

University of Pittsburgh DARPA Bridging the GAP Plus (BG+) Critical Design Review

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Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

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Program Manager: (b)(6) DARPA/BTO

Submitted by:

(b)(4); (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4); (b)(6)

Email: (b)(4); (b)(6)

1 Scientific Design – Preclinical Studies

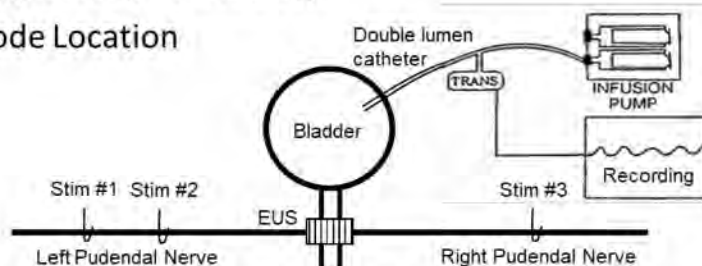
During the (b)(4) of this DARPA BG+ project, our preclinical studies using anesthetized cats (b)(4) have provided us with new information about how to restore bladder, bowel, and sexual functions after spinal cord injury (SCI) by electrical stimulation of pudendal nerves or sacral spinal roots. This information is briefly summarized below to support our current system design and technology development strategy.

1.1 Bladder Function

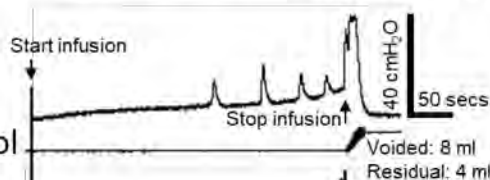
We have successfully demonstrated in anesthetized cats that pudendal nerve stimulation and block by (b)(4) electrodes can achieve a low-pressure (≤ 50 cmH₂O) voiding (see Fig.1). This success indicates that a minimally invasive surgical approach by inserting lead electrodes close to the pudendal nerves can be developed to restore bladder function after SCI. The data/results from this animal study are now published on Experimental Neurology, 2021; 346:113860 (see attachment).

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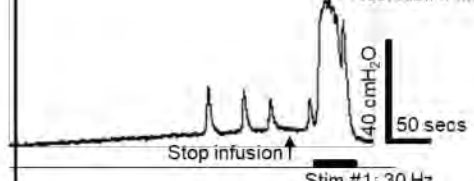
A. Electrode Location



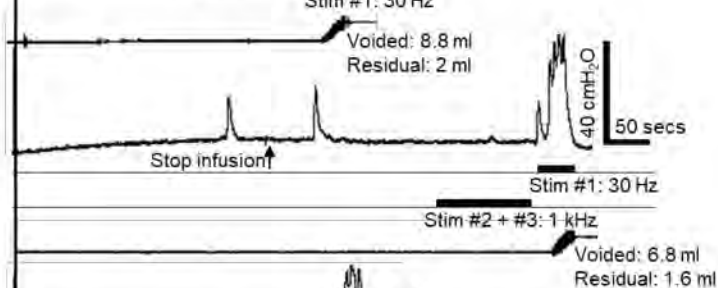
B. Control



C. 30 Hz



D. 30 Hz + 1 kHz



E. 30 Hz

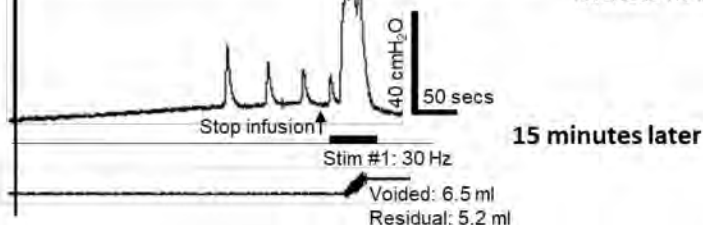


Fig.1: Low-pressure voiding induced by stimulation and 1 kHz post-stimulation block of axons in the pudendal nerves. A. Monopolar wire electrodes were placed on pudendal nerves bilaterally to deliver stimulations at three locations (#1, #2, #3). Stim #1 (30 Hz, 0.2 ms) was delivered to induce a reflex (b)(4) induced by infusion of saline into the bladder without stimulation. C. Stim #1 (30 Hz, 0.2 ms, 9 V) alone produced high-pressure (≈ 60 cmH₂O) voiding. D. When Stim #1 was applied immediately after Stim #2 and #3 delivered the 1 kHz stimulation (1.75 mA, 2 mins), it induced a low-pressure (≈ 40 cmH₂O) voiding. E. In 15 mins after the 1 kHz stimulation, Stim #1 again induced a high-pressure voiding. The black bar under the bladder pressure trace indicates the stimulation duration. Infusion rate = 2 ml/min.

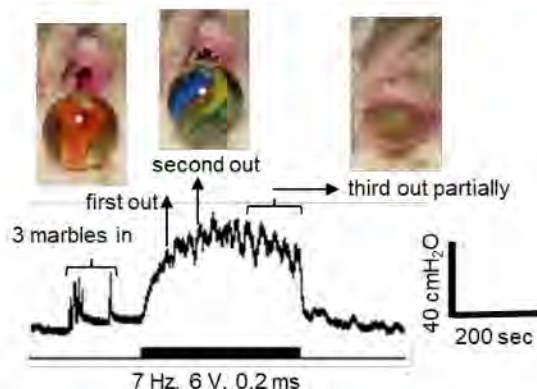
(b)(4)

1.2 Bowel Function

We have successfully demonstrated in anesthetized cats that stimulation of sacral S2 spinal root at 7 Hz is most effective to induce colon contractions, while stimulation of S1/S3 root or pudendal nerve is not effective. In addition, defecation is successfully induced by stimulation of S2 spinal root using a lead electrode (Fig.2). This success indicates that a minimally invasive surgical approach by inserting a lead electrode close to the S2/S3 spinal root in humans can be developed to restore bowel function after SCI. This study is now published on American Journal of Physiology - Gastrointestinal and Liver Physiology 2021; 321: G735-G742. (see attachment).

This preclinical study provides scientific evidence to

A. Cat #1



B. Cat #2

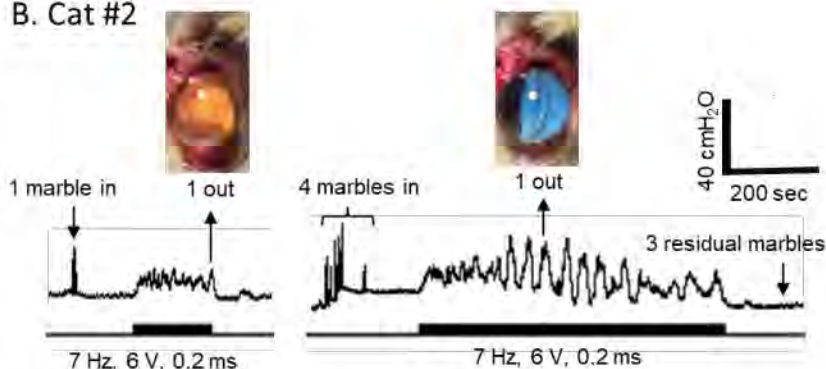


Fig.2: Defecation induced by stimulation of S2 ventral and dorsal roots. A. In cat #1 after inserting three marbles into the rectum, the defecation induced by sacral S2 root stimulation eliminated 2 marbles after 1 minute and 2 minutes of stimulation, respectively, with the last marble partially evacuated during the last 2 minutes of the 6-minute stimulation. B. In cat #2 after inserting 1 marble into the rectum, it was defecated after 2.5 minutes of stimulation. After inserting 4 marbles, stimulation of 11 minutes produced defecation of 1 marble at 4.5 minutes with 3 residual marbles in the rectum. Colon contraction pressure was measured by a large condom catheter with the condom covering the proximal and distal colon.

(b)(4)

(b)(4)

The minimally invasive surgical approach to insert a lead electrode close to the S2/S3 spinal root is routinely used in clinical application to treat overactive bladder by sacral neuromodulation, i.e., the InterStim device (Medtronic, Inc).

electrode for restoring bowel function after SCI.

(b)(4)

1.3 Sexual Function

We have successfully demonstrated in anesthetized male cats that stimulation of sacral S1/S2 spinal root at (b)(4) is most effective to induce the largest (>150 cmH₂O) increase in penile pressure resulting in a strong penile erection (Fig.3), while stimulation of S3 root or pudendal nerve is not effective. This success indicates a minimally invasive surgical approach by inserting a lead electrode close to the S1-S3 spinal root in humans can be developed to restore erectile function after SCI. The data/results from this animal study is now submitted for publication on Neuromodulation: Technology at the Neural Interface (see attachment).

