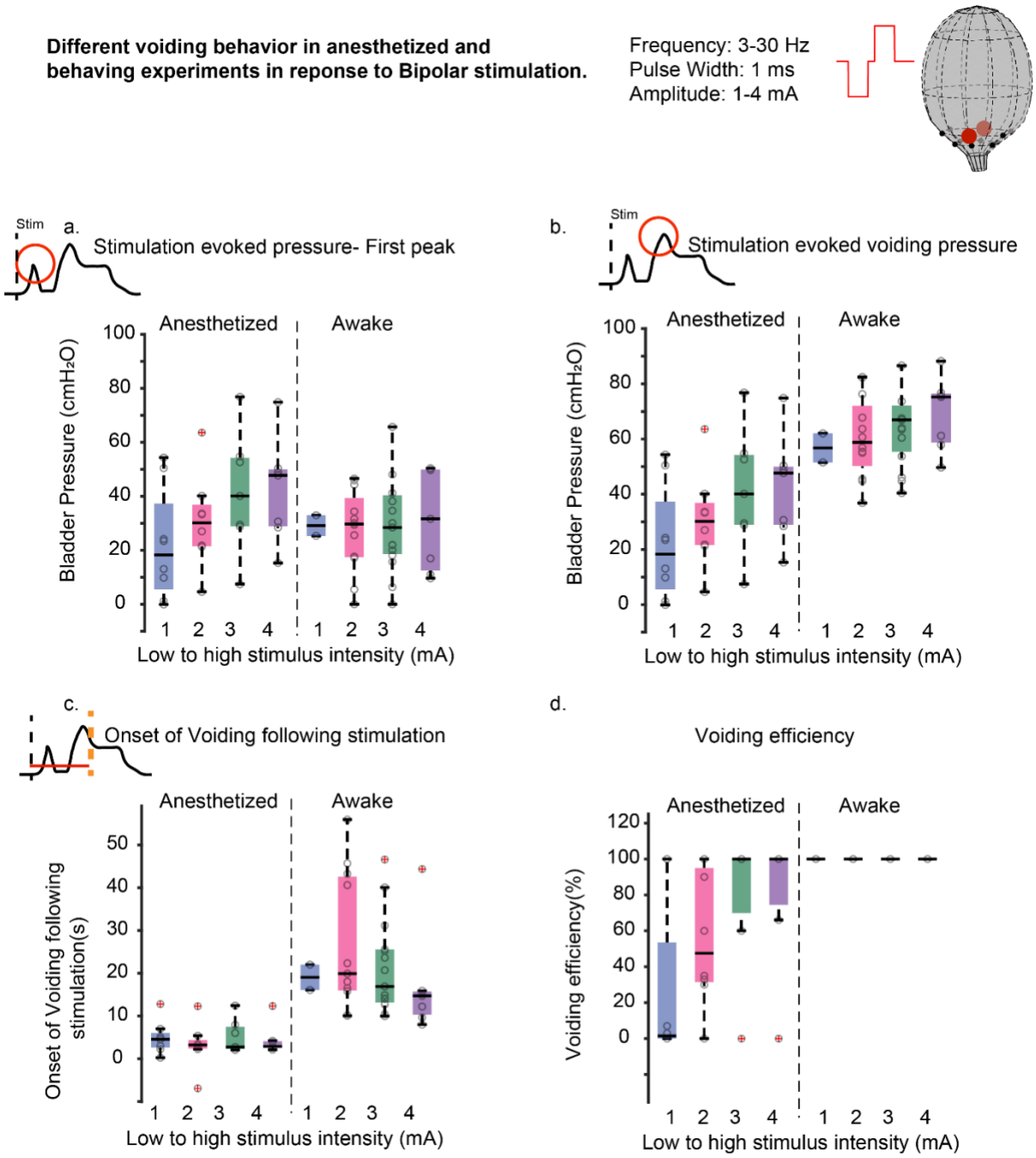

Figure 4.5 Different voiding behavior in anesthetized and behaving experiments in response to bipolar stimulation a) Stimulation evoked pressure response at different stimulus intensities in a behaving trial. b) Cumulative comparison of Δp1 between anesthetized and behaving trials. c) Cumulative comparison of Δp2 between anesthetized and behaving trials. e) Cumulative comparison of time onset between anesthetized and behaving trials. d) Cumulative comparison of voiding efficiency between anesthetized and behaving trials.

4.4.3 Involuntary Voiding in Awake, Behaving Animals

Cat F did not voluntarily void in awake experiments; however, at higher stimulus intensities (>3 mA) cat voided involuntarily, evident from the behavior (not going to the litter box or squatting) and incomplete bladder emptying. Fig 4.6a-b shows an example of bipolar bladder wall stimulation anesthetized and awake behaving trials at increasing stimulus intensities. In the anesthetized trials, the stimulation amplitude increased bladder pressure and voiding efficiency. In behaving trials, bladder pressure increases with stimulus amplitude; however, the cat resisted the stimulation and involuntarily voided at high stimulation amplitude (5 mA). Both $\Delta p1$ and $\Delta p2$ increased with stimulation amplitude. $\Delta p1$ increases with an increase in the stimulation amplitude for both anesthetized (figure 4.6c, $\rho=0.95$, $N=28$, $p<0.05$) and awake trials ($\rho=0.75$, $N=66$, $p<0.05$). Median $\Delta p1$ values are higher for anesthetized trials than awake behaving trials but not statistically significant for 2-4 mA but are lower for 1 mA and 5 mA. However, unlike the other cats, $\Delta p2$ had a higher correlation with stimulus intensity (figure 4.6c, $\rho=0.8$, $N=66$, $p<0.05$), and median $\Delta p2$ was not significantly higher than the bladder pressure changes in anesthetized trials other than 1mA. Voiding efficiency increases as the stimulation amplitude increase for anesthetized trials (figure 4.6d, $\rho=0.94$, $N=28$, $p<0.05$). Since voiding was involuntary in this cat, involuntary voiding efficiency increases with stimulation amplitude ($\rho=0.56$, $N=66$, $p<0.5$). Similar effects were seen with the sequential stimulation.

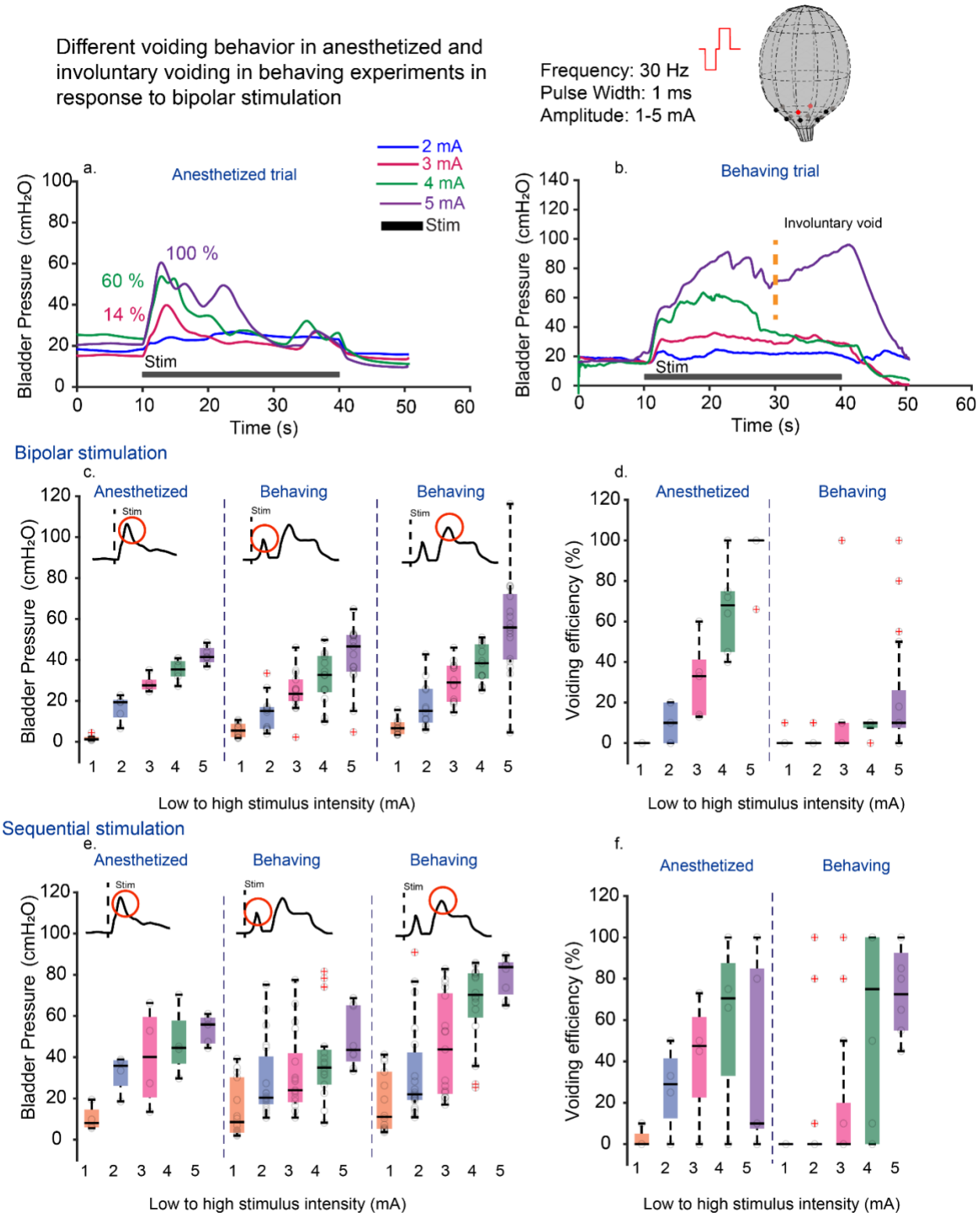

Figure 4.6 Different voiding behavior in anesthetized and involuntary voiding in behaving experiments in response to bipolar and sequential stimulation. a) Stimulation evoked pressure response at different stimulus

intensities in an anesthetized trial. b) Stimulation evoked pressure response at different stimulus intensities in a behaving trial leading to involuntary void in behaving experiments. c) Cumulative comparison of Δp1 and Δp2 between anesthetized and behaving trials in response to bipolar stimulation. d) Cumulative comparison of voiding efficiency between anesthetized and behaving trials in response to bipolar stimulation. e) Cumulative comparison of Δp1 and Δp2 between anesthetized and behaving trials in response to sequential stimulation. f) Cumulative comparison of voiding efficiency between anesthetized and behaving trials in response to sequential stimulation.

For sequential stimulation, Δp1 increases with increase in the stimulation amplitude for both anesthetized (ρ=0.75, N=21, $p<0.05$) and awake behaving trial (ρ=0.53, N=74, $p<0.05$). Median Δp1 values are higher for anesthetized trials than awake behaving trials but not statistically significant for 2-5 mA but are lower for 1mA. Δp2 had higher correlation with stimulus intensity (ρ=0.7, N=74, $p<0.05$) and median Δp2 were not significantly higher than the bladder pressure changes in anesthetized trials other than 5mA. Voiding efficiency increases as the stimulation amplitude increases for anesthetized trials (ρ=0.41, N=21, p=0.06). Since voiding was involuntary in this cat, involuntary voiding efficiency increases with stimulation amplitude (ρ=0.61, N=66, $p<0.5$).

4.4.4 Effects of Stimulus Intensity in Awake, Behaving Animals

Bladder wall stimulation was performed in 7 cats, of which 6 voluntarily voided (cats A, B, C, D, E, G). We observed the effects of stimulus intensity on Δp1, Δp2, and the onset of voiding following the stimulation onset. Data were binned according to minimum stimulation amplitude, which evoked voiding, and maximum stimulation amplitude, which evoked voiding per day, according to the stimulation types. Figure 4.7a depicts an example of bipolar stimulation evoked

pressure traces at the minimum and maximum stimulus intensity of a day. Compared to bladder pressure evoked with minimum stimulation amplitude (2mA), Δp1 and Δp2 were higher at maximum stimulation amplitude. The voiding onset following the stimulation onset was lower for the higher stimulus intensity conveying a stronger desire to void. Figure 4.7b-d summarizes the bipolar stimulation data across 6 cats. Δp1 (figure 4.7b) and Δp2 (figure 4.7c) were significantly higher at maximum stimulation amplitude (N=19, $p<0.05$). The voiding onset following the stimulation onset was significantly lower for the higher stimulus intensity (figure 4.7d, N=19 $p<0.05$).

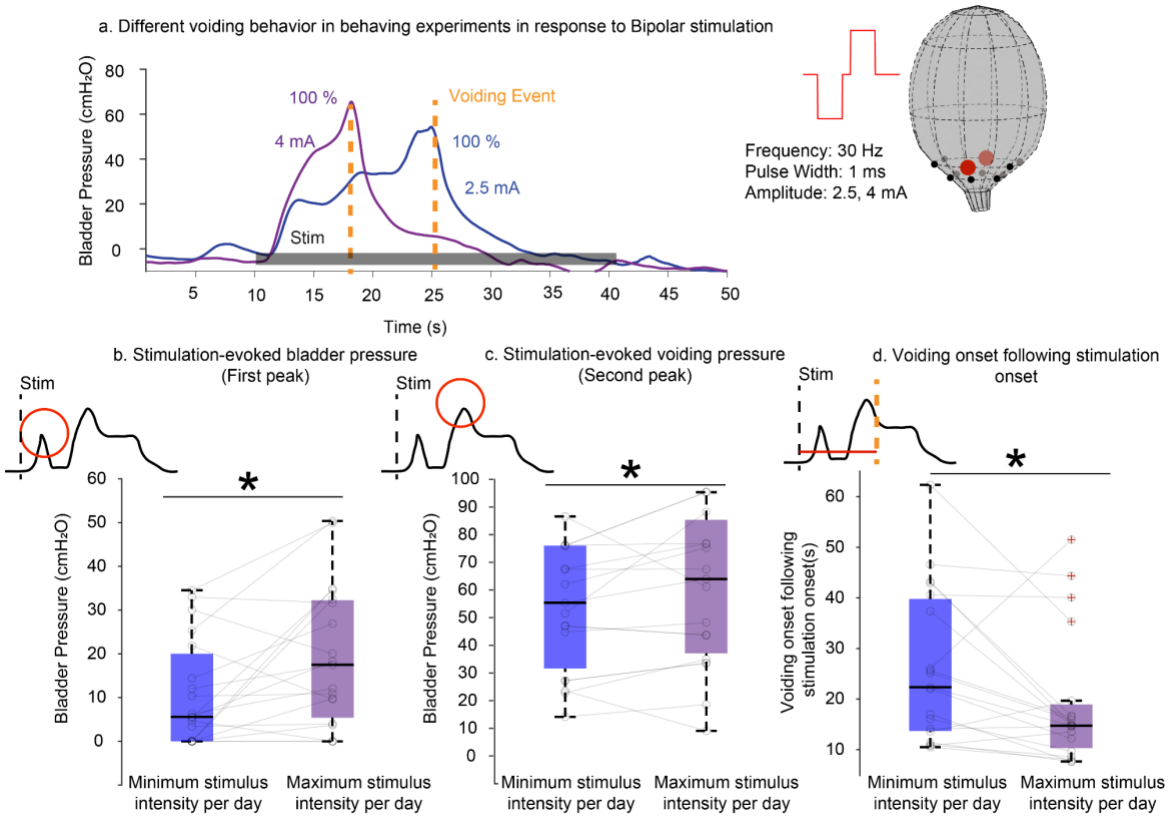

Figure 4.7 Effects of stimulus intensity in awake, behaving animals in response to bipolar stimulation a) Different stimulation-evoked pressure responses at low and high stimulus intensities. b) Stimulation-evoked bladder pressure (Δp1) at low and high stimulus intensity per day. c) Stimulation-evoked bladder pressure (Δp2) at low and high stimulus intensity per day. d) Comparison of time onset at low and high stimulus intensity per day.

