Determining recruitment patterns of continence and micturition reflexes in response to multichannel microstimulation of the sacral dorsal root ganglia.

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Background

Urinary tract complications are a significant problem in spinal cord injury (SCI) patients due to loss of descending control from the brain stem. The most common reason for hospitalization for those living with SCI is from urinary tract infections due to the limitations of existing clinical methods to control or manage bladder function, which greatly contributes to patient healthcare expenses. Social issues surrounding incontinence and voiding control can also lead to decreased quality of life in SCI patients causing these patients to desire improvements in bladder care [1]. Bladder function in SCI patients is a well-researched subject that has led to several promising approaches to improved function through neurotechnologies, but few of these approaches have led to clinical success [2]. Recent approaches to improving bladder control through electrical stimulation have focused on stimulation of sensory pathways, primarily in the pudendal nerve. However, accessing the peripheral pudendal nerve pathways specific to the population of axons required to achieve coordinated bladder function has been proven to be difficult. In order to address this limitation, the overall strategy adopted in the proposal is to enable selective and coordinated activation of pelvic and pudendal afferents by microstimulation through multichannel microelectrode arrays implanted in the sacral dorsal root ganglia (DRG). Other difficulties that occurred in studies surrounding electrical stimulation of the pudendal nerve was the failure to fully activate voiding reflexes as problems occur from a lack of sufficient sensory input to the cord from the bladder (through the pelvic nerve) resulting in incomplete voiding [3]. This may possibly be overcome by electrical stimulation of the pelvic nerve during stimulation of the voiding reflex in the pudendal nerve. In addition to micturition reflexes, understanding continence reflexes are pivotal to developing practical clinical electrical stimulation devices and improving quality of life in SCI patients. Bladder contractions can be suppressed through stimulation of the dorsal nerve of the penis (branch of the pudendal) [4, 5]. We believe that micturition and continence reflexes can be controlled through simultaneous and selective electrical stimulation of reflex pathways travelling through the sacral DRG. By focusing on single channel microstimulation of the sensory afferents from both the pelvic and pudendal nerves in the sacral DRG, we can isolate and locate the specific neurons involved in micturition and continence reflexes. Using coordination of these located single channels involved in the reflexes, we believe that it will be possible to restore normal micturition and continence reflexes through multichannel stimulation. Electrical microstimulation can be clinically effective at improving quality of life and lowering long term healthcare costs in SCI patients if a better design can be implemented to restoring bladder control.

AIM 1: Identify the patterns of the lower urinary tract (LUT) afferent recruitment resulting from microstimulation of the sacral dorsal root ganglia.

The purpose of Aim 1 is to characterize the patterns of peripheral nerve pathways that can be selectively activated through microstimulation of the sacral DRG. Using nerve cuffs placed on different peripheral nerve branches associated with LUT function, we will be able to determine which nerves are activated and which infer with an associated physiological function based on the identity of the nerve.

Hypothesis H1.1: Microstimulation through individual electrodes in the sacral DRG will selectively recruit afferents in isolated peripheral nerve branches at threshold amplitudes.

Methods & Analysis Plan

All experimental protocols have been approved by the University of Pittsburgh’s Institutional Animal Care and Use Committee. Detailed surgical procedures and experimental methods common to the aims were described in previous research [6]. All surgical procedures will be under isoflurane anesthesia. After a sacral laminectomy, microelectrode arrays (32-50 channels per device, 250 µm or 400 µm interelectrode spacing) will be implanted into the S1-S3 DRG unilaterally. Nerve cuff electrodes will also be placed on the pelvic nerve, pudendal nerve, cranial sensory nerve, dorsal penile nerve, caudal rectal nerve, and deep perineal nerve, see Figure 1 [7, 8]. An
additional nerve cuff will be placed on the sciatic nerve to act as a control for recruitment of hindlimb afferents. Fine wire EMG electrodes will be placed in the external urethral and anal sphincters and bladder pressure will be monitored using a catheter placed through the dome of the bladder. After surgical procedures are completed under isoflurane anesthesia, experiments for this aim will be conducted under alpha-chloralose anesthesia to avoid suppression of spinal reflexes. Monitoring the physiological responses is not the goal of Aim 1, however, this data will be used to help guide stimulus parameter selection for Aim 2. Microstimulation will initially be delivered through individual DRG electrodes, and stimulus amplitudes will range from 1 to 30 µA. The electroneurogram (ENG) will be recorded from all instrumented peripheral nerves using a low noise bioamplifier. 5-pole nerve cuffs, recorded as two tripoles, will be placed on the pelvic, pudendal and sciatic nerves to allow measurement of the conduction velocities [9] of the evoked compound action potentials (CAPs). Due to the fine caliber and short branch free lengths of the distal branches of the pudendal nerve, custom bipolar nerve cuff electrodes will be used. Stimulus-triggered averaging will be used to examine peripheral nerve activity for the presence of CAPs. Pulses will be delivered at 10 Hz to reduce the time required for data collection, but also allow sufficient interstimulus time for detection of CAP responses from slowly conducting afferents in distal nerve branches.