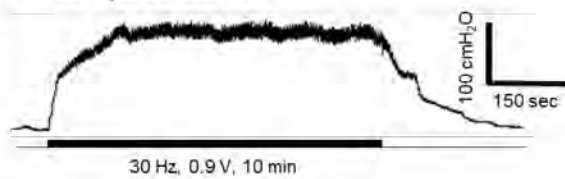
This preclinical study provides scientific evidence to further support the IPG design to

(b)(4)

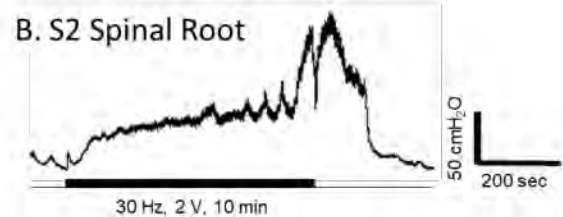
We have also successfully demonstrated in anesthetized female cats that pudendal nerve stimulation can produce periodic contractions and wetness of the vagina (Fig.4). (b)(4)

(b)(4)

A. S1 Spinal Root



B. S2 Spinal Root



C. Sustained Pressure

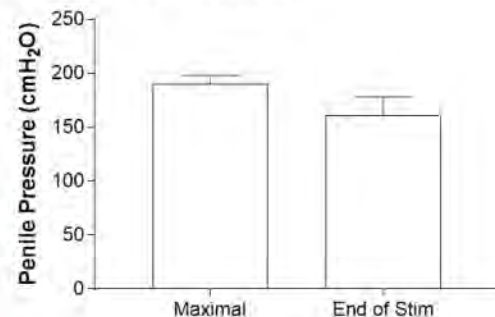


Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure reaching a maximal of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

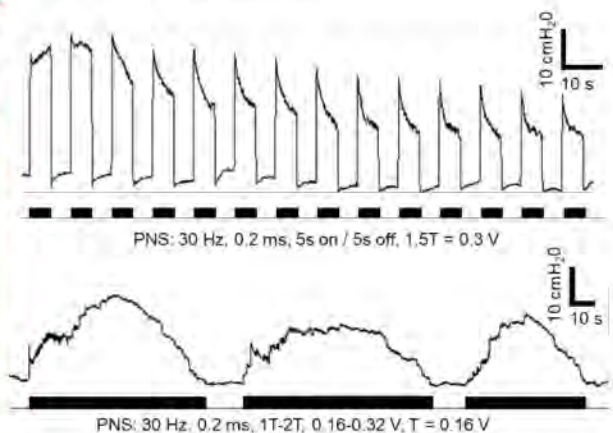


Fig.4: Vagina contractions induced by pudendal nerve stimulation (PNS) in a cat.

(b)(4)

(b)(4)

1.5 System Design and Technology Development Strategy

The Implantable Pulse Generator (IPG)

Based on our preclinical studies, we have finalized our IPG design (b)(4)

(b)(4)

(b)(4)

Strategy for Restoring Bladder, Bowel, and Sexual Functions after SCI

Our preclinical studies in anesthetized cats have shown that pudendal nerve stimulation/block is effective in restoring bladder function (see Fig.1) and female sexual function (Fig.4), (b)(4)

(b)(4)

(b)(4)

2 Engineering Design – System Specification

Based on our preclinical studies conducted in phase 1 of this DARPA BG+ project, we are now developing a (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Fig.7: The implantable stimulation system for restoring bladder, bowel, and sexual functions after SCI.

(b)(4)

(b)(4)



(b)(4)



IPG Specification

Number of channels:

Number of ports:

Output per channel:

Description:

Power supply:

(b)(4)

(b)(4)

(b)(4)

IPG life span is typically driven by the battery's certified life span, with the chosen battery currently certified (b)(4) The electronics and components forming the

(b)(4)

life, documented life testing (b)(4) and high Mean Time Between Failures (MTBF). Our verification testing will include an accelerated age testing to simulate (b)(4)

(b)(4)

This is typically performed with a sampling of PCBA's in a test fixture attached to a multiplexer stressing the boards by cycling them to their maximum outputs then reversing it to zero and repeating it. Data is continuously collected then analyzed for linearity of output and any indication of power output failure, exhibited as non-linear, spikes, roll off or no output etc. More output parameters can also be monitored dependent on test team's objectives to include frequency and wave output shape.

(b)(4)

Charging System

For charging the IPG, a (b)(4) will be used to transfer power from an external charging device to the implant. (b)(4)

(b)(4)

(b)(4)

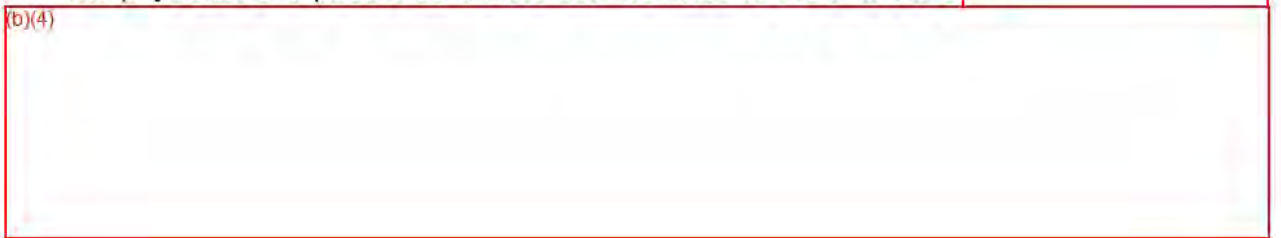


Physician and Patient Controllers

Fig.10 shows the graphic user interface (GUI) designed for physician controller to be used for (b)(4). Fig.11 show the GUI designed for patient controller. The physician controller has the full capacity to program the (b)(4) while the patient controller only has limited control of the (b)(4) activation/deactivation of a function.

The physician and patient controllers communicate to the IPG via a (b)(4)

(b)(4)



(b)(4)

(b)(4)

2.2 (b)(4) Design: (b)(4)

Fig.12 shows the (b)(4) that will be used (b)(4)

(b)(4)

(b)(4) This screening step reduces the likelihood that a patient will receive our IPG but not receive potential benefit from the implant. Patient eligibility is determined (b)(4)

(b)(4)

This clinical observation is indicative of successful blockage of the pudendal nerves by our (b)(4) and thus provides clinical support that the patient's pudendal nerve and external urethral sphincter (EUS) function normally and that this patient is a good candidate for a permanent implantation of the IPG. The (b)(4) will also be evaluated for its ability to initiate bladder contractions and reduce bladder pressure required to induce urine release (secondary endpoints).

(b)(4)

(b)(4)

(b)(4) Fig.10 shows the physician controller GUI to be used to program and control the (b)(4) screening clinical trial that will be conducted during the phase 2 of this DARPA BG+ project. (b)(4)

(b)(4)

(b)(4)

EPG Specification

Number of channels:

Output per channel:

Power supply:

Longevity:

Communication:

Controller:

System latency:

Size:

(b)(4)

2.3 Electrode Design: (b)(4)

(b)(4)

Based on our preclinical studies, (b)(4) electrodes can be used to stimulate the pudendal nerves and/or the sacral roots to restore bladder (Fig.1), bowel (Fig.2), and sexual functions (Fig.3 and Fig.4). (b)(4) electrodes close to the pudendal nerves (Fig.5 and Fig.6). Meanwhile, the (b)(4) surgical approach (b)(4)

(b)(4)

(b)(4) However, electrode migration is always a potential problem for any (b)(4) electrode to be used chronically in humans. To overcome this problem, we (b)(4)

(b)(4) In addition, each (b)(4)

(b)(4)

(b)(4) Therefore, the nerve can always be stimulated by (b)(4)

if the (b)(4) Furthermore, in rare cases where large (b)(4) migration does occur, replacing the migrated (b)(4) with new (b)(4) will only require (b)(4)

(b)(4) Finally, for those patients whose implant works very well but encounters multiple

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4) *Electrode Specification*

Number of contacts:

Contact length:

Contact separation:

(b)(4)

Diameter:

Length:

Longevity:

Features:

(b)(4)

Longevity for (b)(4) Electrode

The (b)(4) contacts are designed and constructed of biocompatible materials that have been proven for implantation and long-term use. The body of (b)(4)

(b)(4)

(b)(4)

Two major testing protocols will be used on our leads, biological and mechanical:

Biological: Biocompatibility testing pursuant to the ISO-10993 standard is currently planned to commence (b)(4) These tests are preceded with a biological evaluation protocol then a

battery of (b)(4) individual tests that include cytotoxicity, sensitization, pyrogenicity and others inclusive of a long-term implantation in an animal model. These tests provide assurances for long term implantation in the human body and verification to the non-reactive condition of the chosen materials.

Mechanical/Age testing: Accelerated aging is a stability test for the leads and its packaging to assure no negative effects are introduced unto the product as a result of the passage of time. These tests will not only provide confirmation and support of the expiration date on the package but will also provide assurances that the materials will not break down and remain stable over time. The acceleration testing is quantified and verified periodically through a series of tests that are designed to test the function of the product over time both mechanical and electrical.

The ASTM F1980 is one standard used that specifically evaluates the aging process of a product along with its package and how it impacts sterility and shelf-life as indicated above.