Similar results were obtained with sequential stimulation (figure 4.8b-d). Δp1 and Δp2 were significantly higher at maximum stimulation amplitude (N=37, $p<0.05$). The voiding onset following the stimulation onset was significantly lower for the higher stimulus intensity (N=37 $p<0.05$). For monopolar stimulation (figure 4.8b-d), median Δp1 is higher for maximum stimulus intensity but is not statistically significant (N=34, p=0.07). Δp2 values were similar at maximum and minimum stimulation amplitude (N=34, p=0.9). However, the voiding onset following the stimulation onset was significantly lower for the higher stimulus intensity (N=34, $p<0.05$).

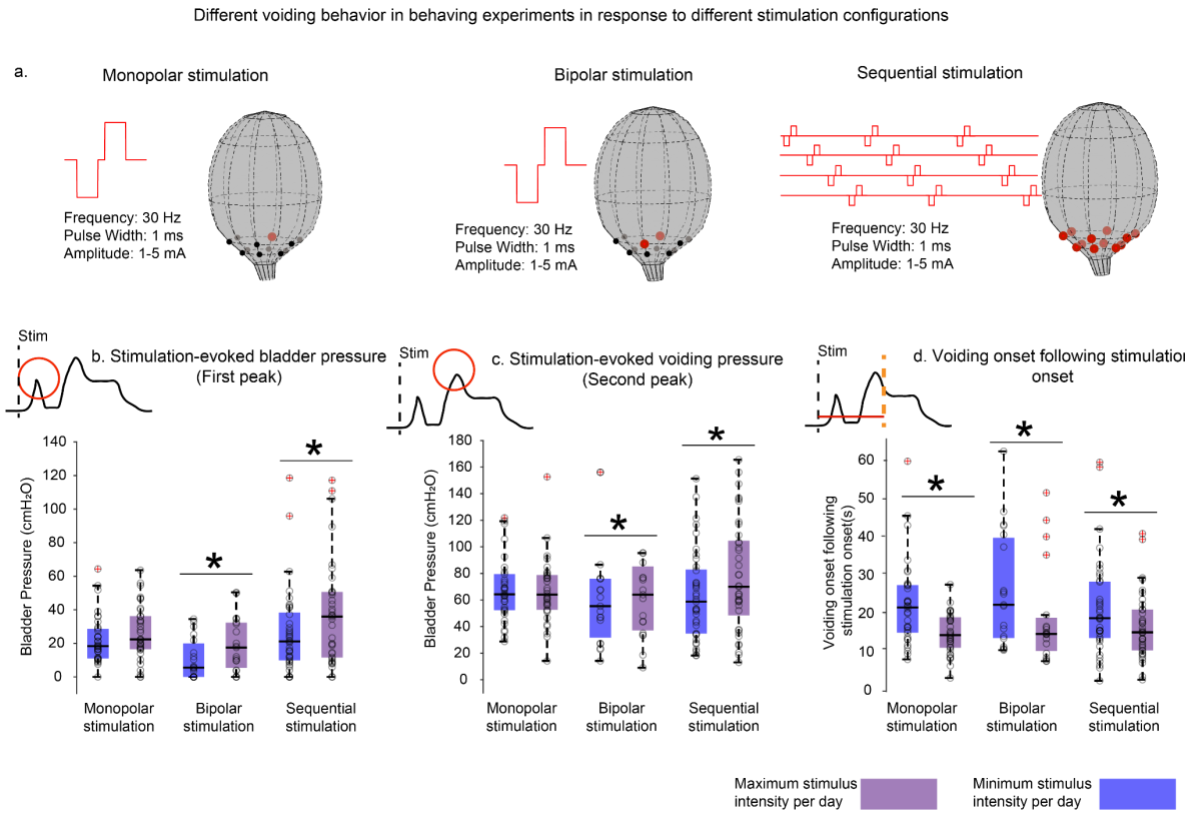

Figure 4.8 Effects of stimulus intensity in awake behaving animals in response to different stimulation configurations a) Illustration of different stimulation configurations, monopolar stimulation [left], bipolar stimulation [middle], sequential stimulation [right]. b) Stimulation-evoked bladder pressure (Δp1) at low and high stimulus intensity per day for monopolar, bipolar, and sequential stimulation. c) Stimulation-evoked bladder pressure (Δp2) at low and high stimulus intensity per day for monopolar, bipolar, and sequential

stimulation. d) Comparison of time onset at low and high stimulus intensity per day for monopolar, bipolar, and sequential stimulation.

4.4.5 Comparison With Pelvic Nerve Stimulation

Pelvic nerve stimulation was performed in 3 cats, of which 2 cats voluntarily voided (cat B, E). We observed the effects of stimulus intensity on Δp1, Δp2, and the onset of voiding following the stimulation onset. Data were binned according to minimum stimulation amplitude, which evoked voiding, and maximum stimulation amplitude, which evoked voiding per day, according to the stimulation types. Fig 4.9a depicts an example of pelvic nerve stimulation evoked pressure traces at the minimum and maximum stimulus intensity on a day. Compared to bladder pressure evoked with minimum stimulation amplitude (0.3 mA), Δp1 and Δp2 were higher at maximum stimulation amplitude (0.6 mA). The voiding onset following the stimulation onset was lower for the higher stimulus intensity conveying a stronger desire to void. Fig 4.9b-d summarizes the pelvic nerve stimulation data across 2 cats. Due to the high thresholds for pelvic nerve stimulation, we could only acquire limited data. Although not statistically significant, Δp1 and Δp2 were higher at maximum stimulation amplitude (N= 6). The voiding onset following the stimulation onset was for the higher stimulus intensity (N=6).

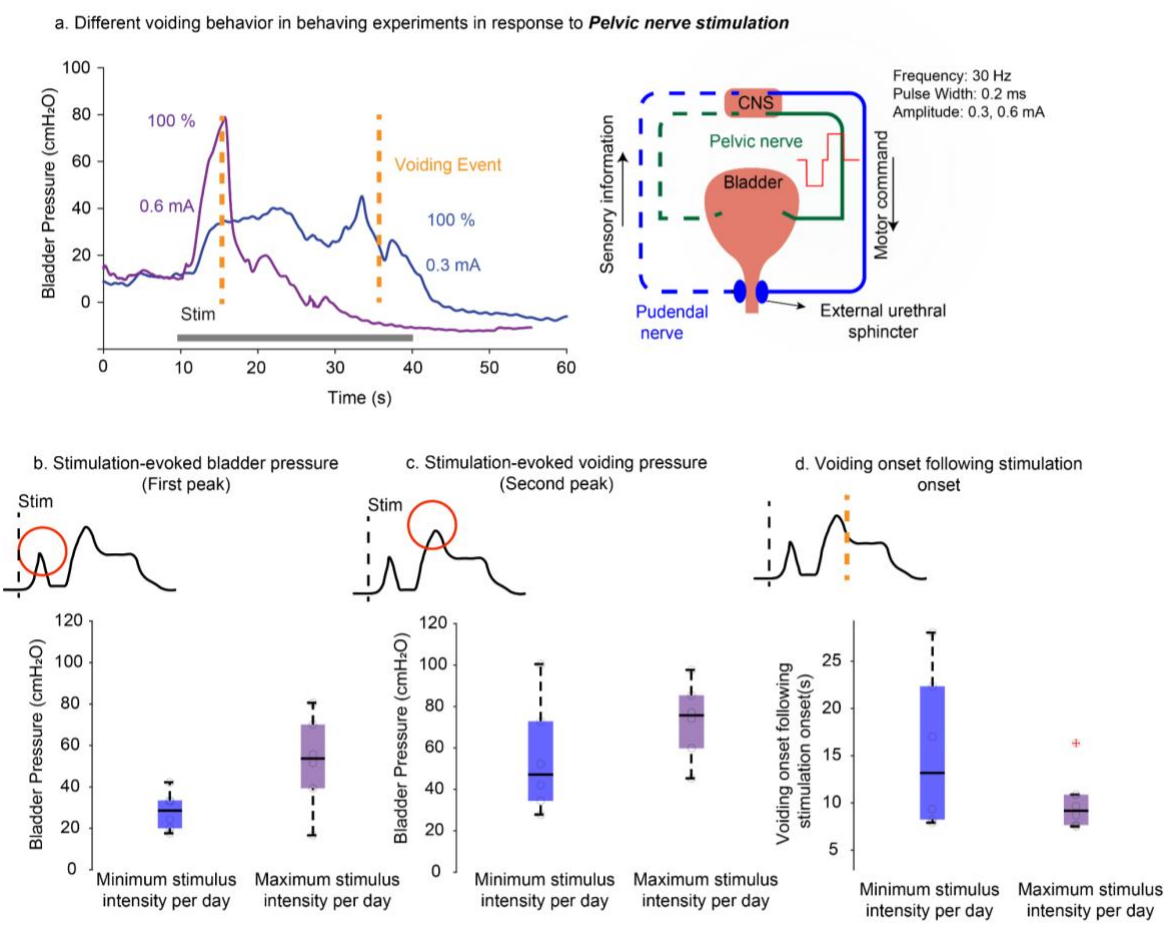

Figure 4.9 Effects of stimulus intensity in awake behaving animals in response to pelvic nerve stimulation a) Different stimulation-evoked pressure responses at low and high stimulus intensities. b) Stimulation-evoked bladder pressure (Δp1) at low and high stimulus intensity per day. c) Stimulation-evoked bladder pressure (Δp2) at low and high stimulus intensity per day. d) Comparison of time onset at low and high stimulus intensity per day.

Cat F did not voluntarily void in awake experiments; however, at higher stimulus intensities (0.6 mA) cat voided involuntarily, evident from the behavior (not going to the litter box or squatting) and incomplete bladder emptying, just like the results of bladder wall stimulation. Fig 4.10a-b shows an example of pelvic nerve stimulation anesthetized and awake behaving trials at increasing stimulus intensities. In the anesthetized trials, the stimulation amplitude increased

bladder pressure and voiding efficiency. In behaving trials, bladder pressure increases with stimulus amplitude; however, the cat resisted the stimulation and involuntarily void at high stimulation amplitude (>0.6 mA). Both Δp1 and Δp2 increase with stimulation amplitude, and Δp1 increases with the stimulation amplitude for both anesthetized and awake behaving trials (figure 4.10c). Median Δp1 values are not significantly different between anesthetized and awake trials. However, Δp2 median values were higher but were not significantly higher than the bladder pressure changes in anesthetized trials. Voiding efficiency increased as the stimulation amplitude for anesthetized trials. Since voiding was involuntary in this cat, involuntary voiding efficiency increased with stimulation amplitude (figure 4.10d).

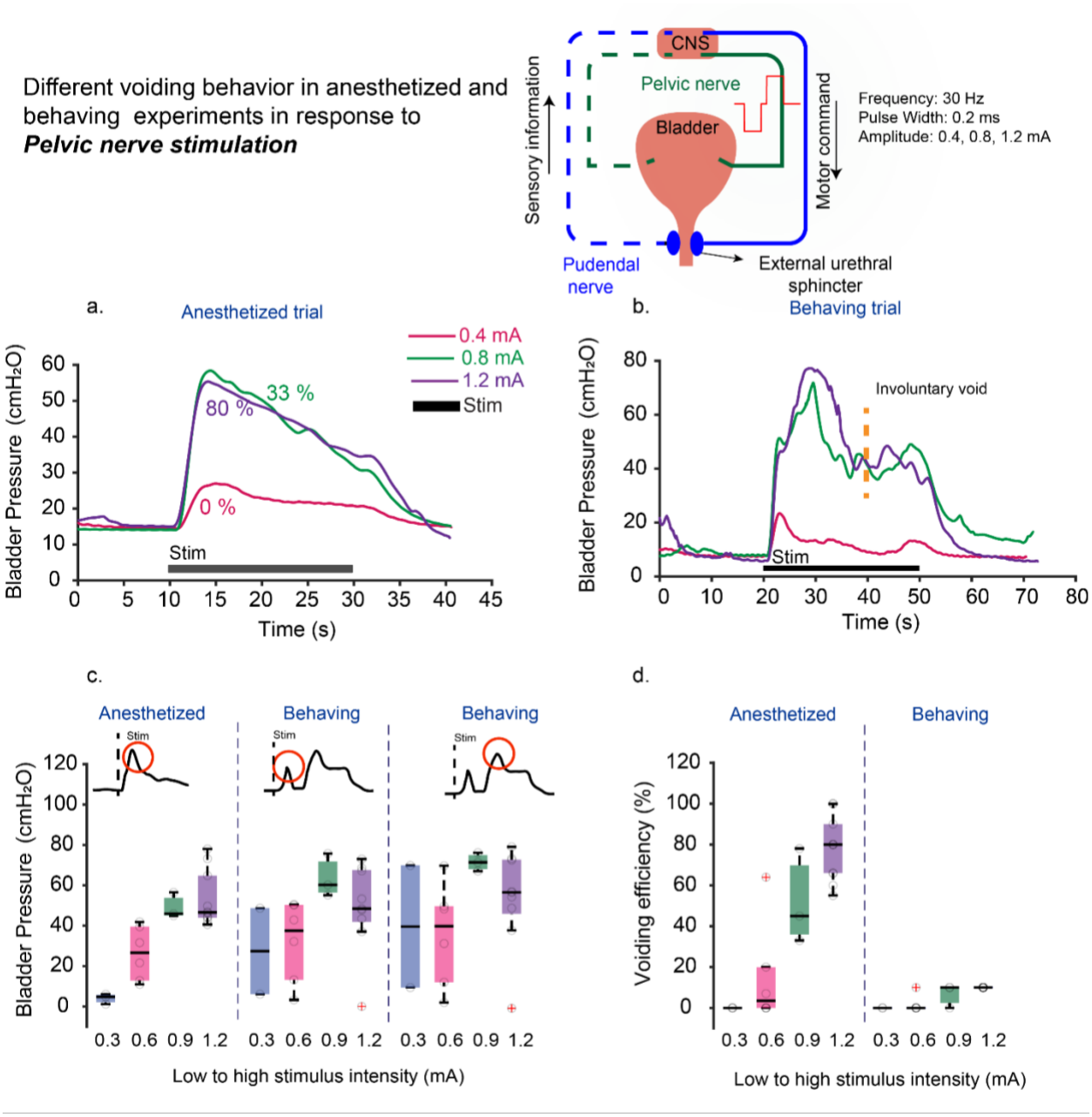

Figure 4.10 Different voiding behavior in anesthetized and involuntary voiding in behaving experiments in response to pelvic nerve stimulation. a) Stimulation evoked pressure response at different stimulus intensities in an anesthetized trial. b) Stimulation evoked pressure response at different stimulus intensities in a behaving trial leading to involuntary void in behaving experiments. c) Cumulative comparison of Δp1 and Δp2 between anesthetized and behaving trials in response to bipolar stimulation. d) Cumulative comparison of voiding efficiency between anesthetized and behaving trials in response to bipolar stimulation.