For H1.1 experiments, an automated stimulus generation and recording system from previous experiments [9] will be used. For every peripheral nerve pathway identified, determination of the presence or absence of a compound action potential will be recorded for every stimulation amplitude and electrode. This allows us to quantify the stimulation amplitude for the lowest threshold of these pathways, the nerves that are recruited at threshold, stimulation ranges and conduction velocities of compound action potentials. Whether single or multiple pathways are recruited at threshold will be recorded. Calculations from this data will determine the percent of stimulation electrodes that activate single pathways to determine “recruitment maps” for peripheral LUT pathways. The analysis will be performed during the experiments themselves.

**AIM 2: Test coordinated multichannel microstimulation of sacral DRG afferents on recruitment of storage and voiding reflexes.**

The results of AIM 1 will allow us to combine and coordinate the single channel microstimulation of sacral DRG afferents involved in the storage and voiding reflexes to elicit a control.

Hypothesis H2.1: Coordinated microstimulation of pelvic and pudendal afferents will elicit a voiding reflex that is independent of bladder volume and thus generate higher voided volumes than pudendal afferent microstimulation alone.

**Methods & Analysis Plan**

Alpha-chloralose anesthesia will be used for all functional testing as it has been demonstrated to lead to less suppression of bladder reflexes than inhaled anesthetics [10]. Experiments will be conducted in the same animals described in the Aim 1 experiments.

For H2.1 experiments, we will used the results from Aim 1 to determine the physiological response to coordinated microstimulation of the pelvic and pudendal nerves. Each trial will have measurements of bladder contraction amplitude and any voided volume. These will be run with stimulation of the different pudendal afferent pathways.
alone, and then in combination with the pelvic nerve. Nerve pathway stimulations will last 20 seconds, following which time voided volumes will be measured. After 2 minute rest saline infusion will begin to refill the bladder for the next trial. Voiding efficiencies will be calculated as the percentage of the initial bladder volume that was voided during the trial. A 20% initial volume will be used to test complete voiding. Paired t-tests will be performed for each of the physiological metrics to determine if the addition of pelvic nerve stimulation changes the response. To reduce free parameters stimulation of pudendal nerve pathways will only be used if they elicit bladder contraction and are selective at maximum amplitude. In each combination only threshold amplitude and maximum selective amplitude will be used. Stimulation frequencies will range from 1-20Hz, consistent with normal physiological range of bladder afferent firing rates [11, 12, 13].

**Rationale and Expected Results**

Understanding continence and micturition reflexes is extremely important in discovering new clinical solutions for bladder control and care in SCI patients. Technical innovation of multichannel microstimulation interfaces in the sacral DRG will be key for success in this project. New software and hardware has been developed to automatically determine recruitment thresholds and conduction velocities of peripheral nerves. This will allow determination of peripheral afferent neuron types that can be recruited and their associated stimulus thresholds. By using this technology we can identify in the sacral DRG the key axons of the pelvic and pudendal nerve responsible for the sensory innervation of the LUT. The antidromic stimulation involved in Aim 1 will allow us to isolate and identify those axons as well as learn about their thresholds and how different microstimulation will cause contraction or suppression of the micturition and continence reflexes. Previous research has shown that these afferent pathways may be vital for the widespread clinical implementation of electrical stimulation devices. Afferent pathways can be utilized to regulate bladder storage and voiding as shown in spinally intact cats [4, 5, 7] and not spinally intact cats [14, 15]. Aim 1 should allow selective activation of the patterns in peripheral nerve pathways in the LUT surrounding the micturition and continence reflexes for determination of their different recruitment thresholds. We expect recruitment thresholds will be at similar amplitudes.

We expect that multichannel stimulation of these reflex pathways at threshold will be selective allowing our desired outcomes. However there is a greater likelihood of non-selective activation when multiple channels are activated, but interleaved stimulus trains could eliminate this effect. Identifying the specific axons and thresholds needed for desired outcome (voiding and storage) will allow continuation to Aim 2. Aim 2 will focus on coordinated microstimulation of sacral DRG involved in the continence and micturition reflexes to elicit contraction and suppression when desired therefore artificially controlling bladder pressure. We believe this coordinated multichannel stimulation will allow the sensory aspects of bladder control to regulate voiding and continence of the bladder upon appropriate stimulation. Stimulation of afferent DRG fibers should have the same capacity to elicit the functional spinal and supraspinal reflexes as the peripheral nerves themselves as long as a sufficient population of sacral DRG afferents can be activated simultaneously and selectively. Stimulation of pudendal afferents elicits a reflexive bladder contraction only when the bladder volume is above a critical volume, which is signaled by stretch sensitive mechanoreceptors [16, 17]. This shows there are multiple channels involved in the contraction of the bladder, and coordinated stimulation can increase the pressure involved in voiding. Increased pressure will ensure completion of bladder emptying and stimulation of continence when voiding is not desired. We expect that selective and simultaneous multichannel microstimulation of sacral DRG afferents will elicit the same physiological responses as normal stimulation of the peripheral nerve in an individual without SCI. Stimulation of the pelvic and pudendal nerves should elicit greater control and complete voiding when compared to just stimulation of the pudendal nerve alone. Following success in these aims, research will be continued to test these results with a feline model of spinal cord injury.
References