Accelerated aging tests simulate 'aging' conditions by exposing the materials to elevated temperatures for periods of time to represent an equivalent real time shelf-life duration. A multiplication factor is used to simulate the passage of time with an average temperature of 55°C. This methodology is a commonly used and accepted methodology. Additionally, precise aging tests achieved by tight temperature tolerances offers enhanced assurance among regulatory agencies and consumers

(b)(4) *Electrode Design*

Our previous studies in awake chronic SCI cats have successfully evaluated (b)(4) electrodes for restoring bladder function. (b)(4)

(b)(4)

(b)(4) Fig.17 shows the (b)(4) electrodes used in our previous animal studies and in

(b)(4) studies. (b)(4)

(b)(4)

(b)(4)

as needed for restoring bladder function. Fig.18 shows our current (b)(4) electrode design for (b)(4) and (b)(4)

Although the human pudendal nerve is surgically (b)(4) accessible for implantation (b)(4)

(b)(4)

(b)(4)

(b)(4)

2.4 Software Development Kit (SDK)

(b)(4)

(b)(4)

3 Regulatory Strategy – FDA IDE Submission (b)(4)

We have had (b)(4) meetings with FDA and incorporated all feedback into our IDE submission (b)(4). This includes FDA feedback on our proposed clinical protocol and informed consent document. We expect FDA IDE approval for our (b)(4) feasibility trial by the end of phase 1 of this DARPA BG+ project to begin our (b)(4) early feasibility study (EFS) in phase 2. Our regulatory strategy for the entire project is outlined below (b)(4).

We plan to submit our IDE application (b)(4)

(b)(4)

We anticipate FDA approval of our IDE (b)(4) and plan to submit our protocol to the University of Pittsburgh's IRB and HRPO as soon as the IDE is approved by FDA.

The original SOW for this EFS included (b)(4) (b)(4) however, based on FDA feedback and many discussions with our clinicians, we now plan to recruit (b)(4)

(b)(4)

(b)(4) In parallel to the EFS trial, we will continue refining and miniaturizing our next generation (b)(4) When fully tested, we plan to submit (b)(4) to FDA to allow the use of (b)(4) with reduced-size circuitry. The (b)(4) EFS trial is scheduled to be conducted from (b)(4) (b)(4) but we will prioritize early enrollment to learn and de-risk (b)(4) development and chronic animal studies. Future (b)(4) meetings for (b)(4) system are tentatively scheduled in the SOW (b)(4)

(b)(4) Results from the (b)(4) EFS trial will be included (b)(4)

(b)(4) when results become available.

(b)(4)

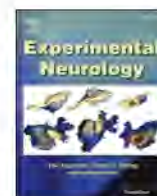
To confirm longer-term performance restoring function in conscious animals, we will conduct chronic animal studies in phase 2. Together, results from our chronic non-GLP animal studies, human EFS, and FDA (b)(4) will inform our GLP study design for the (b)(4) system. Longer-term implantable (b)(4) GLP studies are expected to be conducted between (b)(4)

(b)(4)

The human study with our (b)(4) will be conducted between (b)(4) and (b)(4)

(b)(4)





Research paper

Low pressure voiding induced by stimulation and 1 kHz post-stimulation block of the pudendal nerves in cats

Jialiang Chen^{a,b}, Jianan Jian^a, Jicheng Wang^a, Zhijun Shen^a, Bing Shen^a, William Wang^a, Jonathan Beckel^c, William C. de Groat^c, Christopher Chermansky^a, Changfeng Tai^{a,c,d,*}

^a Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

^b Department of Urology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, PR China

^c Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

^d Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

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ABSTRACT

The goal of this study is to induce low-pressure voiding by stimulation and bilateral 1 kHz post-stimulation block of the pudendal nerves. In anesthetized cats, wire hook electrodes were placed on the left and/or right pudendal nerves. Stimulus pulses (30 Hz, 0.2 ms) were applied to one pudendal nerve to induce a reflex bladder contraction and to produce contractions of the external urethral sphincter (EUS). High frequency (1 kHz) biphasic stimulation was applied to block axonal conduction in both pudendal nerves and block EUS activity. In 4 cats, a catheter was inserted into the distal urethra to perfuse and measure the back pressure caused by the EUS contraction. In another 5 cats, a catheter was inserted into the bladder dome and the urethra was left open to allow voiding. The 1 kHz stimulation (30–60 s, 0.5–5 mA) delivered via a wire hook electrode completely blocked pudendal nerve conduction for ≥ 2 min after terminating the stimulation, i.e., a post-stimulation block. The block gradually disappeared in 6–18 min. The block duration increased with increasing amplitude or duration of the 1 kHz stimulation. Without the 1 kHz block, 30 Hz stimulation alone induced high-pressure (90 cmH₂O) voiding. When combined with the 1 kHz block, the 30 Hz stimulation induced low-pressure (≤ 50 cmH₂O) voiding with a high voiding efficiency (80%). In summary, a minimally invasive surgical approach might be developed to restore voiding function after spinal cord injury by stimulation and block of the pudendal nerves using lead electrodes.

1. Introduction

Normal micturition is elicited by a bladder contraction and a simultaneous relaxation of the external urethral sphincter (EUS), which reduces urethral outlet resistance and results in low-pressure (≤ 50 cmH₂O) voiding. However, after spinal cord injury (SCI) the coordination between bladder and EUS is lost, causing simultaneous contractions of the bladder and EUS, termed detrusor sphincter dyssynergia (DSD). This generates high-pressure (> 50 cmH₂O) in the bladder and prevents complete bladder emptying. High bladder pressure is harmful because it can cause kidney failure if not treated. Currently, DSD is often managed by daily intermittent urethral catheterization so that the bladder can be emptied without generating high bladder pressure. However, repeated urethral catheterization can cause frequent infections of the lower urinary tract (Jamil, 2001). A better treatment for DSD will significantly

improve the quality of life for SCI people who are currently suffering from the problem of bladder emptying (Hallin et al., 2000).

Our previous studies (Tai et al., 2006, 2007a, 2007b; Yang et al., 2013) in cats developed a novel method to prevent DSD by blocking the pudendal nerves bilaterally using 6–10 kHz high frequency biphasic stimulation. Recently, we developed a fully implantable stimulator that successfully produced highly efficient ($> 90\%$) low-pressure (≤ 50 cmH₂O) voiding in awake chronic SCI cats during a 3–4 month period using 10 kHz pudendal nerve block in combination with a 20–30 Hz pudendal nerve stimulation to induce a reflex bladder contraction (Cai et al., 2019; Guo et al., 2021). However, pudendal nerve block by 6–10 kHz stimulation can only be achieved by implantation of cuff electrodes on the nerves (Apovian et al., 2017; Boger et al., 2008; Sojin et al., 2015; Tai et al., 2005), which requires major invasive surgery to expose the pudendal nerves bilaterally. Therefore, a method to block the pudendal

* Corresponding author at: Department of Urology, University of Pittsburgh, 700 Kaufmann Building, Pittsburgh, PA 15213, USA.

E-mail address: cftai@pitt.edu (C. Tai).

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nerve using a lead electrode without a cuff could be very beneficial since a lead electrode could be percutaneously inserted close to the pudendal nerve by a minimally invasive surgical approach.

In this study using spinal cord intact cats under anesthesia, we discovered that high frequency (1 kHz) biphasic electrical stimulation delivered by a wire hook electrode without a cuff could block pudendal nerve conduction during a short period immediately after termination of the stimulation, i.e., a post-stimulation block. In addition, low-pressure voiding could also be induced by applying 30 Hz pudendal nerve stimulation to induce a reflex bladder contraction during the post-stimulation block. These results indicate that a minimally invasive surgical approach might be developed by inserting percutaneous lead electrodes close to the pudendal nerves to achieve pudendal nerve block and treat DSD after SCI.

2. Materials and methods

The experimental protocol and animal use in this study were

approved by the Animal Care and Use Committee at the University of Pittsburgh.