4.5 Discussion

Direct bladder wall stimulation via electrode nets could evoke voiding in both anesthetized and awake behaving trials for the duration of the implant. Cat A had to be terminated at the end of 2 months due to an infection in the back. Cat C was terminated due to the failure of electrodes. This failure resulted from a bond break where the soft silicone conductive polymer connects to the stainless steel wire. In all the cats, the electrode positions were not changed from when it was implanted. To reduce these chances of failures, a new electrode design was made in which, instead of two bond pads (where conductive polymer connects to the stainless-steel wire), 6 bond pads were included to reduce the mechanical stress. This new design was used from cat D-F, and a reduction in electrode failure resulted in experiments until the protocol duration of 3-4 months. Failure of an electrode in the soft silicone mesh was associated with a break in the bonds, which were confirmed during the explant, unlike in previous studies where mechanical displacement of electrodes led to failure both in preclinical[53,80] and clinical studies[32,60].

Awake cats have voluntary control of the urethra, unlike in anesthetized trials[23,96]. Moreover, cats are socially conscious and always void in the litter box. This explains the difference between the voiding responses in anesthetized and awake trials. $\Delta p1$ or the first pressure peak is stimulation-evoked, evident from the sharp rise in bladder pressure at the onset of stimulation in both anesthetized and awake animals. However, the $\Delta p1$ values were lower in awake trials compared to anesthetized trials. This could be due to two reasons: first, in anesthetized trials, the inhibition to the bladder is inactive[92], whereas, in awake behaving trials, the bladder remains at an inhibitory state to maintain continence[41]. Second, unlike in awake, behaving cats, where the cat actively controls the urethra as the bladder pressure increases, which further leads to inhibition, there is no urethral resistance in anesthetized trials. This also explains the time difference in the

onset of voiding from stimulation onset. As pressure rises due to stimulation in anesthetized trials, urine starts flowing out whereas in awake cats, that change in pressure is voluntarily resisted by the cats and depending on the magnitude of bladder pressure, cat goes to the litter box and voids in squatting position which is also a sign of voluntary voiding. In awake behaving cats, second peak pressure (Δp2) is much higher than stimulation evoked pressure in anesthetized trials. This could be due to involvement of reflexes involved in voluntary voiding such as pudendo-vesical (facilitation reflex) in addition to the motor contraction of bladder due to the stimulation[14,15,97]. This can be supported by the results in cat F, where the cat did not voluntarily void; hence Δp2 values were not higher than the pressures in anesthetized trials. Δp2 values were higher than anesthetized trails only at high stimulus intensities, and this could be due to the urethral resistance resulting from cats' conscious control over the urethra.

Voiding efficiency in anesthetized trials increases as the stimulation intensity and bladder pressure increases as the effect of motor activity evoked by stimulation since reflexes in dexdomitor anesthesia are suppressed[92]. However, in awake, behaving trials, voiding was always complete (other than cat F) due to the cats' volitional control, irrespective of stimulation intensity. However, stimulus intensity influenced the time it took for the cat to go to the litter box and void. At higher stimulus intensities, the cat took significantly less time from stimulus onset than at low stimulus intensities. This indicates that stimulus intensity could modulate the sense of urgency in behaving cats. This could be due to two reasons, first, direct stimulation of sensory afferents (via stretch receptors). Second, the higher stimulation evoked pressure generated due to the stimulation of efferent fibers or a mix of both. It is hard to distinguish between these two, but lower stimulation intensities resulted in less urgent voiding behavior at stimulation. This could indicate the ability of electrode nets to recruit sensory neurons in the bladder wall[75]. It could be due to the stimulation or

a result of both. Varying stimulus intensities might result in differential recruitment of afferent and efferent fibers within the bladder wall and neural circuits involved in micturition[59]. Our observations suggest that the spinal-intact awake cat's voiding behavior in response to DBWS is governed by enhanced sensory reflexes and active urethral sphincter control. With different motor and sensory reflexes, the sensation or urge to void can be modulated[98]. Hence, the voiding behavior can be distinct across stimulus intensities[98–101].

Direct bladder wall stimulation through electrode nets can generate substantial magnitudes of bladder contraction is evident from the voiding behavior in anesthetized and awake behaving trials. In cat F, stimulation at high stimulus intensities led to involuntary voiding, which was evident from the cat's incomplete voiding and non-squatting behavior. These stimulus intensities could overcome the voluntary control of the urethra. In no case, the stimulus intensities led to leg movement or uncomfortable behavior. High stimulus intensities that lead to involuntary voiding strengthen the fact that stimulation through electrode nets is focused on the bladder without co-activation of the urethra, which was a major challenge in the literature. At lower stimulus intensities, the cat could control voiding in awake, behaving cats but voided in anesthetized trials. This proves the ability of DBWS through electrode nets, leading to functional voiding and bladder pressures.

Peripheral nerve stimulation (PNS) of the pelvic nerve is known to elicit efficient voiding behaviors. When stimulated at threshold amplitude and 30 Hz frequency, the pelvic nerve results in functional voiding. Pelvic nerve stimulation activates both the parasympathetic efferent pathway causing bladder contraction and the sensory afferent pathway, which conveys the sense of bladder fullness to supra-spinal centers[42]. The results were similar to DBWS with the limited data acquired

in these experiments with pelvic nerve stimulation. This indicates the modulation of sensory and motor parasympathetic fibers in the bladder wall.

This type of neural interface would ideally be useful in a pathological model where the bladder is underactive or atonic; however, we did not test it in a diseased model. In pathological models, the stimulation amplitude might increase as it does in sacral nerve stimulation[55], and that is a limitation of this work. With intact spinal reflexes in awake behaving cats, it might be the case that sensory reflexes are playing a major role, In pathological models, these reflexes are severed and reorganized[44,102], so the effects of stimulation might not be the same. Hence, this neural interface should be tested in pathological models. Moreover, in awake, behaving experiments, a cat's desire to void plays a major role which could be affected by the social environment even after training to void in the experimental setting. For instance, Cat F never voided voluntarily with stimulation or during filling cystometry. However, it immediately voided when it was taken back to its own housing. So, the voluntary control and social environment could have biased these results.

We show that DBWS through electrode nets could generate comfortable, complete voiding in long-term functional outcomes in implanted animals. However, these studies must be performed with pathophysiological models of conditions where the bladder is underactive or atonic.

5.0 Neural Mechanisms of Direct Bladder Wall Stimulation

5.1 Overview

Direct bladder wall stimulation (DBWS) has been attempted for decades to restore bladder function in people with spinal cord injury and other voiding dysfunctions with various pathological conditions. These efforts led to some successes and failures depending on the pathological conditions. However, the neural mechanisms of DBWS are poorly understood. To understand the mechanisms of DBWS, we conducted terminal experiments in 6 anesthetized cats. First, we compared the stimulation-evoked bladder pressure responses under Isoflurane (suppressed reflexes) and α-chloralose anesthesia (active reflexes) and found that bladder pressures under α-chloralose were significantly higher than those under Isoflurane. We then used pharmacological agents to characterize the local neuronal population on the bladder surface. We induced atropine and found that stimulation-evoked bladder pressures were significantly reduced after atropine, indicating the neurogenic nature of the stimulation evoked bladder pressure responses. We then induced hexamethonium and found that stimulation-evoked bladder pressures result from mixed activation of preganglionic and postganglionic fibers in the bladder wall and that at higher stimulus intensities, we can generate bladder pressures in the absence of preganglionic input. We then induced propranolol and found that DBWS doesn't affect inhibitory neurons in the bladder wall.

Next, we transected the pelvic nerves bilaterally to eliminate reflex activity with its afferent arm in the pelvic nerve that might be activated by stimulation and found that robust bladder contractions could still be generated at higher stimulus intensities but were suppressed at lower stimulus intensities indicating the role of pelvic-to-pelvic reflexes in generating bladder

contractions. No considerable effect was found after pudendal nerve transection in our isovolumetric studies. This mechanistic data suggest that robust bladder contractions can be generated by stimulating the bladder wall even in the absence of inputs from the central nervous system and without presynaptic activation of the pelvic ganglia, mimicking the case of pelvic nerve denervation, which can occur in conditions such as diabetic neuropathy and sacral spinal cord injury.

5.2 Introduction

LUT functions are carried out in the coordination of multiple neural reflexes involving central and local pathways. Though direct bladder wall stimulation (DBWS) has been attempted for decades, the mechanisms are not clear. Previously, we have shown that stimulating the bladder base with a soft silicone electrode interface can generate robust bladder contractions. At the reported stimulus intensities, it is understood that the response is mediated through neural structures within the bladder wall. While it is possible to directly stimulate the smooth muscle bundles to excite the bladder, the stimulus intensities required are higher[73]. Hence, DBWS might work through complex neurogenic mechanisms rather than just myogenic mechanisms. Sympathetic and parasympathetic outflow from the lumbar and sacral spinal cord regularly interact through the pelvic plexus in the periphery[103]. The same descending information continues into intramural ganglia located on the bladder wall[95]. This outflow can either inhibit or excite the bladder, depending on the state of fullness. In the absence of descending outflow from the brain, such as in spinal cord injury, the sacral cord and postganglionic innervation to the bladder remain intact and regulate bladder function[104].

Further, in the absence of input from the sacral cord, intramural innervation can regulate bladder function[70]. Retrograde tracing studies show that many of these intramural ganglia are located around the ureterovesical junction at the base of the bladder[71,72,105]. The number of postganglionic neurons found on the bladder surface decreases depending on the level of spinal cord injury[72]. It is, therefore, necessary to characterize the neuronal population on the bladder surface activated through electrical stimulation of the bladder base.

In pathological conditions, neural reflex pathways of LUT are reorganized[1]. Increased urethral resistance resulting from co-activation of the urethral sphincter has been reported as a major challenge for bladder wall stimulation[26,61]. In healthy animals, reflexes such as the pudendo-vesical reflex or the augmentation reflex[97,106] significantly contribute to achieving complete bladder emptying. In the absence of these urethral-to-bladder reflexes, higher stimulation amplitudes might be necessary to achieve the same emptying. It is, therefore, important to understand the contribution of these reflexes involved in DBWS.

We hope to gain a mechanistic understanding of the neural pathways involved in DBWS. We used different anesthesia, pharmacological agents, and selective nerve transections to determine mechanisms mediating DBWS. While pharmacological agents are reversible, they are often incapable of blocking both afferent and efferent innervation in a nerve. Hence, bilateral transection of pelvic and pudendal nerves is necessary for abolishing a set of reflexes.

5.3 Materials and Methods

All experiments are approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC). Terminal acute experiments will be performed on 6 cats (4 males and 2 females).

5.3.1 Surgical Preparation

Anesthesia was induced with inhaled Isoflurane in a closed chamber. Throughout the surgery, anesthesia was maintained via inhaled Isoflurane (1%-2%). Animals were artificially ventilated at 12-14 breaths per minute throughout the procedure. Animals were monitored continuously for heart rate, SpO2, blood pressure, and body temperature on a vital monitoring system (SurgiVet). A pressure-sensing catheter (AD Instruments) was inserted in the carotid artery for blood pressure monitoring. Warm air heating pads were used to maintain temperature, and IV fluids (Nacl-Dextrose) were administered continuously. A midline abdominal incision was made to expose the bladder. The pelvic nerve was identified bilaterally, and the right pelvic nerve was instrumented with a bipolar nerve cuff (Microleads Inc.). The electrode net was placed on the bladder for stimulation. A dual-lumen pressure catheter was inserted via the bladder dome to record bladder pressure and infuse saline into the bladder. Bladder pressure was recorded from this catheter with a pressure transducer connected to a Trans-Bridge amplifier (WPI Inc.).

5.3.2 Electrode Design and Placement

12 electrodes were placed on the bladder base in two concentric circles of 6, each separated by 2.0 mm vertically, as shown in figure 5.1. These electrodes were anchored with a soft silicone mesh with a convoluted structural design over the bladder, allowing the bladder to accommodate volume changes while maintaining electrode stability.

5.3.3 Determination of Isovolumetric Bladder Volume

In every experiment, the bladder was manually filled with saline through the pressure catheter until it began to leak. The volume at which the bladder starts leaking was considered the maximum bladder capacity, and the volume was maintained between 70-90% of maximum bladder capacity for optimally placing the electrodes.

5.3.4 Data Collection and Instrumentation

The analog output from the pressure amplifier was recorded with an Analog-Digital I/O headstage through the Grapevine Neural Interface Processor (Ripple Inc.). The electrode array on the bladder was connected to a high current stimulator (Ripple Inc.) with a compliance voltage of 30V and a maximum current generation capacity of 15 mA (Ripple Inc.) to stimulate the electrodes on the bladder surface. This stimulator was interfaced with a Grapevine neural interface processor (Ripple Inc.) controlled through MATLAB (MathWorks Inc.). A nanostim stimulation headstage was connected to the pelvic nerve cuff for stimulation.

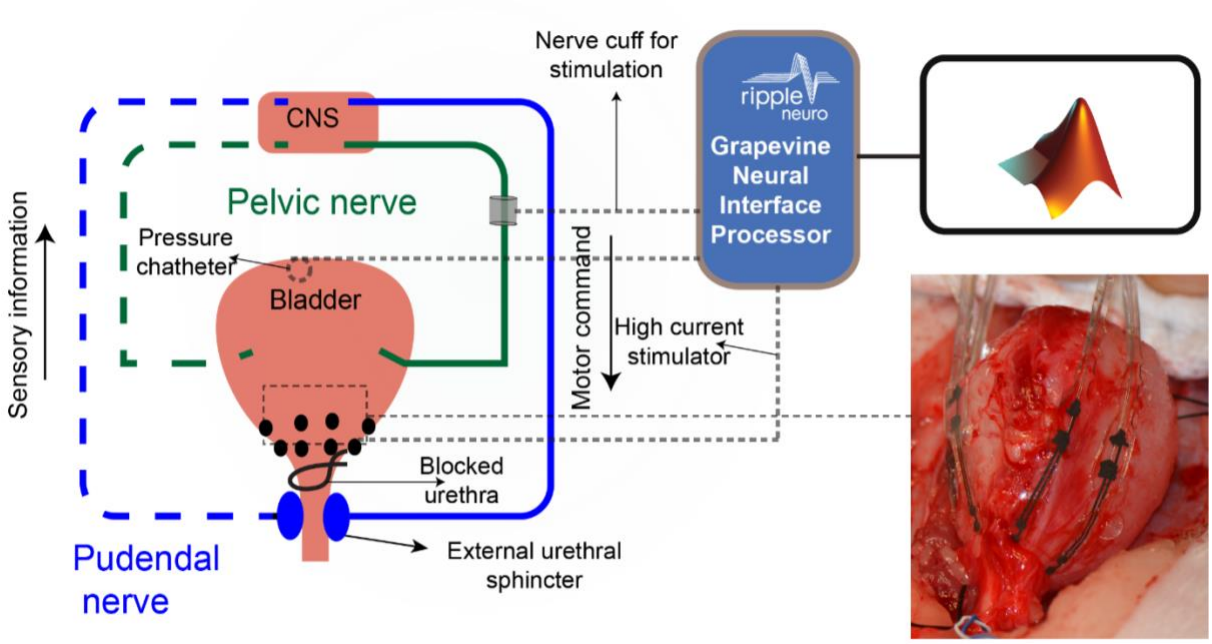

Figure 5.1 Experimental setup

5.3.5 Experiment Protocol

Electrical stimulation: Biphasic charged balanced square pulses were used for electrical stimulation with constant pulse width and frequency of 1 ms and 30 Hz, respectively. Stimulation amplitude was variable from (0.2 mA – 6 mA) depending on the stimulation type and the study. Electrodes on the bladder were stimulated via one of the following three stimulation configurations. 1) Monopolar stimulation – stimulating a single electrode with its return path through the large diameter ground electrode placed on the abdomen. 2) Bipolar stimulation- stimulating a single electrode with its return path from one of the 11/12 electrodes. 3) Sequential stimulation- Sequentially stimulating all 12 electrodes with a return path on the abdomen or stimulating bipolar pair of electrodes on the bladder surface. For comparison of anesthetics and nerve transection studies- Monopolar and bipolar stimulation were grouped in the data analysis as

single paired stimulation. So, Stimulation for these studies was either single pair (Monopolar, bipolar) or sequential stimulation. For the pharmacology section of the study, only monopolar stimulation was performed. The pelvic nerve was stimulated using pulse width and frequency of 0.2 ms and 30 Hz, respectively. The stimulation amplitude was varied from 0.1 mA to 0.6 mA.