2.1. Surgical procedures

A total of 9 cats (8 females and 1 male, 3.4 ± 0.2 kg, 7–12 months old, Liberty Research, Waverly, NY) were anesthetized with isoflurane (2–5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. The left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847 V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, the ureters were isolated, tied, and cut for external drainage. In the first experimental group of 4 female cats, a plastic catheter (7F) was inserted via a small cut in the proximal urethra to a location 2–3 cm distal to the

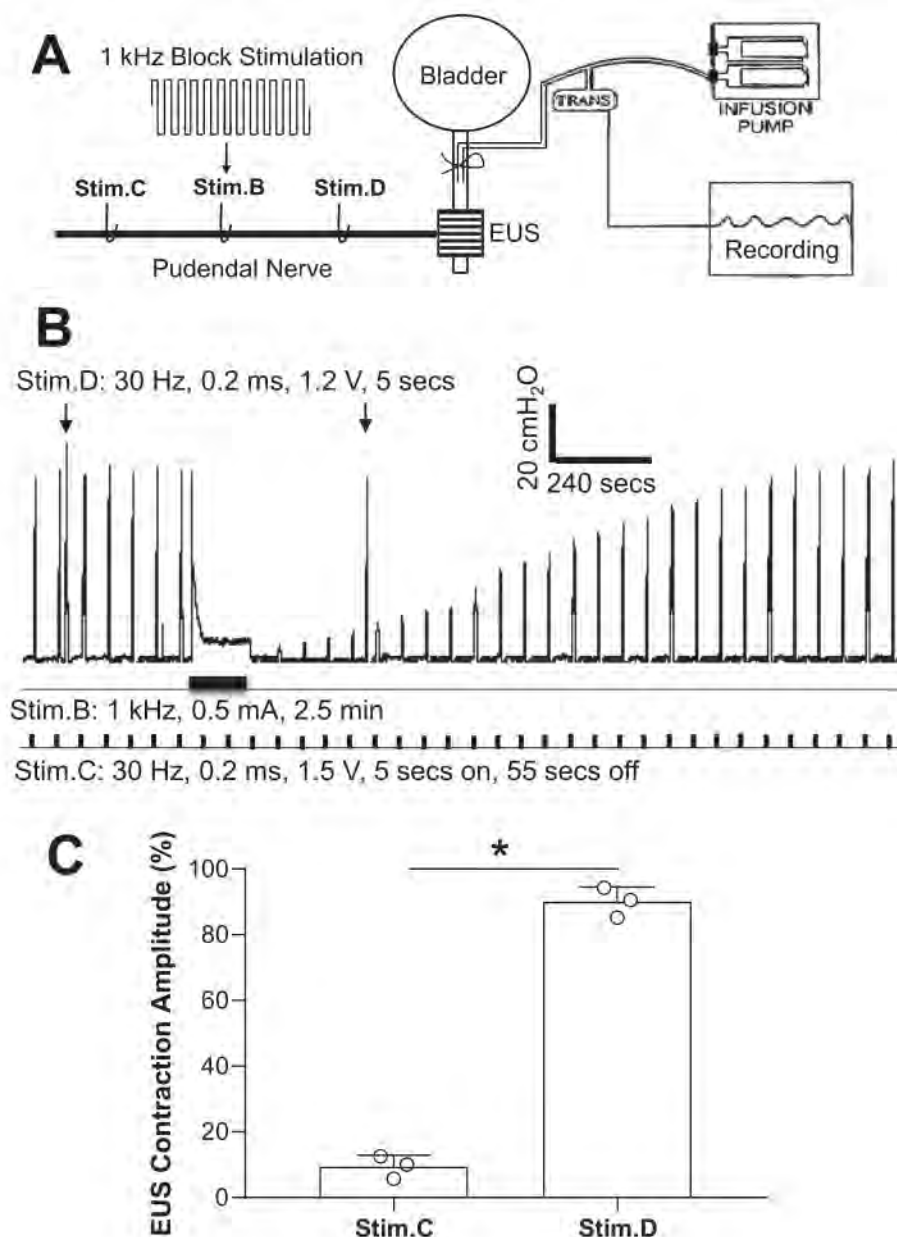


Fig. 1. A. Experimental setup for pudendal nerve block elicited by 1 kHz stimulation with a wire electrode. B. Complete nerve block occurred after the end of the 1 kHz biphasic continuous stimulation. The arrows indicate the EUS contraction pressures induced by the 30 Hz stimulation at the distal site (Stim.D). The thick bars under the EUS pressure trace indicate the stimulation durations for Stim.B and Stim.C. After ending the 1 kHz Stim.B, the EUS contraction can still be induced by the distal Stim.D but the contractions induced by the central Stim.C are completely blocked initially and then gradually recover, indicating that nerve conduction block occurs locally at Stim.B and the EUS is not fatigued. C. The normalized EUS contraction amplitude induced by Stim.C or Stim.D during the post-stimulation period. $N = 3$ nerves. * indicates a significant difference ($p = 0.0026$, paired t -test). EUS -External Urethral Sphincter.

bladder neck and secured by a ligature. The urethra was slowly perfused with saline via the catheter to measure the intraurethral back pressure caused by urethral contractions (Fig. 1A). The pudendal nerves were dissected bilaterally via 3- to 4-cm incisions in the sciatic notch lateral to the tail for placement of monopolar wire hook electrodes (Fig. 1A). A 16-gauge needle was inserted in the shoulder muscle as the reference electrode. The nerve and electrodes were covered by mineral oil during the study. In the second experimental group of 5 cats (4 female and 1 male), a double lumen plastic catheter (7F) was inserted into the bladder via a small puncture on the bladder dome and secured by a ligature so that voiding could occur without a physical urethral outlet obstruction. One lumen of the catheter was connected to a pump to infuse the bladder with saline, and the other lumen filled with saline was attached to a pressure transducer to record intravesical pressure (Fig. 4A). A funnel was used to collect the voided fluid into a beaker that was attached to a force transducer to record the voided volume. The pudendal nerves were exposed bilaterally for placement of either monopolar ($N = 2$ cats) or bipolar ($N = 3$ cats) wire hook electrodes (Fig. 4A). The nerve and electrodes were covered by saline or mineral oil during the study to confirm that low-pressure voiding could be achieved with the electrode surrounded by media of either low (saline) or high (oil) impedance.

2.2. Experimental protocol

In the first group of 4 cats, the urethra was slowly (1 ml/min) perfused to test the pudendal nerve block induced by a 1 kHz biphasic stimulation waveform (see Fig. 1A). In the first cat, three monopolar stainless-steel hook electrodes (diameter 0.5 mm and 5 mm long) were placed on either the right or left pudendal nerve with 3–5 mm between the electrodes (Fig. 1A). The blocking electrode (Stim.B) always delivered the 1 kHz block stimulation that was generated by a computer running LabView program and delivered via a constant current stimulus isolator (A395, World Precision Instruments, Sarasota, FL). The central electrode (Stim.C) delivered short bursts of stimulation (30 Hz, 0.2 ms, 5 s on and 55 s off) at an amplitude of 3 times the threshold (T) amplitude for inducing anal twitch to produce maximal EUS contractions. The 30 Hz stimulation was generated by an electrical stimulator (S88, Grass Medical Instruments, Quincy, MA) and delivered via a constant voltage stimulus isolator (SIU5, Grass Medical Instruments). The distal electrode (Stim.D) delivered a single short burst (5 s) of stimulation (30 Hz, 0.2 ms, 3 T) to confirm that Stim.B suppressed the EUS contractions induced by Stim.C via a local nerve block instead of causing EUS fatigue. The 1 kHz stimulation (Stim.B) at various stimulation amplitudes and durations was explored in the first cat to determine the appropriate range of stimulation parameters for further testing.

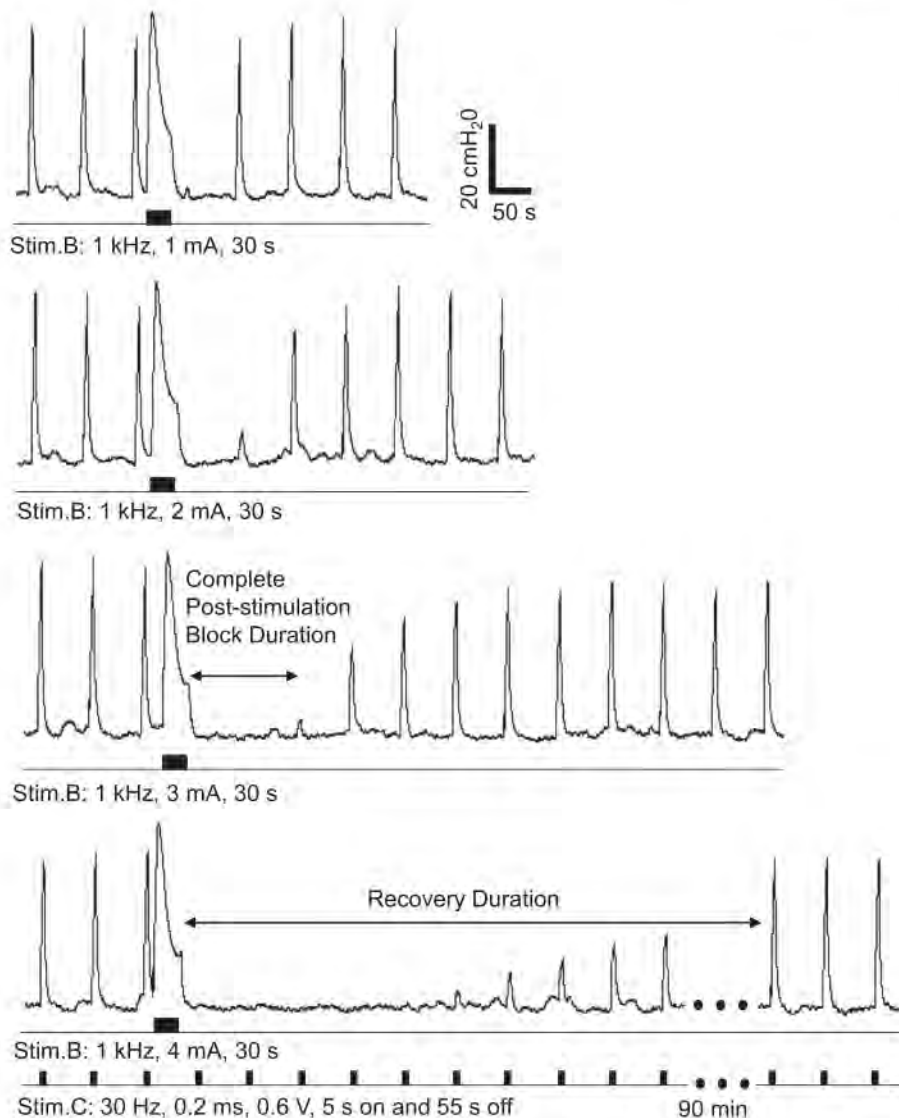


Fig. 2. Block stimulation (Stim.B) produced a complete post-stimulation block of duration ≥ 2 min as the stimulation amplitude gradually increased from 1 mA to 3 mA. At 4 mA amplitude, Stim.B produced a prolonged post-stimulation block with a recovery period of 110 min. The black bar under each urethral pressure trace indicates the stimulation duration. The duration of Stim.C shown at the bottom by short black bars applies to all urethral pressure traces. The amplitude for Stim.C which is at 3 times the threshold (T) amplitude for inducing visible anal twitch induces maximal contractions of the external urethral sphincter.

In the next 3 cats, two monopolar hook electrodes (Stim.C and Stim.B) were placed initially on left pudendal nerve with 3–5 mm between the electrodes (Fig. 1A). Stim.C delivered short bursts of stimulation (30 Hz, 0.2 ms, 3 T, 5 s on and 25/55 s off) to induce periodic EUS contractions during which Stim.B (1 kHz, 30 s) was applied at an increasing amplitude starting at 1 mA with 1 mA increase each time until a complete post-stimulation block of duration ≥ 2 min was observed. This duration was considered to be sufficient to allow complete voiding. Complete block was defined as a reduction in the Stim.C evoked EUS contraction pressure to <10 cmH₂O that would allow low-pressure voiding to occur (see third trace in Fig. 2). After each application of the 1 kHz stimulation, a waiting period was given to allow the EUS contractions induced by Stim.C to recover to the level preceding the application of the 1 kHz stimulation. If a complete post-stimulation block of duration ≥ 2 min was not observed before the amplitude of 1 kHz stimulation reached the level causing hindlimb movement, the duration of 1 kHz was doubled to 60 s and the test was repeated (Fig. 3). Then, the Stim.D electrode (Fig. 1A) was added to confirm Stim.B suppressed the EUS contractions induced by Stim.C via a local nerve block instead of causing EUS fatigue. After testing the left pudendal nerve, the electrodes were moved to the right pudendal nerve and the same test was repeated. However, on the right pudendal nerve the amplitude of 1 kHz stimulation was increased above the level that produced complete post-stimulation block of ≥ 2 min duration to elicit a more prolonged (>30 min) recovery duration that is defined as the time for the response to recover to at least 80% of the level prior to the application of 1 kHz stimulation (see fourth trace in Fig. 2).

In the second group of 5 cats with the bladder catheterized through the bladder dome, multiple voiding CMGs were performed initially to determine the control values for bladder capacity, voiding pressure, voiding efficiency, and post-void residual volume. Then, in the following repeated CMGs the bladder was infused to a volume close to the control capacity without triggering reflex voiding. With the bladder partially full, pudendal nerve stimulation (Stim #1 in Fig. 4A: 30 Hz, 0.2 ms, 3–4 T) was first applied to induce a pudendal-to-bladder reflex contraction for voiding. Our previous studies (Cai et al., 2019; Guo et al., 2021; Tai et al., 2006, 2007a, 2007b; Yang et al., 2013) in both normal and chronic spinal cord injured cats showed that 30 Hz stimulation was effective in inducing large reflex bladder contractions. In the next CMG with the bladder partially full, the 1 kHz stimulation (1 mA, 30 s) was applied bilaterally to the pudendal nerves via Stim #2 and Stim #3 (see Fig. 4A) to induce a post-stimulation block. Immediately following the termination of the 1 kHz block stimulation, the Stim #1 was applied to induce a bladder contraction for voiding. If the voiding pressure was not reduced, the same block/stimulation protocol was repeated in the

following CMGs with bladder partially full, but the 1 kHz stimulation was applied at the maximal amplitude without triggering hindlimb movement or at an increased stimulation duration starting at 1 min and with 1 min increments each time. Once a low-pressure (≤ 50 cmH₂O) voiding was achieved, only the Stim #1 was applied in the following repeated CMGs to monitor the recovery of the voiding pressure. At the end of each CMG, post-void residual volume was determined by withdrawing fluid from the bladder catheter.

2.3. Data analysis

In the first group of cats, the amplitude and duration for the 1 kHz stimulation to induce a complete post-stimulation block of duration ≥ 2 min were determined, and the related block and recovery durations were measured. The amplitudes of the EUS contractions induced during the post-stimulation period by Stim.D and Stim.C within a 30 s interval as shown in Fig. 1B were compared by normalizing them to their control amplitudes before the 1 kHz stimulation. In the second group of cats, the bladder volume, voiding pressure, voiding efficiency, and post-void residual volume were measured for each CMG. The bladder volume was measured as the voided volume + the post-void residual volume. The bladder volume and post-void residual volume were normalized to the control bladder capacity in the same animal. The data from different animals are presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined using repeated-measures one-way ANOVA followed by Dunnett's multiple comparison or using Welch's or paired *t*-test.

3. Results

3.1. Post-stimulation block of the pudendal nerve by 1 kHz biphasic stimulation waveform

As shown in Fig. 1, Stim.B (1 kHz, 0.5 mA, 2.5 min) successfully blocked the periodic EUS contractions induced by short bursts of Stim.C (30 Hz, 0.2 ms, 1.5 V, 5 s on and 55 s off) during a period after terminating the 1 kHz stimulation, i.e., a post-stimulation block. Since the EUS contractions induced by Stim.D (30 Hz, 0.2 ms, 1.2 V, 5 s) were not changed while the contractions induced by Stim.C were blocked, it is clear that Stim.B blocked pudendal nerve conduction instead of causing EUS fatigue that should affect the responses induced by both Stim.C and Stim.D. On average the EUS contraction amplitude induced by Stim.D during the post-stimulation period was maintained at about 90% of its control amplitude measured before the 1 kHz stimulation while the contraction amplitude induced by Stim.C was significantly ($p = 0.0026$, paired *t*-test) reduced to only 10% of its control (Fig. 1C). The large difference between the reductions in the amplitude of the two evoked responses indicates a local nerve block occurred at the Stim.B and that EUS fatigue which should reduce the responses induced by Stim.C and Stim.D equally was not involved.

The duration of 1 kHz post-stimulation block was dependent on the amplitude and duration of the stimulation. As shown in Fig. 2, the block duration was increased by increasing the amplitude of 1 kHz stimulation (30 s) from 1 mA to 4 mA. At the amplitude of 3 mA, the duration for a complete post-stimulation block reached 2 min with a full recovery occurring at 6 min (see the third trace in Fig. 2). When the amplitude of 1 kHz stimulation increased to 4 mA, the complete block duration increased to 6 min, but the time for complete recovery increased dramatically to 100 min (see the fourth trace in Fig. 2). In the other 2 cats, a prolonged recovery (58 min or 60 min) was induced by 1 kHz stimulation at the amplitude/duration combination of 4 mA/30 s or 2 mA/60 s, respectively.

Fig. 3 summarizes the amplitude and duration required for 1 kHz stimulation to induce a complete post-stimulation block of duration ≥ 2 min, the corresponding duration of the complete block, and the time for full recovery from block ($N = 6$ nerves from 3 cats). In one cat, 1 kHz stimulation of 30 s duration failed to induce a complete post-stimulation

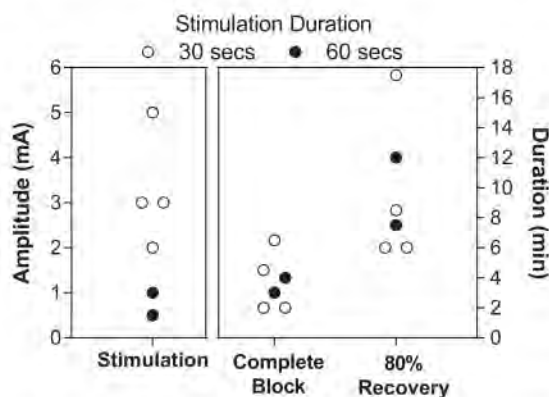


Fig. 3. The amplitude and duration (30 s or 60 s) required for 1 kHz stimulation to produce a complete post-stimulation block of duration ≥ 2 mins, the corresponding duration for the complete block, and the duration for an 80% recovery from the block. Stimulation amplitude is shown on the left Y-axis. Duration for the complete block and 80% recovery is shown on the right Y-axis. $N = 6$ nerves from 3 cats.