Anesthesia: In the first 3 animals (cats A, B,C) bladder wall stimulation was performed with Isoflurane and then was switched to α-chloralose (65 mg/kg) until the reflexes recovered. Similar stimulation was performed after switching to α-chloralose. All the other parts of the experiment were done with α-chloralose anesthesia. For the 3 experiments where Isoflurane vs. α-chloralose studies were not performed, animals were switched to α-chloralose immediately after the surgery.

Pharmacology: After acquiring the control data, a similar stimulation protocol was performed after inducing the atropine (0.5 mg/kg) through the femoral vein and similar stimulation protocol was performed (cats E, F). After recovery from atropine, control data was acquired again, followed by hexamethonium (2 mg/kg) induction. A similar stimulation protocol was performed (Cats A-F). After recovery from hexamethonium, control data were acquired, followed by induction of propranolol (1 mg/kg). A similar stimulation protocol was repeated (Cats A-C, E, F). Control data were acquired before injecting every drug.

Nerve transection: In 3 animals (cats A, E, F), control data were acquired at varying stimulus intensities with sequential and single pair stimulation. Following that, bilateral pelvic nerve transection was performed. After 30 mins wait period, a similar stimulation protocol was performed. In 2 animals (cats B, D), after acquiring the control data, bilateral pudendal nerve transection was performed. A similar stimulation protocol was performed. The bilateral pelvic

nerve was transected, and a similar stimulation protocol was repeated. A summary of experiments conducted in each cat is shown in table 5.1.

Table 5.1: Summary of experiments conducted in each cat. X indicates that the particular study was conducted in the cat.

Cat	Isoflurane vs. α-chloralose	Atropine	Hexamethonium	Propranolol	Pelvic nerve transection only	Pudendal nerve transection followed by pelvic nerve transection
A	X		X	X	X	
B	X		X	X		X
C	X		X	X		
D			X			X
E		X	X	X	X	
F		X	X	X	X	

5.3.6 Data Analysis and Statistics

All data analysis was performed in MATLAB (Mathworks Inc). The pressure change was calculated as a difference between the pressure value before 100 ms from the onset of stimulation and the highest value reached during the stimulation time window. Pressure changes greater than 5 cmH_2O indicated that a given electrode was a responder and was used for further statistical analysis. Six animals were used in this study. The data is non-normal, and hence non-parametric tests were performed. Pair-wise group comparisons were tested using Friedman test and the post-

hoc for paired comparisons were tested using Wilcoxon signed rank test with a $p<0.05$. Unequal group comparisons were tested using Kruskal-Wallis test and the post-hoc for unequal group were tested using Wilcoxon rank sum test with a $p<0.05$. If data is not significant but still has a pattern, median values were reported in the results. Furthermore, linear mixed models analysis was performed for each test and is reported as supplementary statistics in Appendix B.

5.4 Results

5.4.1 Isoflurane vs. α-chloralose

To determine the role of spinal reflexes in bladder pressures evoked by DBWS, we leveraged that different anesthesia has distinct effects on lower urinary tract functions. Different anesthesia has different effects on bladder pressures evoked during filling cystometrograms in felines[92]. Distension-evoked bladder pressure changes were reported significantly higher for α-chloralose anesthesia) than Isoflurane as the spinal reflexes are preserved in α-chloralose, unlike Isoflurane. To examine the contribution of reflexes, we stimulated the bladder surface with isoflurane and after inducing α-chloralose in 3 animals (cat A, B, C). We stimulated the bladder surface at multiple amplitudes (2, 4 mA) using both monopolar and bipolar stimulation paradigms as well as sequential stimulation (0.5, 1, 2 mA) keeping the pulse width and frequency constant at 1 ms and 30 Hz, respectively.

Data from monopolar and bipolar stimulation electrodes were combined as we found that there was no significant difference between the evoked pressures (see chapter 3, figure 3.6). We

also used a sequential stimulation paradigm and analyzed these data separately as this method evokes much larger changes in bladder pressure.

Figure 5.2b shows an example of the pressure changes evoked by bipolar DBWS with isoflurane and α-chloralose at 2 and 4 mA. Bladder pressure evoked with α-chloralose were higher than evoked pressure with isoflurane. We calculated the difference between bladder pressure with isoflurane and α-chloralose as a metric of evaluating the effect of anesthesia.

For monopolar or bipolar stimulation, grouped together, we first examined the effect of both the anesthesia on bladder pressure for each electrode across all amplitudes and cats (fig 5.2c). We calculated the pressure difference for each electrode with isoflurane and after α-chloralose and found that the median difference in bladder pressure was significantly higher than 0 ($p = 6.10e-18$, Wilcoxon-signed rank test, fig. 5.2d) indicating that α-chloralose led to increase in bladder pressure. Next, we wanted to see if this difference in bladder pressure was affected by stimulation amplitude and found that there was no significant difference ($p=0.9651$ Friedman test, fig. 5.2e). We also performed an LMM analysis and found that anesthesia had a significant effect on bladder pressure, however, no significant effect of amplitude was found (fig. 5.2c, Appendix Table B1). However, the interaction term between anesthesia and amplitude – the effect of the anesthesia taking into consideration stimulation amplitude – was not significant (Appendix Table B1), consistent with the result in fig. 5.2e. Lastly, cat identity was not a significant factor (Appendix Table B1).

We repeated these analyses for the sequential stimulation paradigms. Fig. 5.2f shows the distribution of pressures with isoflurane and α-chloralose for each stimulation amplitude across all stimulation paradigms and cats. The median difference in bladder pressure between isoflurane and α-chloralose was significantly higher than 0 ($p=6.1e-18$, Wilcoxon-signed rank test, fig. 5.2g)

indicating that α-chloralose lead to increase in bladder pressure. Similar to the monopolar and bipolar experiments, there was no significant effect of amplitude on the bladder pressure difference ($p=0.368$, Friedman test, fig. 5.3h). The LMM analysis was repeated for these data (Appendix Table B2), showing the same results.

Overall, these data suggest that stimulation-evoked pressure were significantly higher with α-chloralose anesthesia indicating the contribution of central reflexes in evoked bladder pressure. Importantly, even with isoflurane, functionally relevant pressures could be generated at higher amplitudes.

We also compared bladder pressures evoked by stimulating the pelvic nerve at different stimulus intensities (amplitude: 0.1, 0.2, 0.3, 0.4, 0.5 mA, frequency: 30 Hz, pulse width: 1 ms). Although not statistically significant ($n=3$), similar results were found where the bladder pressures were higher for α-chloralose anesthesia ($n=3$, figure 5.8).

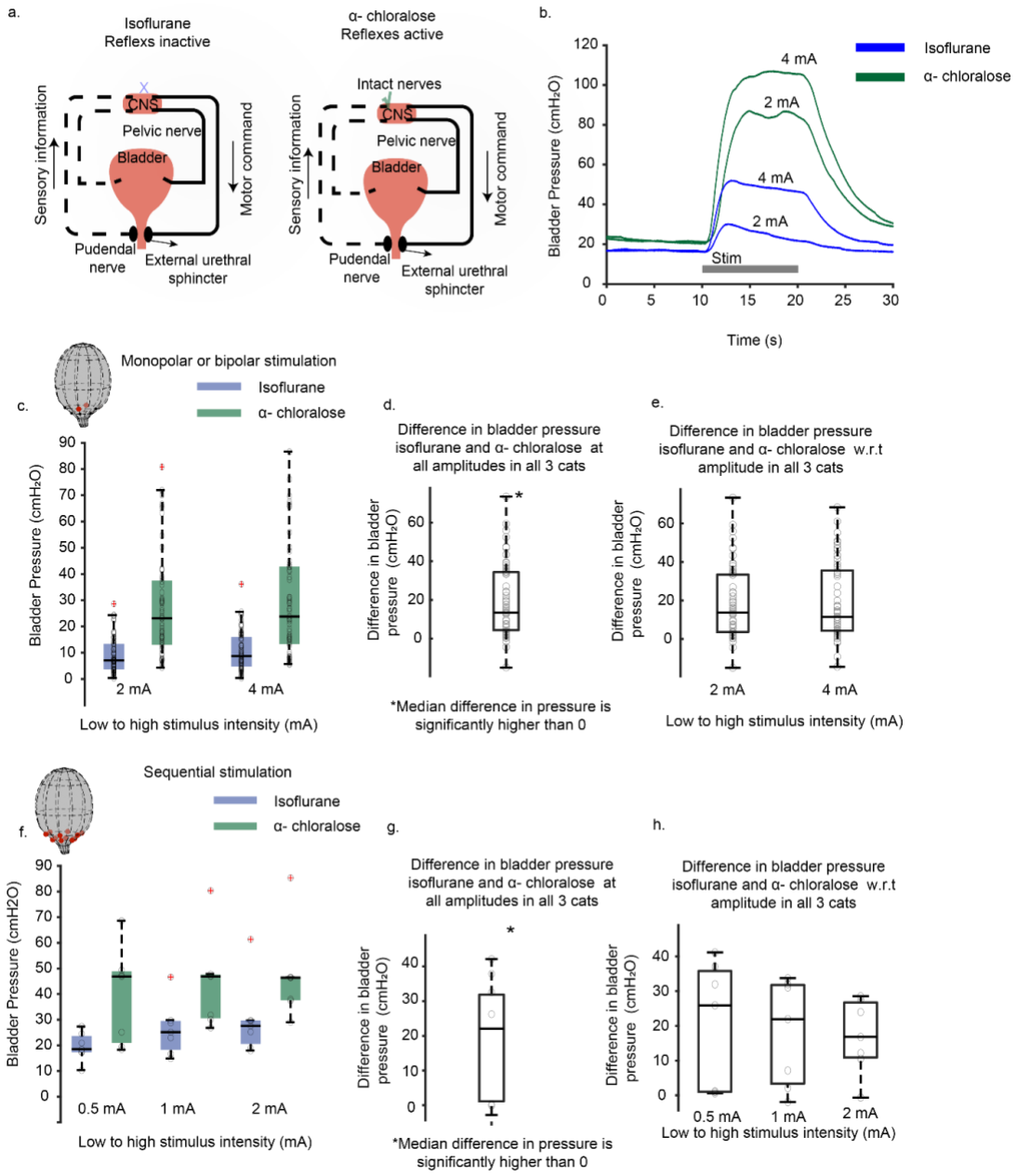

Figure 5.2 Isoflurane vs. α-chloralose. a) Illustration of diminished reflexes with Isoflurane and preserved reflexes with α-chloralose. b) Stimulation evoked pressure responses at different stimulation amplitudes with Isoflurane and α-chloralose. c) Comparison of stimulation-evoked pressures with Isoflurane and α-chloralose to monopolar or bipolar stimulation across all amplitudes and all cats. d) Difference in stimulation-evoked

pressures with Isoflurane and α-chloralose to monopolar or bipolar stimulation across all amplitudes and all cats. e) Differences in bladder pressure between Isoflurane and α-chloralose w.r.t. amplitudes. f) Comparison of stimulation-evoked pressures with Isoflurane and α-chloralose to sequential stimulation across all amplitudes and all cats. g) Difference in stimulation-evoked pressures with Isoflurane and α-chloralose to sequential stimulation across all amplitudes and all cats. h) Differences in bladder pressure following nerve transection w.r.t. amplitudes.

5.4.2 Nerve Transection

5.4.2.1 Pelvic Nerve Transection

Next, we wanted to know whether reflexes mediated by pelvic nerve afferents played a significant role in stimulation-evoked bladder pressure changes through DBWS. To examine this contribution, we stimulated the bladder surface before and after bilateral transection of the pelvic nerve in 3 animals (cat 3, 7,8). We stimulated the bladder surface at multiple amplitudes (0.2, 0.5, 1, 2, 4 mA) using both monopolar and bipolar stimulation paradigms keeping the pulse width and frequency constant at 1 ms and 30 Hz, respectively. Data from monopolar and bipolar stimulation electrodes were combined as we found that there was no significant difference between the evoked pressures (see chapter 3, figure 3.6). We also used a sequential stimulation paradigm and analyzed these data separately as this method evokes much larger changes in bladder pressure. Figure 5.3b shows an example of the pressure changes evoked by sequential stimulation before and after the nerve transection at 0.2, 0.5, and 1 mA. Transection of the pelvic nerve led to changes in bladder pressure in all cases. This included both decreases in the maximum pressure, as well as changes in the pressure during sustained stimulation. Importantly however, the pressures generated after pelvic nerve transection at the higher stimulation amplitudes remained high enough to be

functionally relevant. We calculated the difference between bladder pressure before and after nerve transection as a metric of evaluating the effect of nerve transection.

For monopolar or bipolar stimulation, grouped together, we first examined the effect of nerve transection on bladder pressure for each electrode across all amplitudes and cats (fig 5.3c). We calculated the pressure difference for each electrode before and after transection and found that the median difference in bladder pressure was significantly higher than 0 ($p = 1.9e-8$, Wilcoxon-signed rank test, fig. 5.3d) indicating that pelvic nerve transection led to decrease in bladder pressure. Next, we wanted to see if this difference in bladder pressure was affected by stimulation amplitude and found that there was no significant difference ($p=0.11$, Kruskal-Wallis test, fig. 5.3e). We also performed a LMM analysis and found that nerve transection and stimulation amplitude had a significant effect on bladder pressure. In this case, the significant effect of stimulation refers to the fact that increasing the stimulation amplitude itself increased the bladder pressure with the pelvic nerves were intact and transection (fig. 5c, Appendix Table B3). However, the interaction term between transection and amplitude – the effect of the transection taking into consideration stimulation amplitude – was not significant (Appendix Table B3), consistent with the result in fig. 5e. Lastly, cat identity was not a significant factor (Appendix Table B3).

We repeated these analyses for the sequential stimulation paradigms. Fig. 5f shows the distribution of pressures before and after transection for each stimulation amplitude across all stimulation paradigms and cats. The median difference in bladder pressure before and after was significantly higher than 0 ($p=0.006$, Wilcoxon-signed rank test, fig. 5.3g) indicating that pelvic nerve transection led to a decrease in bladder pressure. Similar to the monopolar and bipolar experiments, there was no significant effect of amplitude on the bladder pressure difference ($p=0.5$

Kruskal-Wallis test, fig. 5.3h). The LMM analysis was repeated for these data (Appendix Table B 4) showing the same results.

Overall, these data suggest that having an intact pelvic nerve contributes to increased bladder pressures in response to bladder wall stimulation, perhaps through a reflex mechanism. However, these effects were relatively small and were not affected by the stimulation amplitude itself. Most importantly, even after nerve transection, functionally meaningful bladder pressures can still be generated.

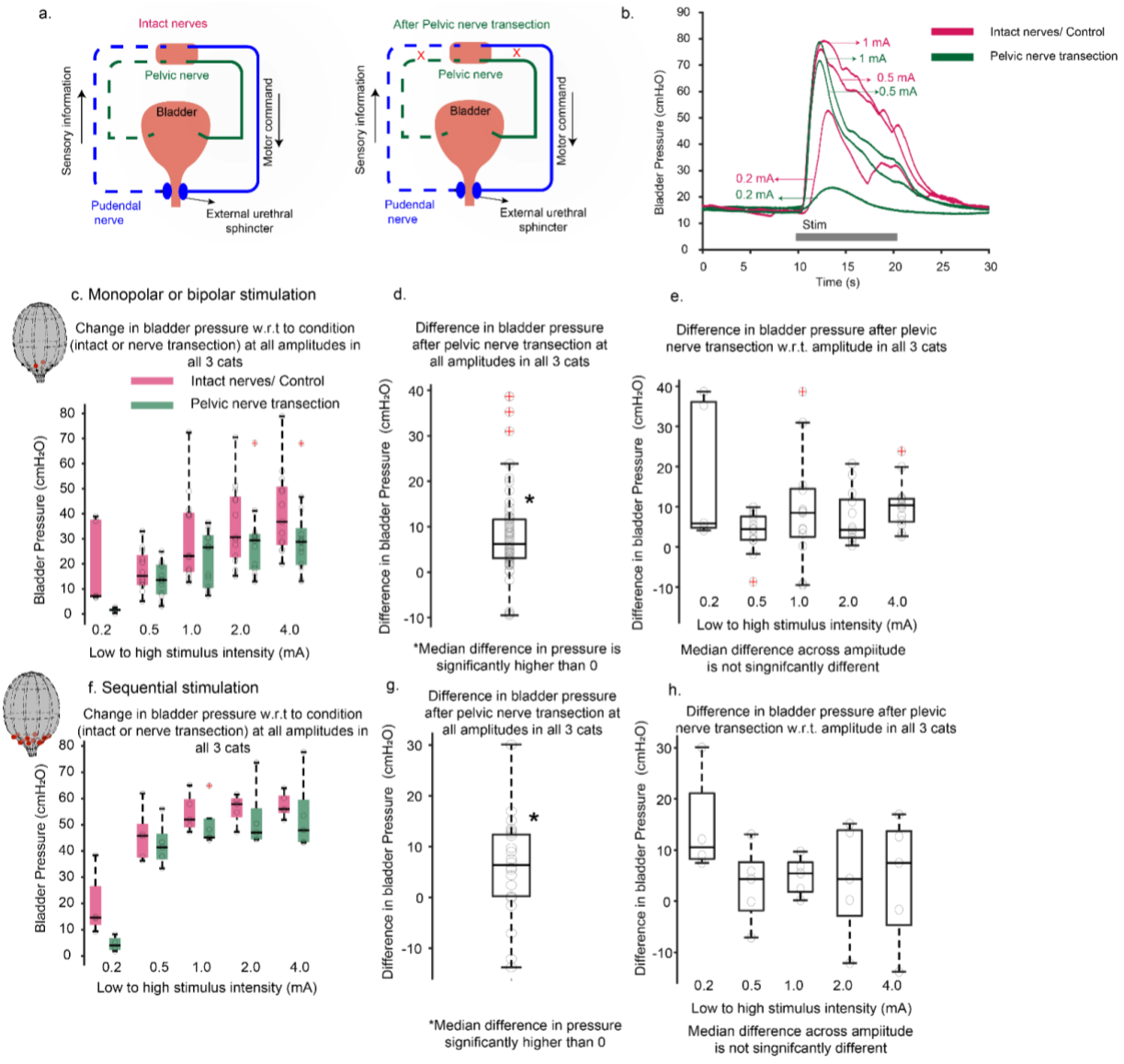

Figure 5.3 Effects of pelvic nerve transection a) Illustration of intact reflexes and severed reflexes after pelvic nerve transection. b) Stimulation evoked pressure responses at different stimulation amplitudes with intact nerves after bilateral pelvic nerve transection. c) Comparison of stimulation-evoked pressures before and after pelvic nerve transection at different stimulation amplitudes in response to monopolar or bipolar stimulation. d) Difference in stimulation-evoked pressures with intact nerves and after pelvic nerve transection in response to monopolar or bipolar stimulation across all amplitudes and all cats. e) Differences in bladder pressure following nerve transection w.r.t. amplitudes. f) Comparison of stimulation-evoked pressures before and after pelvic nerve transection at different stimulation amplitudes in response to sequential stimulation. g) Difference in stimulation-evoked pressures with intact nerves and after pelvic nerve transection in response to sequential stimulation at across all amplitudes and all cats. h) Differences in bladder pressure following nerve transection w.r.t. amplitudes

5.4.2.2 Pudendal Nerve Transection

Next, we wanted to know whether reflexes mediated by pudendal nerve afferents played a significant role in stimulation-evoked bladder pressure changes through DBWS. To examine this contribution, we stimulated the bladder surface before and after bilateral transection of the pudendal nerve in 2 animals (cat B, D). Following that, we also transected the bilateral pelvic nerve to see if bladder pressure significantly changed when the bladder is decentralized from parasympathetic and somatic inputs.

We stimulated the bladder surface at multiple amplitudes (0.2, 0.5, 1, 2, 4 mA) using both monopolar and bipolar stimulation paradigms keeping the pulse width and frequency constant at 1 ms and 30 Hz, respectively.

Data from monopolar and bipolar stimulation electrodes were combined as we found that there was no significant difference between the evoked pressures (see chapter 3, figure 3.6). We also used a sequential stimulation paradigm and analyzed these data separately as this method

evokes much larger changes in bladder pressure. Figure 5.4b shows an example of the pressure changes evoked by sequential stimulation before and after the nerve transection at 0.2 and 0.5 mA. Transection of the pudendal nerve led to a decrease in bladder pressure in both cases, however, following the bilateral pelvic nerve transection, this decrease in bladder pressure was higher. Importantly however, the pressures generated after pudendal nerve transection as well as with both pudendal and pelvic nerve transection at the higher stimulation amplitudes remained high enough to be functionally relevant. We calculated the difference between bladder pressure before and after nerve transection as well as the difference between bladder pressure before and after when both pudendal and pelvic nerves were transected as a metric of evaluating the effect of nerve transection.

For monopolar or bipolar stimulation, grouped together, we first examined the effect of nerve transection on bladder pressure for each electrode across all amplitudes and cats (fig 5.4c). We calculated the pressure difference for each electrode before and after transection and found that the median difference in bladder pressure was not significantly higher than 0 after pudendal nerve transection ($p = 0.85$, Wilcoxon-signed rank test, fig. 5.4d), however, median difference in bladder pressure was not significantly higher than 0 after the pelvic nerve transection ($p = 0.0014$, Wilcoxon-signed rank test, fig. 5.4d) indicating that pudendal nerve transection did not affect the bladder pressure whereas bladder pressure decreased when pelvic nerves were transected following the pudendal nerve transection.

Next, we wanted to see if this difference in bladder pressure for both the cases i.e. after the pudendal nerve transection and pudendal and pelvic nerve transection was affected by stimulation amplitude and found that there was no significant difference after pudendal nerve transection ($p=0.85$ Kruskal-Wallis test, fig. 5.4e) as well as when both the pudendal nerve and pelvic nerve were transected ($p=0.23$ Kruskal-Wallis test, fig. 5.4f).We also performed an LMM analysis and

found that nerve transection did not affect the bladder pressure whereas stimulation amplitude had a significant effect on bladder pressure. In this case, the significant effect of stimulation refers to the fact that increasing the stimulation amplitude itself increased the bladder pressure with both the pudendal and pelvic nerves were intact and following the nerve transection (fig. 5.4c, Appendix Table B5). However, the interaction between transection and amplitude – the effect of the transection taking into consideration stimulation amplitude – was not significant (Appendix Table B5), consistent with the result in fig. 5.4e. Lastly, cat identity was not a significant factor (Appendix Table B5).

We repeated these analyses for the sequential stimulation paradigms. Fig. 5f shows the distribution of pressures before and after transection for each stimulation amplitude across all stimulation paradigms and cats. The median difference in bladder pressure was not significantly higher than 0 after pudendal nerve transection ($p = 0.54$, Wilcoxon-signed rank test, fig. 5.4f) as well as after the pelvic nerve transection ($p = 0.07$), Wilcoxon-signed rank test, fig. 5.4f) indicating that nerve transection did not affect the bladder pressure whereas bladder pressure decreased when pelvic nerves were transected following the pudendal nerve transection. Similar to the monopolar and bipolar experiments, there was no significant effect of amplitude on the bladder pressure difference in both cases i.e., after the bilateral pudendal nerve transection ($p=0.46$ Kruskal-Wallis test, fig. 5.4g) as well as after both pudendal and pelvic nerve transection ($p=0.2$ Kruskal-Wallis test, fig. 5.4g). The LMM analysis was repeated for these data (Appendix Table B6), showing the same results.

Overall, these data suggest that having an intact pelvic nerve contributes to increased bladder pressures in response to bladder wall stimulation, it could be that intact pudendal nerve

plays no significant role. This data was collected only in 2 cats, so data might not be enough to make a substantial claim however claim. However, these effects were relatively small and were not affected by the stimulation amplitude itself. Most importantly, even after nerve transection, functionally meaningful bladder pressures can still be generated when the bladder is decentralized from its somatic and parasympathetic connections.

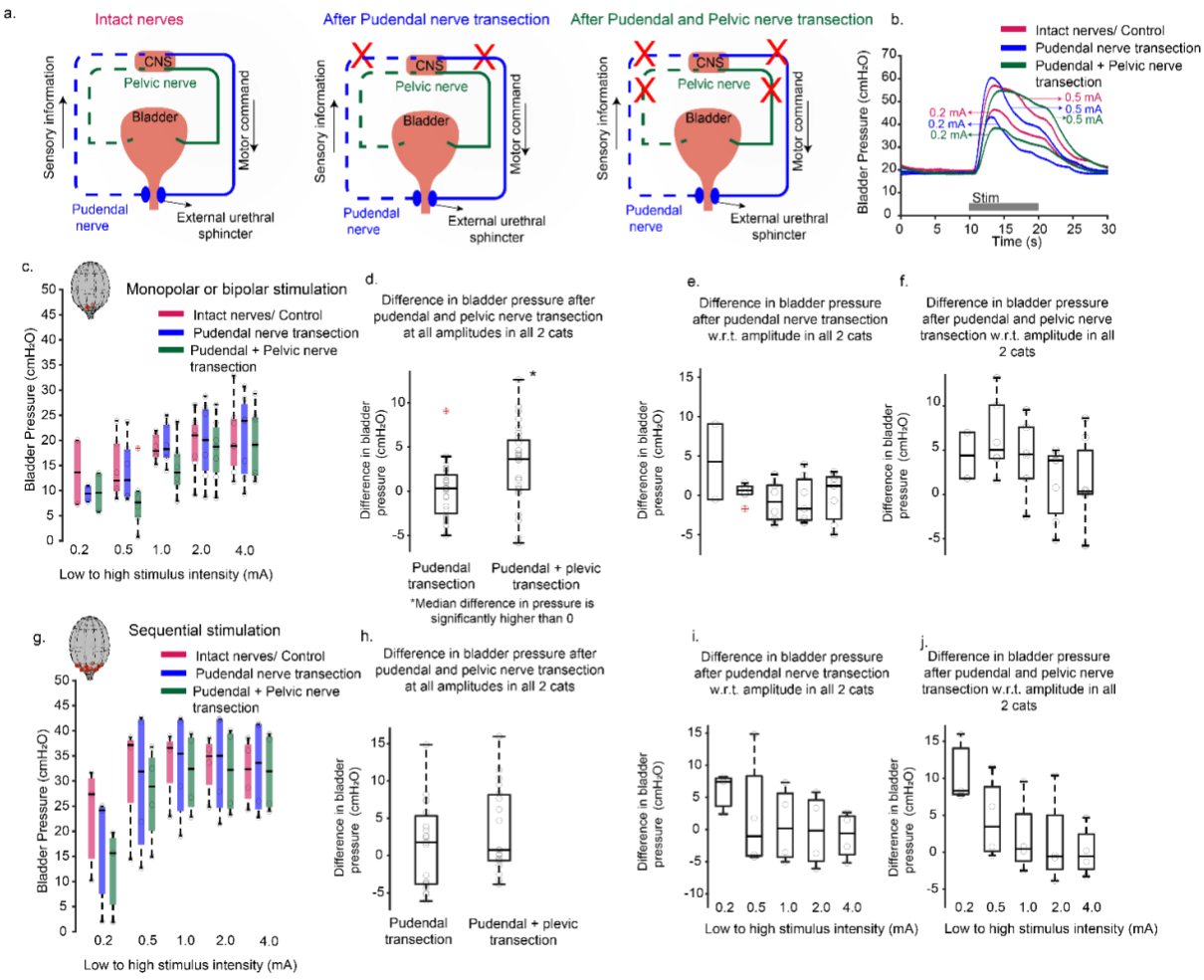

Figure 5.4 Effects of pudendal nerve transection followed by pelvic nerve transection . a) Illustration of intact reflexes and severed reflexes after pudendal nerve transection and pudendal and pelvic transection. b) Stimulation evoked pressure responses at different stimulation amplitudes with intact nerves after bilateral pudendal and pudendal+pelvic nerve transection. c) Comparison of stimulation-evoked pressures before and

after pudendal and pelvic nerve transection at different stimulation amplitudes. d) Difference in stimulation-evoked pressures with intact nerves and after pudendal and pelvic nerve transection in response to monopolar or bipolar stimulation across all amplitudes and all cats. e) Differences in bladder pressure following pudendal nerve transection w.r.t. amplitudes. f) Differences in bladder pressure following pudendal and pelvic nerve transection w.r.t. amplitudes. g) Comparison of stimulation-evoked pressures before and after pudendal and pelvic nerve transection at different stimulation amplitudes in response to sequential stimulation. h) Difference in stimulation-evoked pressures with intact nerves and after pudendal and pelvic nerve transection in response to sequential stimulation across all amplitudes and all cats. i) Differences in bladder pressure following pudendal nerve transection w.r.t. amplitudes. j) Differences in bladder pressure following pudendal and pelvic nerve transection w.r.t. amplitudes.