A. Electrode Location

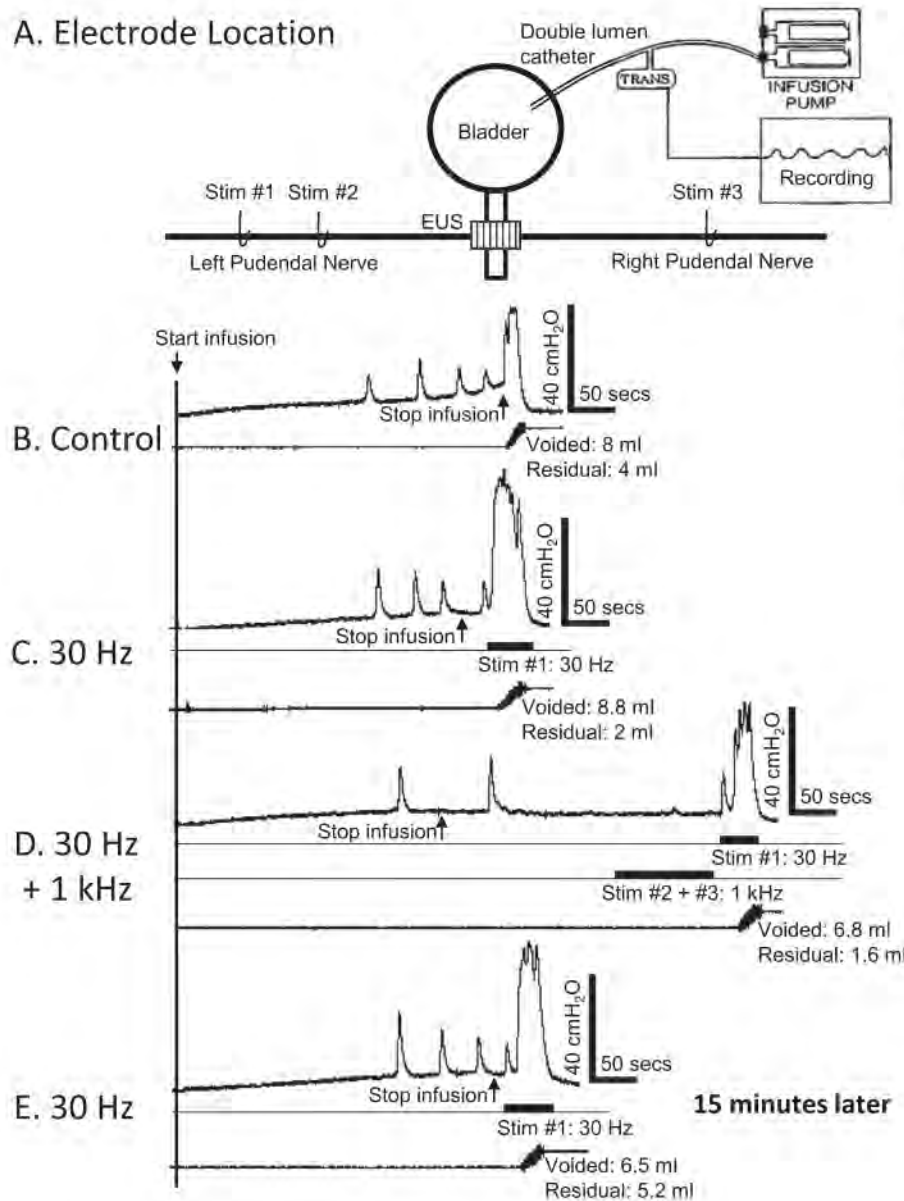


Fig. 4. Low-pressure voiding induced by stimulation and 1 kHz post-stimulation block of axons in the pudendal nerves. **A.** Monopolar wire electrodes were placed on pudendal nerves bilaterally to deliver stimulations at three locations (#1, #2, #3). Stim #1 (30 Hz, 0.2 ms) was delivered to induce a reflex bladder contraction after Stim #2 and #3 delivered a short burst of 1 kHz stimulation to produce a post-stimulation block of the pudendal nerves. EUS - external urethral sphincter. **B.** Low-pressure voiding induced by infusion of saline into the bladder without stimulation. **C.** Stim #1 (30 Hz, 0.2 ms, 9 V) alone produced high-pressure (≈ 60 cmH₂O) voiding. **D.** When Stim #1 was applied immediately after Stim #2 and #3 delivered the 1 kHz stimulation (1.75 mA, 2 mins), it induced a low-pressure (≈ 40 cmH₂O) voiding. **E.** In 15 mins after the 1 kHz stimulation, Stim #1 again induced a high-pressure voiding. The black bar under the bladder pressure trace indicates the stimulation duration. Infusion rate = 2 ml/min.

block of duration ≥ 2 min before its amplitude increased to the level causing hindlimb movement. In this cat after increasing the stimulation duration to 60 s, the 1 kHz stimulation-induced complete block occurred at a much lower stimulation amplitude (0.5–1 mA, see the black dots in Fig. 3) than the 30 s stimulation ($p = 0.0236$, Welch's *t*-test). These results indicate that there is a combination of amplitude and duration for 1 kHz to induce a complete post-stimulation block of duration ≥ 2 min that can gradually disappear within 20 min (Fig. 3). However, if the amplitude and/or the duration are increased further, a prolonged recovery of 1–2 h can occur (see the fourth trace in Fig. 2).

3.2. Low-pressure voiding induced by 1 kHz block and 30 Hz stimulation

Fig. 4 shows that when using monopolar hook electrodes in a saline pool the 30 Hz pudendal nerve stimulation combined with 1 kHz post-stimulation block successfully induced low-pressure (≤ 50 cmH₂O) voiding. During the control CMG (Fig. 4B), slow infusion of the bladder triggered a micturition reflex producing a low-pressure (≈ 40 cmH₂O) voiding. With the bladder partially full (Fig. 4C), Stim #1 (30 Hz) alone induced voiding at a high pressure (≈ 60 cmH₂O). However, the same

Stim #1 successfully induced low-pressure (≈ 40 cmH₂O) voiding when applied immediately after termination of the 1 kHz axonal blocking stimulation (1.75 mA, 2 min) at both Stim #2 and #3 (see Fig. 4D). The high-pressure (≈ 60 cmH₂O) voiding returned in about 15 min after termination of the 1 kHz blocking stimulation (Fig. 4E).

Fig. 5 shows that low-pressure voiding can also be induced by a combination of 1 kHz block and 30 Hz stimulation using bipolar hook electrodes in a mineral oil pool. However, the reversal from low-pressure voiding to high-pressure voiding was slower due to a stronger effect of the 1 kHz post-stimulation block in this experiment. As shown in Fig. 5A–B, the micturition reflex elicited by filling the bladder induced low-pressure (< 50 cmH₂O) voiding; while Stim #1 (30 Hz) alone induced high-pressure (≈ 100 cmH₂O) voiding. After applying 1 kHz stimulation (2 mA, 1 min) to both Stim #2 and #3, the response to Stim #1 was completely blocked for about 10 min, after which it recovered to successfully induce low-pressure (< 50 cmH₂O) voiding (see Fig. 5C). The low-pressure voiding induced by Stim #1 alone lasted for at least an hour after the 1 kHz blocking stimulation (Fig. 5D–E); but at 3 h, the high-pressure voiding (70 cmH₂O) returned (Fig. 5F). Note that there was no leakage of fluid from the bladder during the period immediately