5.4.3 Pharmacological Evaluation

5.4.3.1 Effects of Atropine

To evaluate if the bladder pressure evoked through DBWS was a result of detrusor muscle stimulation (myogenic) or the intramural nerve network (neurogenic)in the bladder wall, we induced atropine which blocks the neuromuscular junction for cholinergic transmission in 2 animals (cat E, F). We stimulated each electrode on the bladder (monopolar stimulation) at 1, 2, and 4 mA keeping frequency and pulse width constant at 30 Hz and 1 ms, respectively. Fig 5.5b depicts an example of stimulation evoked bladder pressure before and after the atropine induction at stimulation amplitude 1, 2, and 4 mA. The stimulation-evoked bladder pressure significantly reduces after induction of atropine at all the stimulation intensities. We calculated the difference between bladder pressure before and after induction of atropine as a metric of evaluating the effect of nerve transection.

We first examined the effect of atropine on bladder pressure for each electrode across all amplitudes and cats (fig 5.3c). We calculated the pressure difference for each electrode before and after atropine induction and found that the median difference in bladder pressure was significantly higher than 0 ($p = 7.7e-8$, Wilcoxon-signed rank test, fig. 5.3d) indicating that induction of atropine led to decrease in bladder pressure. Next, we wanted to see if this difference in bladder pressure was affected by stimulation amplitude and found that there was no significant difference ($p=0.116$, Friedman test, fig. 5.3e). We also performed a LMM analysis and found that induction of atropine has significant effect on bladder pressure. However, the amplitude has no significant effect on the bladder pressure (fig. 5.5c, Appendix Table B7). The interaction term between drug and amplitude – the effect of the drug taking into consideration stimulation amplitude – was not significant (Appendix Table B7), consistent with the result in fig. 5e. Lastly, cat identity was not a significant factor (Appendix Table B7).

We would expect that at these stimulation amplitudes, the pressure would have completely reduced, but it could be that atropine failed to completely block the neuromuscular junction. However, a significant decrease in bladder pressure indicates that majority of pressure change due to bladder wall stimulation was mediated by the nerve network. We also expected that at higher amplitudes, the pressure difference will be lower than at lower amplitudes, but it could be that 4 mA was not high enough to evoke bladder pressure change with direct activation of the muscle. Overall, these data suggest that bladder pressure changes due to stimulation on the bladder surface are a result of both myogenic (direct activation of muscle) and neurogenic activation (activation of nerve network).

We also compared bladder pressures evoked by stimulating the pelvic nerve at different stimulus intensities (amplitude: 0.1, 0.2, 0.4 mA, frequency: 30 Hz, pulse width: 1 ms). Although

not statistically significant (n=2), similar results were found where the bladder pressures were reduced after atropine induction (figure 5.9a).

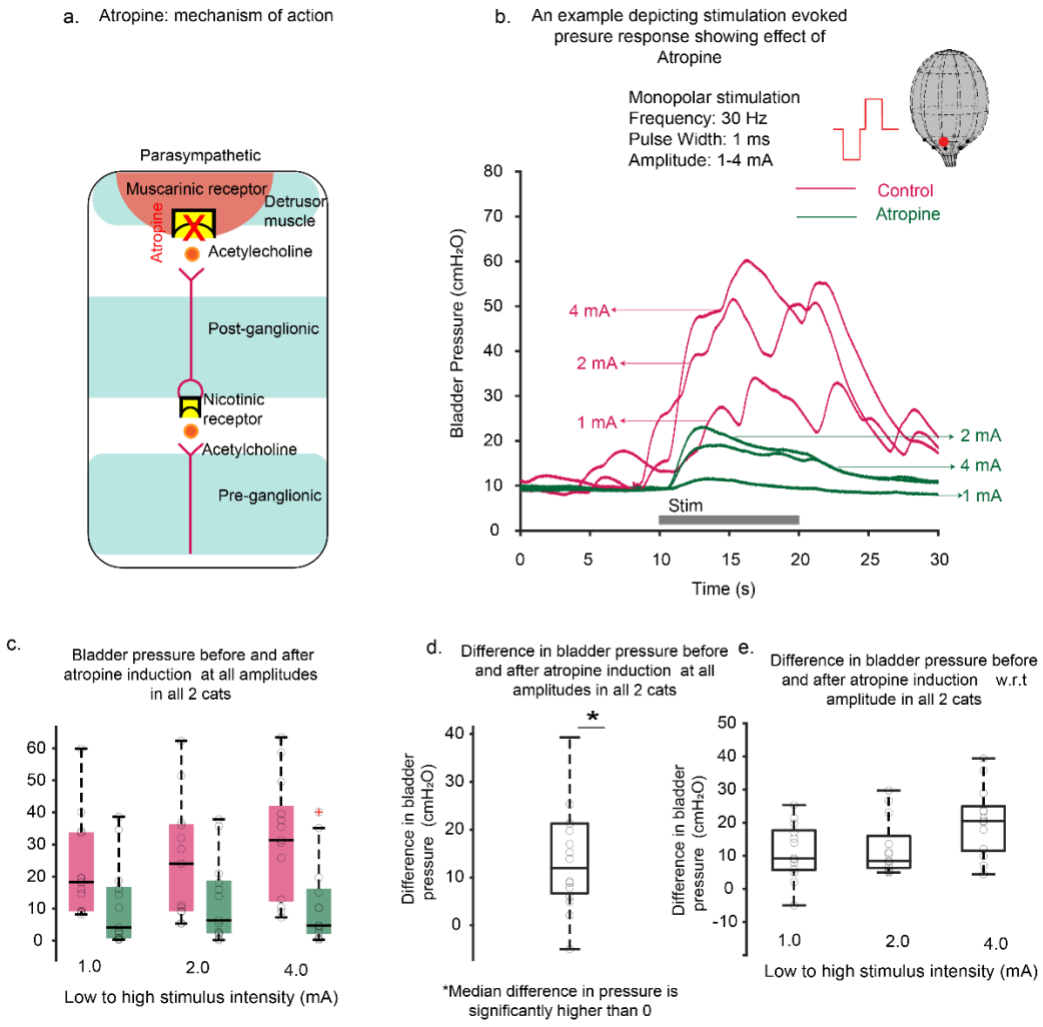

Figure 5.5 Effects of atropine on bladder pressure

a) Illustration of atropine mechanism of action. b) An example depicting stimulation-evoked pressure response showing effects of atropine at different amplitudes. c) Comparison of stimulation-evoked pressures before and after atropine induction at different stimulation amplitudes in response to monopolar stimulation in all cats. d) Difference in stimulation-evoked pressures before and after atropine induction in response to monopolar stimulation across all amplitudes and all cats. e) Differences in bladder pressure following atropine induction w.r.t. amplitudes.

5.4.3.2 Effects of Hexamethonium

To evaluate the type of neurons recruited (postganglionic and preganglionic efferent) via bladder wall stimulation, we induced hexamethonium, which blocks the intramural ganglia by binding to the nicotinic receptor in 6 animals (cat A-F). We stimulated each electrode on the bladder (monopolar stimulation) at 1, 2, and 4 mA keeping frequency and pulse width constant at 30 Hz and 1 ms, respectively. Fig 5.6b depicts an example of stimulation evoked bladder pressure before and after the hexamethonium induction at stimulation amplitude 1, 2, and 4 mA. The stimulation evoked bladder pressure reduced after induction of hexamethonium at all the stimulation intensities.

We first examined the effect of hexamethonium on bladder pressure for each electrode across all amplitudes and cats (fig 5.6c). We calculated the pressure difference for each electrode before and after hexamethonium induction and found that the median difference in bladder pressure was significantly higher than 0 ($p = 2.2e-17$, Wilcoxon-signed rank test, fig. 5.6d) indicating that induction of hexamethonium led to decrease in bladder pressure. Next, we wanted to see if this difference in bladder pressure was affected by stimulation amplitude and found that there was a significant difference ($p=0.005$, Friedman test, fig. 5.6e). There was no significant difference between 1 mA and 2 mA (adjusted $p=0.9$, Wilcoxon signed rank test), however significant difference was found between pressure changes between 2 mA and 4 mA (adjusted $p=0.0024$, Wilcoxon signed-rank test).We also performed a LMM analysis and found that induction of hexamethonium and amplitude has significant effect on bladder pressure (fig. 5.5c, Appendix Table B8).The interaction term between drug and amplitude – the effect of the drug taking into consideration stimulation amplitude – was not significant (Appendix Table B8), inconsistent with our finding above and could be due to high variance in the data due to different

electrode sites on the bladder surface. Lastly, cat identity was not a significant factor (Appendix Table B8).

We hypothesized that stimulation-evoked bladder pressure is mediated by mixed activation pre-ganglionic and post-ganglionic fibers. Overall, these data suggest support for our hypothesis in 2 ways. First, after the hexamethonium induction, functionally meaningful pressures could still be generated. Second, at higher amplitude, the difference in pressure after hexamethonium induction was lower than that of 1 mA and 2 mA. This could indicate, higher recruitment of post-ganglionic fibers.

We also compared bladder pressures evoked by stimulating the pelvic nerve at different stimulus intensities (amplitude: 0.1, 0.2, 0.4 mA, frequency: 30 Hz, pulse width: 1 ms). Although not statistically significant (n=5), similar results were found where the bladder pressures were reduced after hexamethonium induction at all the stimulus intensities (figure 5.9b).

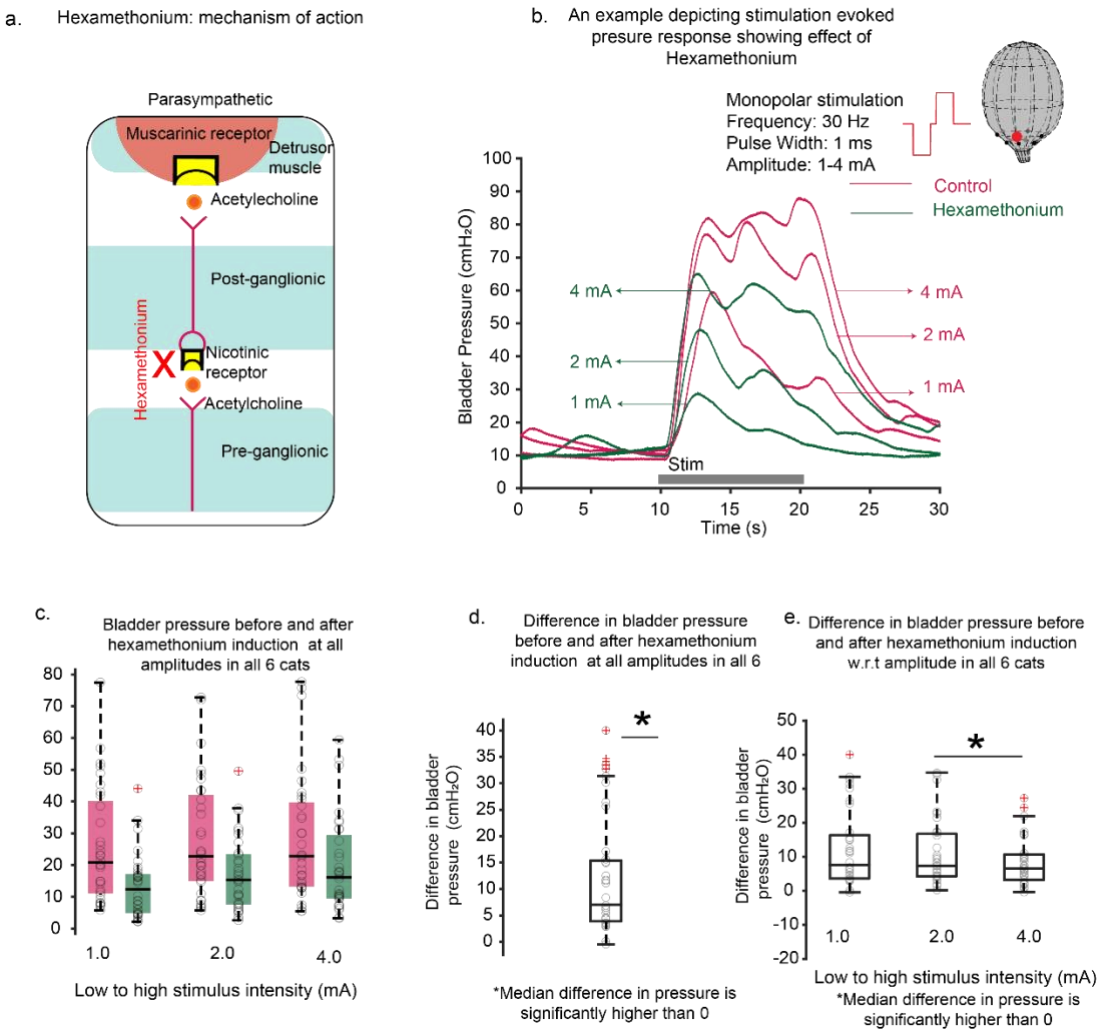

Figure 5.6 Effects of hexamethonium on bladder pressure *:* a) Illustration of hexamethonium mechanism of action. b) An example depicting stimulation-evoked pressure response showing effects of hexamethonium at different amplitudes. c) Comparison of stimulation-evoked pressures before and after hexamethonium induction at different stimulation amplitudes in response to monopolar stimulation in all cats. d) Difference in stimulation-evoked pressures before and after hexamethonium induction in response to monopolar stimulation across all amplitudes and all cats. e) Differences in bladder pressure following hexamethonium induction w.r.t. amplitudes.

5.4.3.3 Effects of Propranolol

Since the bladder wall consists of excitatory and inhibitory neurons, we wanted to know if DBWS influences inhibitory neurons. To evaluate that, we induced propranolol which blocks the adrenergic receptor binding site for norepinephrine, blocking the inhibitory neuronal input to the detrusor muscle in 5 animals (cats A, B, C, E, F). We stimulated each electrode on the bladder (monopolar stimulation) at 1, 2, and 4 mA keeping frequency and pulse width constant at 30 Hz and 1 ms, respectively. Fig 5.7b depicts an example of stimulation evoked bladder pressure before and after the propranolol induction at stimulation amplitude 1, 2, and 4 mA. The stimulation-evoked bladder pressure increases after induction of propranolol at all the stimulation intensities. We calculated the difference between bladder pressure before and after induction of propranolol as a metric of evaluating the effect of nerve transection.

We first examined the effect of propranolol on bladder pressure for each electrode across all amplitudes and cats (fig 5.7c). We calculated the pressure difference for each electrode before and after propranolol induction and found that the median difference in bladder pressure was significantly higher than 0 ($p = 1.2e\text{-}12$, Wilcoxon-signed rank test, fig. 5.7d) indicating that induction of propranolol led to increase in bladder pressure. Next, we wanted to see if this difference in bladder pressure was affected by stimulation amplitude and found that there was no significant difference ($p=0.962$, Friedman test, fig. 5.7e). We also performed an LMM analysis and found that induction of propranolol and amplitude has a significant effect on bladder pressure. In this case, the significant effect of stimulation refers to the fact that increasing the stimulation amplitude itself increased the bladder pressure with and without propranolol induction (fig. 5.7c, Appendix Table B9). The interaction term between drug and amplitude – the effect of the drug taking into consideration stimulation amplitude – was not significant (Appendix Table B9),

consistent with the result in fig. 5e. Lastly, cat identity was not a significant factor (Appendix Table B9).