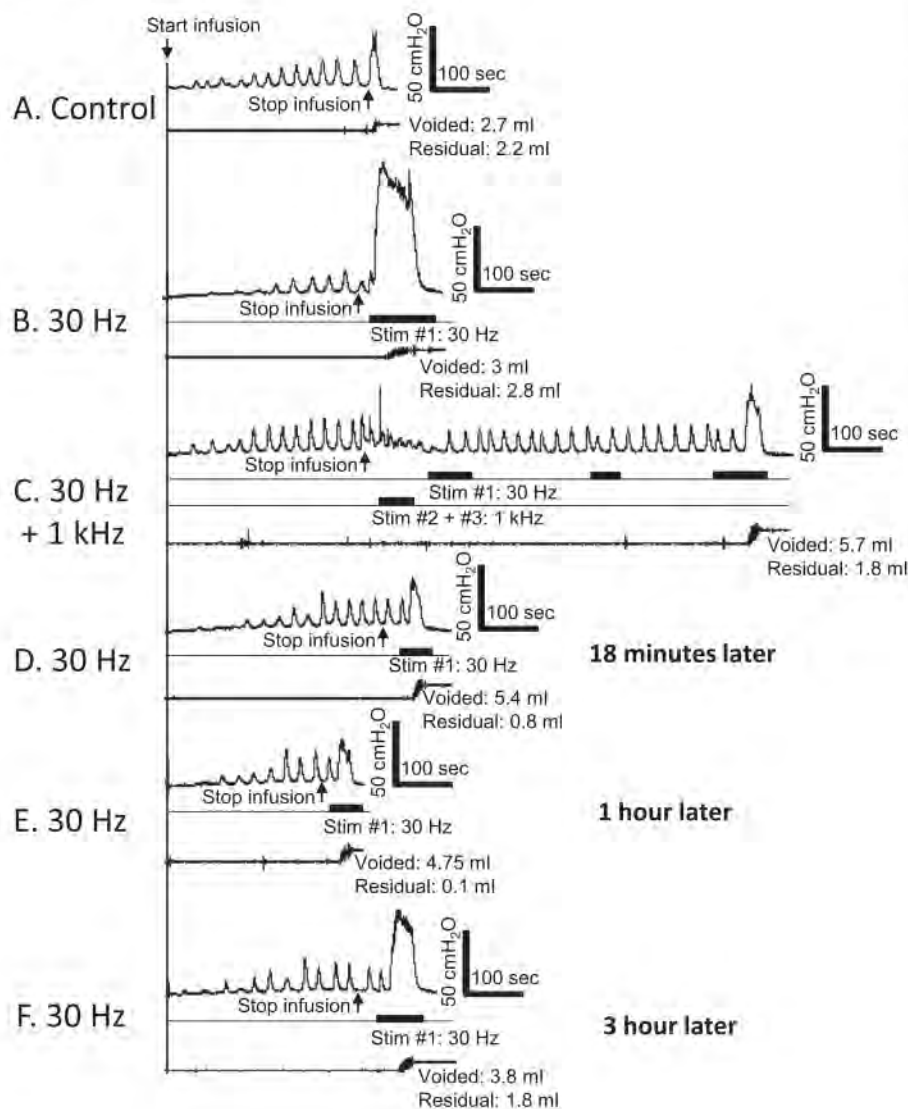


Fig. 5. Long-lasting effect of 1 kHz post-stimulation block of axons in the pudendal nerves. A. Control cystometrogram showing a low-pressure (<50 cmH₂O) voiding produced by a micturition reflex induced by infusion of saline into the bladder. B. Stim #1 (30 Hz, 0.2 ms, 0.48 V) alone produced a high-pressure (≈100 cmH₂O) voiding. C. After the 1 kHz stimulation (2 mA, 1 min) at Stim #2 and #3, Stim #1 lost the ability to induce a bladder contraction for about 10 min, after which Stim #1 induced a low-pressure (≈50 cmH₂O) voiding. D-E. The low-pressure voiding induced by Stim #1 lasted for at least 1 h. F. At 3 h after the 1 kHz stimulation, the voiding pressure induced by Stim #1 increased to about 70 cmH₂O. The black bar under the bladder pressure trace indicates the stimulation duration. Infusion rate = 1 ml/min.

following termination of the 1 kHz blocking stimulation although many non-voiding contractions occurred during this period (Fig. 5C).

Fig. 6 summarizes the results from 5 cats. On average, in the control condition the micturition reflex induced by filling the bladder produced low-pressure (≈40 cmH₂O) voiding (Fig. 6A) and a 50% voiding efficiency (Fig. 6B) with a post-void residual volume of 50% (Fig. 6C) of the control bladder capacity. A slightly higher voiding efficiency, but not significantly different from control, and a lower post-void residual volume were produced by 30 Hz stimulation alone (Fig. 6 B–C) at a significantly ($p = 0.0149$) higher voiding pressure (90 cmH₂O, Fig. 6A). However, 30 Hz stimulation combined with 1 kHz post-stimulation block significantly ($p = 0.0203$) increased voiding efficiency (80%, Fig. 6B) and reduced post-void residual volume (20%, Fig. 6C) but at a significantly ($p = 0.0303$) lower pressure (≈40 cmH₂O) that was same as in the control condition (Fig. 6A). After a recovery period ranging from 15 mins to 3 h (see Fig. 4 and Fig. 5), the voiding pressure induced by 30 Hz stimulation alone increased to about 70 cmH₂O (Fig. 6A) while voiding efficiency declined (50%, Fig. 6B) and post-void residual volume significantly ($p = 0.0354$) increased (50%, Fig. 6C) above that measured immediately after the 1 kHz stimulation. On average a similar amount of saline was infused into the bladder under the different conditions (Fig. 6D).

4. Discussion

This study shows that application of 1 kHz stimulation to the pudendal nerve by a wire electrode elicits a block of axonal conduction that persists following the stimulation, i.e., a post-stimulation block (Fig. 1) and that the block duration increases with increasing amplitude and duration of the stimulation (Fig. 2 and Fig. 3). All previous studies (Apovian et al., 2017; Boger et al., 2008; Soin et al., 2015; Tai et al., 2005) applied 5–10 kHz stimulation that requires a cuff electrode to successfully block the nerve conduction during the stimulation. The 5–10 kHz stimulation also requires a very long stimulation duration (>10 min) to induce a post-stimulation block (Wang et al., 2020). However, our recent study using cuff electrodes (Shapiro et al., 2020) discovered that a post-stimulation block could be induced by low-frequency (100 Hz – 1 kHz) stimulation of a short duration (30–60 s). The current study further demonstrates for the first time that a cuff electrode is not needed, and that post-stimulation block can be induced by 1 kHz stimulation using a monopolar or bipolar wire electrode, raising the possibility that a lead electrode without a cuff might also be useful in clinical applications to block nerve conduction. This result is significant because instead of a major invasive surgical procedure to implant the cuff electrodes a minimally invasive surgical approach could be developed to insert lead electrodes close to the pudendal nerves to

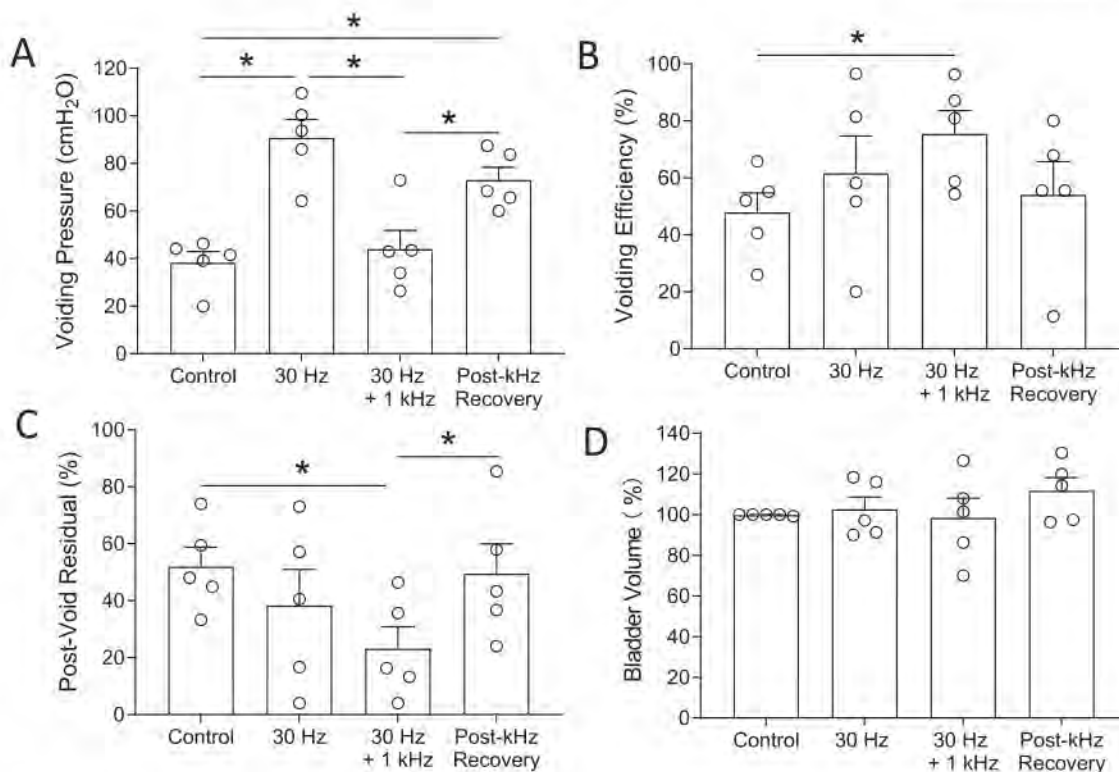


Fig. 6. Summary of the urodynamic parameters for control condition, 30 Hz stimulation alone (30 Hz), 30 Hz stimulation after 1 kHz stimulation (30 Hz + 1 kHz), and recovery from the 1 kHz blocking effect (Post-kHz recovery). A. Voiding pressure. B. Voiding efficiency. C. Post-void residual. D. Bladder volume infused. * indicates a significant difference ($p < 0.05$, ANOVA). $N = 5$ cats.

achieve pudendal nerve block. More importantly, during the bilateral post-stimulation block of the pudendal nerves, low-pressure reflex voiding can be induced by 30 Hz stimulation of afferent axons in the pudendal nerve (Figs. 4–6). Nerve block used in combination with stimulation of pudendal nerve afferent axons has a possible clinical application to reduce voiding pressure and improve voiding efficiency after SCI (Cai et al., 2019; Gup et al., 2021; Yang et al., 2013). Because high-pressure voiding after SCI can damage the kidneys, the low-pressure voiding induced by pudendal nerve stimulation and block (Figs. 4–6) would contribute to the long-term preservation of upper and lower urinary tract function and thus be a significant advance in the care of people with SCI.