The goal of this experiment was to see if DBWS was also recruiting inhibitory fibers in the bladder wall. Although there was a significant increase in bladder pressure after propranolol induction which would indicate that we were in fact stimulating inhibitory neurons as well, but to support this claim, we would expect a higher difference in bladder pressure at a higher amplitude. Even with pelvic nerve stimulation (fig. 5.9c), the bladder pressure was increased after propranolol induction but not as high as bladder wall stimulation. Hypogastric nerve regulates the bladder compliance and blocking adrenergic transmission in general increased the pressure. Also, it is a possibility that we were also stimulating inhibitory neurons in the bladder wall. We can't make a substantial claim with this approach and these data that weather the change in bladder pressure was due to deactivation inhibitory neurons in the bladder wall or the overall bladder compliance. But it seems that it was an effect of both.

Although not statistically significant (n=4), similar results were found where the bladder pressures were increased after propranolol induction at all the stimulus intensities (figure 5.9c).

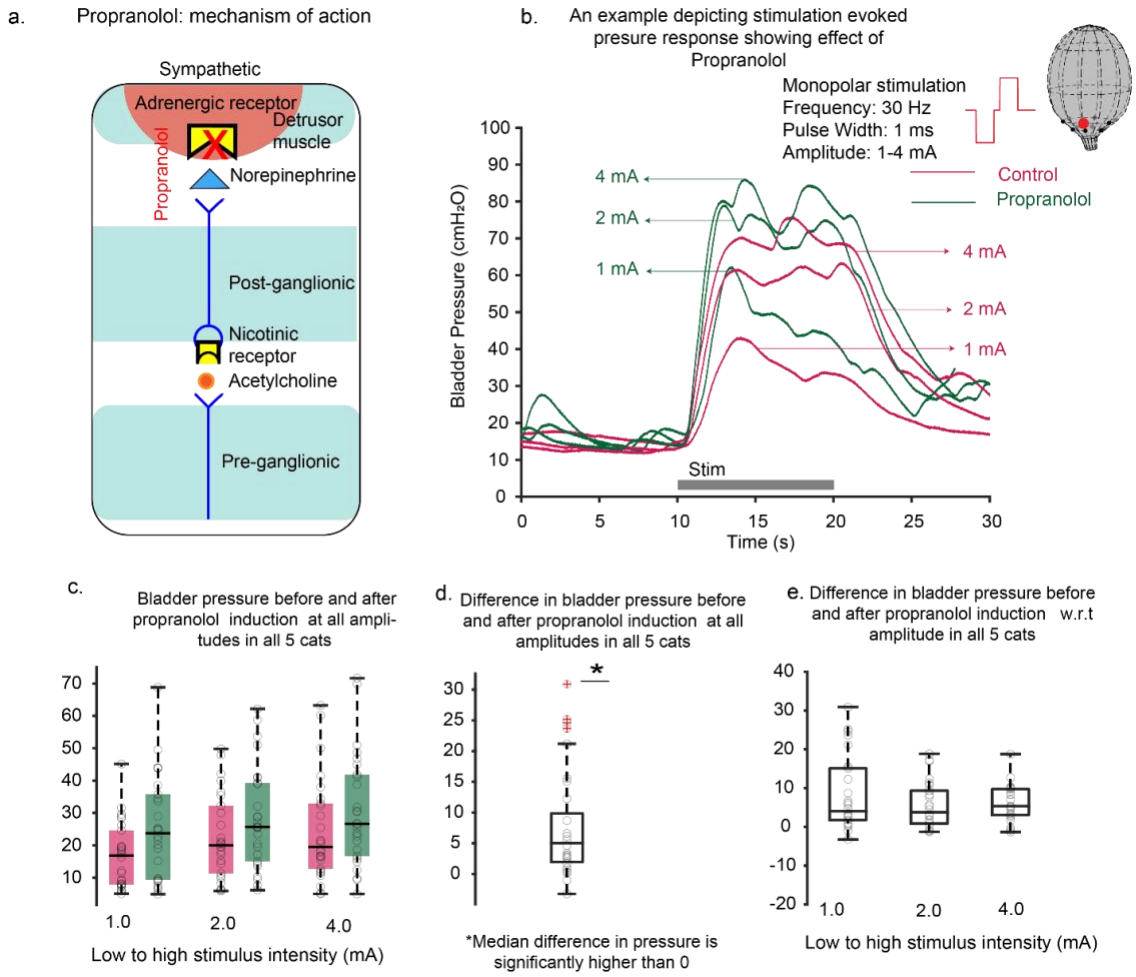

Figure 5.7 Effects of propranolol on bladder pressure a) Illustration of propranolol mechanism of action. b) An example depicting stimulation-evoked pressure response showing the effects of propranolol at different amplitudes. c) Comparison of stimulation-evoked pressures before and after propranolol induction at different stimulation amplitudes in response to monopolar stimulation in all cats. d) Difference in stimulation-evoked pressures before and after propranolol induction in response to monopolar stimulation across all amplitudes and all cats. e) Differences in bladder pressure following propranolol induction w.r.t. amplitudes.

5.5 Discussion

Micturition reflexes are suppressed in isoflurane anesthesia and are known to facilitate only the local reflexes within the bladder[92]. This preparation is useful to evaluate the bladder and urethral activity in isolation from influences of the central nervous system. On the other hand, α-chloralose has been used to evaluate the entire micturition reflex comprising the bladder, urethra, spinal cord, and brainstem [107,108]. Our study assessed the effects of varying stimulus intensities of bladder wall stimulation in isoflurane and α-chloralose anesthesia and found that central-mediated reflexes play a significant role in producing the voiding behavior. The α-chloralose preparation resulted in higher contraction amplitude than Isoflurane, which was consistent in single-pair and sequential electrode configurations. While it is understood that bladder wall stimulation under Isoflurane continued to produce an effect in the absence of reflexes, it is tempting to argue that pelvic-to-pelvic or pelvic-to-pudendal reflexes within the sacral spinal cord might have contributed to increased function in α-chloralose preparation.

Further, since all our stimulation trials were performed at isovolumetric bladder condition with an occluded urethra, increased contraction amplitude might arise from an excitatory pelvic-to-pelvic reflex[109,110] rather than contributions from flow-sensitive afferents from the urethra[14]. In conclusion, active input from the spinal centers during bladder wall stimulation under α-chloralose anesthesia ultimately facilitates higher contraction amplitude. These reflexes were further investigated in experiments involving nerve transections and pharmacology.

The pelvic nerve is primarily responsible for transducing the contraction of the bladder wall. Since all of our understanding of pelvic reflexes are mainly from peripheral nerve stimulation experiments[15,42,103,111,112], we were interested in evaluating the role of these pelvic reflexes in DBWS. A series of nerve transection experiments initially involved pelvic nerve transection.

Unsurprisingly, pelvic nerve-mediated responses were the primary drivers of stimulation-evoked increases in bladder pressure across varying stimulus amplitudes in nerve-intact controls. This was confirmed by decreased contraction amplitude at lower stimulation amplitudes observed after bilateral pelvic nerve transections in 3 cats but were similar at higher stimulation amplitudes. Changing the stimulation electrode configuration from single pair electrode to multi-electrode sequential stimulation or varying the stimulation amplitude did not change the pattern of decrease we observed after pelvic nerve transections. The pelvic nerve facilitates a pelvic-to-pelvic reflex that is primarily excitatory to bladder function. A plausible explanation to the decreased functional outcome is that this excitatory reflex was abolished after nerve transection. There is evidence of local pelvic ganglia-mediated reflexes that are excitatory in nature[39,40,103]; however, our investigation did not look at dissecting the pelvic ganglia and pelvic spinal reflexes separately. Hence, it is understood from this limited yet simple approach that pelvic nerve-mediated spinal or local reflexes contribute to some part of the increase in the functional outcome of bladder wall stimulation.

To further ascertain the role of pelvic nerve reflexes and the contribution of reflexes from the pudendal nerve, which are responsible for excitatory and inhibitory urethral-to-bladder reflexes, we initially transected the pudendal nerve followed by the pelvic nerve. If the role of pudendal reflexes in bladder wall stimulation was significant, transection of the pudendal nerve in the first step would increase or decrease in contraction amplitude. However, the transection of bilateral pudendal nerves did not exhibit significant changes in bladder pressure, even at higher stimulus intensities. This was followed by a transection of the pelvic nerves, which exhibited the same decrease at low stimulation amplitudes in pressure as previously discussed. While these results further ascertain the role of pelvic nerve reflexes in contributing to increases in bladder

pressure, it also eliminates the pudendal reflexes from the picture. It can be argued that our experimental setup is entirely based on isovolumetric bladder preparations where pudendal reflexes are absent. This might be true for excitatory reflexes, where flow-sensitive afferents help sustain bladder contraction. However, inhibitory reflexes such as the guarding reflex are active under the isovolumetric condition where urethral flow is not required[112,113]. In conclusion, it is evident from our transection experiments that somatic components of the urethra, mediated by the pudendal nerve, do not play a significant role in bladder wall stimulation compared to the pelvic nerve, which facilitates an excitatory response leading to an increased functional outcome. While the complete transection of the pelvic nerve does not abolish the stimulation-evoked responses in our trials, it significantly decreases the response at lower stimulus intensities where transduction of the contraction is due to sensory neural structures.

Parasympathetic postganglionic axons in the pelvic nerve release acetylcholine in the neuromuscular junction of the bladder wall. Hence, cholinergic transmission is the primary neurotransmitter mechanism by which bladder wall contraction occurs. While this is true in healthy bladders, other neurotransmitter mechanisms, such as purinergic, are often upregulated in pathological conditions. We set out to ascertain if the cholinergic mechanism remains the primary driver of the stimulation-evoked increase in bladder pressure. Atropine, a muscarinic receptor antagonist, was chosen to study the effect of muscarinic receptor blockade on stimulation-evoked responses. Similar to pelvic nerve transection experiments, muscarinic receptor blockade eliminated most of the responses to bladder wall stimulation rendering it ineffective for generating voiding-like reflexes. Unlike the transection experiments, the pressure generated post-blockade was minimal (~ 5cmH_2O). While this is not surprising, it is safe to conclude that the presence of muscarinic receptors is crucial for bladder wall stimulation to work effectively. Upregulation and

increased sensitivity of muscarinic receptors are one of the primary drivers of bladder activity in pathological conditions such as spinal cord injury, diabetes mellitus, and overactive bladder[114,115]. Hence, our neuromodulation technique's translational significance might rely on the health of muscarinic receptors in people who experience the pathologies mentioned above.

The increased sensitivity to electrical stimulation around the ureterovesical junction influenced the distribution of stimulation electrodes on our electrode nets. We hypothesized that this sensitivity was partly driven by the density of muscarinic fibers and intramural ganglia within the bladder smooth muscle. To evaluate this idea within our experimental setup, we introduced hexamethonium, a ganglionic blocker, to study the effect of ganglionic transmission on muscarinic activity. Since we only tested the highest dose reported for complete blockade[116–118], we report an interesting trend across stimulus intensities post-blockade (fig.5.6d). The greatest decrease in stimulation-evoked contraction amplitude was noticed at the lowest stimulus intensity (~ 1mA) with an increasing trend at higher stimulus intensities (2-4 mA). Such an effect is possible only when different mechanisms drive functional outcomes at varying stimulus intensities. While it is understood that neurogenic and myogenic responses together drive contraction of the smooth muscle layers within the bladder wall, this difference in response across varying stimulus intensities indicates that lower currents tend to rely on neural structures to transduce and sustain bladder contraction, probably making them the most vulnerable to a ganglionic blocker. However, at higher currents, it is perhaps the myogenic structures or postganglionic efferent fibers which are coactivated, rendering them the least vulnerable to a ganglionic blocker like hexamethonium. While some electrodes produced the greatest reduction in stimulation-evoked response, other electrodes remained unaffected by hexamethonium. Hence, the pharmacological investigations in these experiments suggest that bladder wall stimulation work through a mixture of muscarinic

receptors mediated by the pelvic nerve relaying through the intramural ganglia of the bladder wall, and bladder contractions can still be generated at high stimulus intensities in the absence of the ganglionic transmission.

A series of pharmacological and transection experiments involving muscarinic and ganglionic blockers along with pelvic and pudendal transection resulted in delineating the mechanism of action by which bladder wall stimulation results in complete emptying of the bladder. While this effort focused on somatic (pudendal components) and parasympathetic (pelvic nerve components), we were curious if adrenergic components within the bladder wall contributed to the generation of bladder function during bladder wall stimulation. Hence, we used a reversible adrenergic blocker, propranolol, in our study. Propranolol reversibly induced overactivity of the detrusor resulting from the loss of inhibitory input from the adrenergic components within the bladder wall. However, propranolol did not affect the voiding reflex generated by bladder wall stimulation, evident from no effect of stimulus intensity in percentage increase in bladder pressure from propranolol.

Our study delineates the role of muscarinic receptors and the neural apparatus involved in transducing the contraction of the bladder during bladder wall stimulation with our novel electrode nets neural interface for DBWS.

5.6 Supplementary Figures

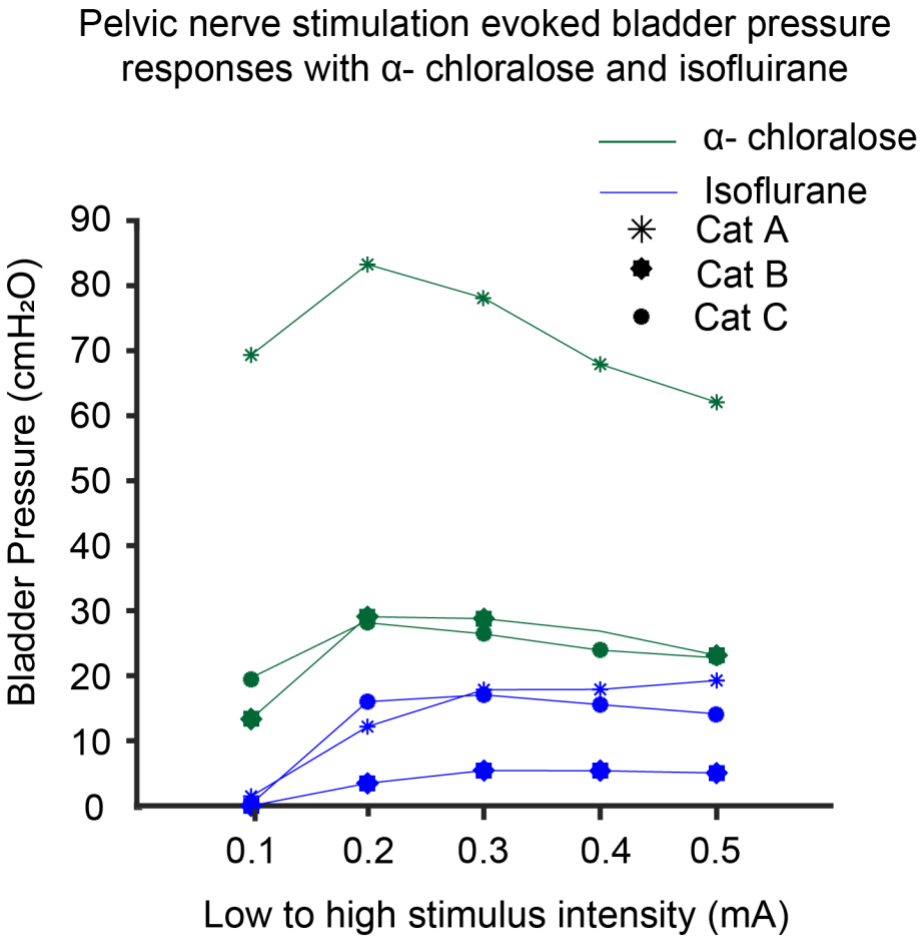

Figure 5.8 Comparison of Pelvic nerve stimulation evoked bladder pressures after Isoflurane and α-chloralose

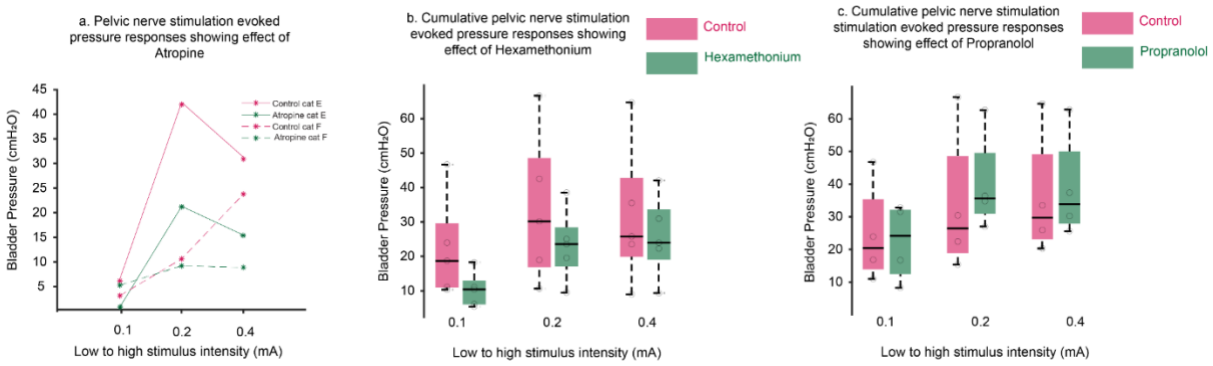

Figure 5.9 Comparison of stimulation evoked bladder pressures upon Pelvic nerve stimulation before and after inducing atropine, hexamethonium, and propranolol a) Stimulation evoked pressure responses at different stimulation amplitudes with control, and after atropine induction in response to pelvic nerve stimulation b) Stimulation evoked pressure responses at different stimulation amplitudes with control and after hexamethonium induction in response to pelvic nerve stimulation c) Stimulation evoked pressure responses at different stimulation amplitudes with control and after propranolol induction in response to pelvic nerve stimulation.

6.0 Summary of Results and Future Work

This dissertation was aimed at developing a novel neural interface to improve bladder function. Bladder function is affected by many pathological conditions such as spinal cord injury, multiple sclerosis, Parkinson's disease, and peripheral neuropathies[2,3,8,119,120]. We targeted scenarios that are characterized by a bladder that is underactive or atonic. Despite being one of the most significant, but often unrecognized consequences of these diseases or injuries, the current standard solution for bladder emptying is intermittent catheterization, which has significant side effects[5]. Electrical stimulation of the nervous system has been used to intervene at different locations in the lower urinary tract to generate bladder emptying[10] and there have been numerous demonstrations of successfully emptying the bladder in both animal and human studies. However, clinical translation of systems that reliably empty the bladder are virtually nonexistent. Here, we aimed to revive an old technique referred to as direct bladder wall stimulation (DBWS), which was actively attempted from 1960-2000[46,121]. Despite successful animal and human studies, efforts were discontinued due to a variety of technical and physiological.

6.1 Dissertation Summary

The thesis was briefly introduced in chapter 1 and a review of the relevant anatomy and physiology of the lower urinary tract was provided in chapter 2. In chapter 3, we reviewed the history of DBWS and identified three specific challenges that have prevented the technique from being more successful. First, current spread caused direct and reflexive activation of urethral

structures, activation of the legs, and co-recruitment of other pelvic organs. This current spread arises from a number of factors including electrode design, electrode size, electrode placement on the bladder, and the high electrical stimulation amplitude commonly required for DBWS. Second, the placement of electrodes on the detrusor muscle itself was challenging due to its unique structure and dynamic function, leading to displacement of electrodes and damage to the tissue. Third, injuries that lead to hyperreflexia in the external urethral sphincter and pelvic floor, such as suprasacral spinal cord injury, complicate DBWS as stimulation can more easily lead to direct or indirect increases in outlet pressure, preventing complete bladder emptying.

In chapter 4, we aimed to design a stretchable soft silicone electrode net that could be placed on the bladder and could conform to the bladder during volume changes. We started by electrically stimulating different bladder areas and measuring bladder pressure. We found that the bladder base was the most sensitive to electrical stimulation and that electrodes confined to the base of the bladder were sufficient to generate robust bladder contractions. We postulate that this was due to a high density of postganglionic neurons at the bladder base that require lower stimulation amplitude to be effective compared to stimulating the detrusor muscle directly. Since the electrodes were limited to the base of the bladder and stimulation amplitudes were low, co-activation of nearby tissue structures were also reduced. We further evaluated if we could use bipolar stimulation to reduce the co-activation of nearby tissue structures and found that activation of the external urethral sphincter, pelvic floor, abdominal muscles, and legs were significantly reduced. However, even monopolar stimulation using the small electrode sizes and electrode-tissue contact afforded by the novel electrode design generated off-target muscle activity that was not necessary functionally relevant. Based on these findings we designed a stretchable soft silicone mesh that could be placed around the bladder body to anchor the electrodes directly with the base

of the bladder. We believe that this design overcomes the technological limitations of historical DBWS attempts.

In chapter 5, we implanted this optimized neural interface in healthy, behaving cats to evaluate its performance in permanent implants. Even though the potential application of DBWS are to restore bladder function in pathological conditions where the bladder is underactive or atonic, testing in healthy and awake animals offers the advantage of studying 1) whether DBWS can evoke bladder pressures and voiding in unanesthetized animals, 2) intact sensory reflexes and their role in voiding during DBWS, 3) conscious sensations evoked by DBWS, and 4) the longevity of this neural interface. We found that DBWS via soft silicone electrode nets could evoke voiding in both anesthetized and awake behaving trials for the duration of the implant (3-4 months). Two cats had to be terminated prior to the expected time points; the first was due to the external surgical complications leading to an exposed wound after two months and the second was due to the failure of the electrode array itself. Specifically, the electrode failure resulted from a bond break where the soft silicone conductive polymer connects to the stainless steel extension wire. However, in all the cats, the electrode positions were not changed from when it was implanted. This demonstrates the positional stability of this neural interface, in contrast to previous electrode designs where electrode displacement due to repeated changes in bladder volume was a common problem. Direct bladder wall stimulation evoked comfortable and complete voiding in these animals when then were both anesthetized and awake for the duration of the implant. Comfortable voiding in awake, behaving animals supports the argument that co-activation of nearby tissue structures was limited with this interface. Interestingly, data from awake, behaving trials indicated a significant role for sensory reflexes through DBWS, presumably through activation of the pelvic nerve afferents. This

suggests that the stimulation through these electrodes could recruit the sensory fibers conveying a sense of fullness to the brain.

In chapter 6, we examined the neural mechanisms by which DBWS generates bladder contractions. Using different anesthesia, we found that active input from the spinal centers during bladder wall stimulation under α-chloralose anesthesia ultimately facilitates higher contraction amplitude, indicating the role of spinal reflexes. However, robust bladder contractions could be generated at higher stimulation amplitudes even with isoflurane, which suppresses spinal reflexes. These reflexes were further investigated in experiments involving nerve transections and pharmacological manipulation. Unsurprisingly, pelvic nerve-mediated responses were the primary drivers of stimulation-evoked increases in bladder pressure across varying stimulus amplitudes in nerve-intact controls. This was confirmed by decreased contraction amplitude at lower stimulation amplitudes observed after bilateral pelvic nerve transection in 3 cats; however, contraction amplitudes were similar at higher stimulation amplitudes. Since a pelvic-to-pelvic reflex exists that increases activity in pelvic efferents, a plausible explanation for the decreased functional outcome is that this excitatory reflex was abolished after nerve transection. However, even without this excitatory reflex, robust bladder contractions could be generated. To further ascertain the role of pelvic nerve reflexes and the contribution of reflexes from the pudendal nerve, which are responsible for both excitatory and inhibitory urethral-to-bladder reflexes, we initially transected the pudendal nerve followed by the pelvic nerve. If the role of pudendal reflexes in bladder wall stimulation was significant, transection of the pudendal nerve first would increase or decrease in contraction amplitude. However, transection of the pudendal nerves bilaterally had no significant effect on bladder pressure. This was followed by the transection of the pelvic nerves, which exhibited the same decrease at low stimulation amplitudes as observed previously. These data

suggest that DBWS at the bladder base can generate robust contractions in the absence of central reflexes, such as in spinal cord injury (supraspinal reflex component), peripheral neuropathies, and other neurodegenerative diseases leading to the underactive bladder[119,122,123].

Using pharmacological agents, we also dissected the neuronal populations activated through DBWS. Using atropine, we blocked transmission at the neuro-muscular junction. As expected, this led to a significant decrease in bladder pressure, indicating that DBWS primarily acts by recruiting axons that ultimately activate the detrusor muscle, rather than by direct activation of muscle cells themselves. Since the bladder wall contains intramural ganglia, a high density of which are at the bladder base, we wanted to know if DBWS was activating preganglionic or postganglionic efferents. We delivered hexamethonium, which blocks ganglionic transmission, and observed a decrease in bladder pressure. However, DBWS continued to generate bladder contractions, demonstrating that stimulation activates a mixed population of pre and postganglionic efferent fibers. This result indicates that bladder wall stimulation can be used to generate bladder contractions even if the preganglionic innervation of the bladder is damaged.

6.2 Conclusion and Future Work

Overall, this dissertation revists the concept of direct bladder wall stimulation and develops a soft-silicone electrode nets as a novel interface that overcomes many of the significant limitations present in earlier designs. Nevertheless, this neural interface has some remaining, such as a weak bond between the biocompatible conductive polymer and metal lead wire, as wellas the highly variable resistance in the conductive ink. Another major challenge to any translational effort will be surgical deployment of the device. Ideally, a minimally invasive approach could be developed

as the studies here involved complete exposure of the bladder, which is highly invasive surgery. Although this device is not yet ready for clinical translatability, the concepts developed here fill a significant gap in the literature on DBWS. This systematic approach and data could be leveraged to inform the design of a translatable neural interface for DBWS. After further optimizing the electrodes themselves, the next most important effort will be to deploy these electodes in pathological conditions resulting in underactive bladder to understand the future scope of DBWS. Particular attention should be directed towards monitoring and minimizing off-target activation in these experiments to confirm the selectivity that was observed with these novel electrodes. With continuously evolving technological advancements, the development of flexible electronics, and electrode designs, we believe DBWS could be a successful neurostimulation intervention capable of eliminating the need for intermittent catheterization.

Appendix A : Supplementary Statistics for Chapter 4

Appendix Table A1: X-strip

		P-value
Fixed factors	Location	0.033
	Amplitude	0.001
	Location*Amplitude	0.117
Random factor	Cat	0.715
Dependent variable	Bladder pressure	-

Appendix Table A2: X-strip with respect to aspects

		P-value
Fixed factors	Aspect	0.118
Random factor	Cat	0.647
Dependent variable	Bladder pressure	-

Appendix Table A3: Effect of frequency

		P-value
Fixed factors	Frequency	0.001
	Amplitude	0.001
	Frequency*Amplitude	0.003
Random factor	Cat	0.445
Dependent variable	Bladder pressure	-

Appendix Table A4: Measure of Current spread (pressure)

		P-value
Fixed factors	Amplitude	0.193
	StimType	0.137
	StimType*Amplitude	0.793
Random factor	Cat	0.350
Dependent variable	Bladder pressure	-

Appendix Table A5: Measure of current spread (EUS)

		P-value
Fixed factors	Amplitude	0.644
	StimType	0.769
	StimType*Amplitude	0.814
Random factor	Cat	0.855
Dependent variable	EUS EMG MAV	-

Appendix Table A6: Measure of current spread (EAS)

		P-value
Fixed factors	Amplitude	0.001
	StimType	0.001
	StimType*Amplitude	0.001
Random factor	Cat	0.382
Dependent variable	EAS EMG MAV	-

Appendix Table A7: Measure of current spread (Gluteal muscle)

		P-value
Fixed factors	Amplitude	0.502
	StimType	0.024
	StimType*Amplitude	0.677
Random factor	Cat	0.719
Dependent variable	Gluteal muscle EMG	-

Appendix Table A8: Instrumented net

		P-value
Fixed factors	Amplitude	0.208
Random factor	Cat	0.544
Dependent variable	Bladder pressure	-

Appendix B : Supplementary Statistics for Chapter 5

Appendix Table B1: Effects of Anesthesia (monopolar or bipolar stimulation)

		P-value
Fixed factors	Anesthesia	0.001
	Amplitude	1.0
	Anesthesia*Amplitude	1.0
Random factor	Cat	0.326
Dependent variable	Bladder pressure	-

Appendix Table B2: Effects of Anesthesia (sequential stimulation)

		P-value
Fixed factors	Anesthesia	0.001
	Amplitude	0.030
	Anesthesia Transection*Amplitude	0.912
Random factor	Cat	0.332
Dependent variable	Bladder pressure	-

Appendix Table B3: Effects of pelvic nerve transection (monopolar or bipolar stimulation)

		P-value
Fixed factors	Transection	0.001
	Amplitude	0.001
	Transection*Amplitude	0.489
Random factor	Cat	0.332
Dependent variable	Bladder pressure	-

Appendix Table B4: Effect of pelvic nerve transection (sequential stimulation)

		P-value
Fixed factors	Transection	0.001
	Amplitude	0.001
	Transection*Amplitude	0.489
Random factor	Cat	0.332
Dependent variable	Bladder pressure	-

Appendix Table B5: Effects of pudendal nerve transection (monopolar or bipolar stimulation)

		P-value
Fixed factors	Transection	0.077
	Amplitude	0.001
	Transection*Amplitude	0.896
Random factor	Cat	0.491
Dependent variable	Bladder pressure	-

Appendix Table B6: Effects of pudendal nerve transection (sequential stimulation)

		P-value
Fixed factors	Transection	0.072
	Amplitude	0.001
	Transection*Amplitude	0.763
Random factor	Cat	0.482
Dependent variable	Bladder pressure	-

Appendix Table B7: Effects of atropine (monopolar stimulation)

		P-value
Fixed factors	Drug	0.001
	Amplitude	0.466
	Drug*Amplitude	0.441
Random factor	Cat	0.504
Dependent variable	Bladder pressure	-

Appendix Table B8: Effects of hexamethonium (monopolar stimulation)

		P-value
Fixed factors	Drug	0.001
	Amplitude	0.047
	Drug*Amplitude	0.539
Random factor	Cat	0.122
Dependent variable	Bladder pressure	-

Appendix Table B9: Effects of propranolol (monopolar stimulation)

		P-value
Fixed factors	Drug	0.001
	Amplitude	0.047
	Drug*Amplitude	0.539
Random factor	Cat	0.122
Dependent variable	Bladder pressure	-

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