However, it is important to recognize that the present study was performed in anesthetized spinal cord intact cats with normal lower urinary tract reflexes that produced relatively efficient reflex voiding at low pressures in response to bladder filling (Fig. 4B). This is due to coordinated bladder and EUS activity that produces a relaxation of the EUS during the bladder voiding contraction. After SCI the EUS relaxation is lost and the EUS contracts during voiding, a condition termed DSD, which increases urethral outlet resistance, impedes urine flow, increases voiding pressure and impairs complete bladder emptying. Block of the efferent input to the EUS by 1 kHz pudendal nerve stimulation would prevent DSD after SCI and normalize voiding. In the present study DSD was mimicked by applying pudendal nerve stimulation at 30 Hz, which caused a reflex bladder contraction and a simultaneous contraction of the EUS resulting in a high-pressure voiding. The efficacy of 1 kHz blocking stimulation in reducing the high pressure during the voiding induced by 30 Hz stimulation in the present study predicts that the blocking stimulation would be similarly effective in reversing the DSD-induced inefficient high-pressure voiding after SCI. It is worth noting that the 30 Hz stimulation combined with 1 kHz block significantly increased voiding efficiency over the control condition but not over the 30 Hz stimulation alone (Fig. 6B). However, 30 Hz stimulation alone

produced a very high voiding pressure reaching about 90 cmH₂O on average (Fig. 6A) that can increase voiding efficiency but is also harmful to the kidney and useless for a long-term clinical application. Therefore, the 1 kHz nerve block is very important because it allows a low-pressure voiding (40 cmH₂O as shown in Fig. 6A) to be induced by the 30 Hz stimulation.

This study examined two mechanisms (nerve block and EUS muscle fatigue) that might contribute to the prolonged suppression of neurally evoked EUS contractions by 1 kHz stimulation. The possibility that 1 kHz induces muscle fatigue instead of a local nerve block is not supported by the observation that when the response induced by Stim.C is blocked (see Fig. 1B–C) the response induced by Stim.D (see Fig. 1A) at a site distal to the blocking electrode is not blocked (see Fig. 1B–C). This method (Stim.C/Stim.D) has been used in many previous studies by different neurophysiologists to distinguish local nerve block from muscle fatigue (Bowman and McNeal, 1986; Kilgore and Bhadra, 2004). The initial muscle response to 1 kHz stimulation in our current study is similar to that elicited by 10 kHz stimulation (Tai et al., 2005) that is known to block axonal conduction without producing muscle fatigue (Bowman and McNeal, 1986; Kilgore and Bhadra, 2004). Therefore, the initial fast decline in response (see Fig. 1B) may not necessarily be muscle fatigue. Furthermore, the EUS is striated muscle that is more fatigue resistant than skeletal muscle. A previous study showed that prolonged (40 min) electrical nerve stimulation (12–24 Hz) of maximal intensity failed to relax the EUS and allow passage of urine (Brindley et al., 1974). This result is completely different from the result in our current study showing a complete relaxation of EUS (Fig. 1 and Fig. 2) that allowed low-pressure efficient voiding to occur (Figs. 4–6). In addition, it is certainly not typical that a short duration (30 s) neurally evoked EUS contraction produces complete EUS fatigue lasting 5 min followed by a very long (1.5 h) period for a full recovery of the EUS contractions (see the bottom trace in Fig. 2). Therefore, we believe that the prolonged 1 kHz induced suppression of the EUS contractions

observed in our current study is due to axonal block and not due to muscle fatigue.

More importantly, the 1 kHz post-stimulation block is reversible within minutes if an appropriate combination of stimulation amplitude and duration is used (Figs. 3–4). This reversibility is needed because the pudendal nerve needs to be functional after each voiding to maintain continence during urine storage. There is an optimal combination of stimulation amplitude and duration for achieving a short duration (~2 min) post-stimulation block to allow a low-pressure voiding to occur while also allowing the block to recover within 20 min (Fig. 3). In future clinical application, this optimal combination of stimulation amplitude and duration will have to be determined for each individual subject after implanting the pudendal nerve stimulation and block device. If the 1 kHz stimulation is too strong or lasts too long, axonal conduction in the pudendal nerve will need a long time to recover from the block (see fourth trace in Fig. 2), which will result in a prolonged period of low-pressure voiding (Fig. 5). It is worth noting that during the prolonged period of pudendal nerve block there was no leakage of fluid from the bladder although many small amplitude non-voiding contractions were occurring during this period (Fig. 5). This result indicates that a basal urethral outlet resistance was maintained during the prolonged pudendal nerve block that prevented leakage from the bladder. Furthermore, the 1 kHz stimulation elicited an initial strong EUS contraction (Fig. 2) but did not elicit a large reflex bladder contraction before it blocked the pudendal nerve (Fig. 4D and Fig. 5C), which eliminates the possibility for the 1 kHz stimulation to generate high bladder pressures in a long-term clinical application causing kidney damage.

Wire electrodes were used in this study to block the pudendal nerve. The electrical current can spread over a large area around the electrode because a nerve cuff was not used to limit the current spread. Since the sciatic nerve is very close to the pudendal nerve in cats, leg movement caused by the spreading current prevented further increasing the amplitude of the 1 kHz stimulation. Although this may not be an issue in clinical applications due to the larger distance between the sciatic nerve and the pudendal nerve in humans, our study shows that the limitation of using an increased stimulation amplitude can be overcome by increasing the stimulation duration to achieve the nerve block at a lower intensity (Fig. 3). To reduce the spread of the electrical current, a bipolar lead electrode configuration may be preferred over a monopolar lead electrode configuration in clinical applications to block a peripheral nerve.

The nerve block by continuous kHz stimulation has been approved by FDA for human application to treat obesity (Apovian et al., 2017) or amputation limb pain (Soin et al., 2015). Therefore, we expect that the 1 kHz post-stimulation block of pudendal nerve is safe for restoring voiding function after SCI because it will only be applied 5–8 times per day and a few minutes each time for voiding. This study has shown that the 1 kHz block is safe in acute application since it is always reversible (Figs. 1–2). However, for a long-term clinical application the safety of the 1 kHz block still needs to be determined in a chronic animal study using a fully implantable device.

To prevent DSD and improve voiding after SCI, the commercially available Brindley stimulator (Brindley, 1977; van Kerrebroeck et al., 1996) requires sacral posterior root rhizotomy, which is destructive and irreversible and results in the loss of reflex sexual and defecation functions that are very important residual reflexes for SCI people. The long-term goal of our project is to develop a novel fully implantable pudendal neuromodulation device to restore lower urinary tract functions for SCI people without sacral posterior root rhizotomy. Our approach will preserve the spinal reflex functions of the bowel and sexual organs, and more importantly provide the opportunity for SCI people to benefit from advances in neural regeneration and repair techniques in the future.

The mechanism of post-stimulation block induced by 1 kHz stimulation is currently unknown. Our recent modeling studies (Zhong et al., 2021a, 2021b) indicate that the changes induced by high-frequency stimulation in intracellular and extracellular sodium and potassium

concentrations could cause the post-stimulation block. As shown in this study the recovery following stimulation is slow requiring many minutes because after ending the stimulation the ion pump can only slowly restore the intracellular and extracellular sodium and potassium concentrations to normal levels. More animal studies are needed to better understand the mechanism underlying post-stimulation block.

Previous studies (Bowman and McNeal, 1986; Crosby et al., 2017; Kilgore and Bhadra, 2004; Tai et al., 2005) found that a stimulation frequency of 1 kHz is too low to produce nerve conduction block during the stimulation. However, these studies never investigated the possibility of post-stimulation block after termination of the stimulation, which the current and more recent studies showed can occur at frequencies >5 kHz (Bhadra et al., 2018; Wang et al., 2020; Yang et al., 2017) as well as at 100–1000 Hz (Shapiro et al., 2020). In addition, previous studies (Solomonow et al., 1983a, 1983b) also showed that muscle fatigue or partial nerve block could be induced during a 600 Hz stimulation, but the post-stimulation effect was not investigated. Therefore, our result showing post-stimulation block by 1 kHz stimulation is not in conflict with previous studies.

In summary, this study in anesthetized normal cats demonstrated for the first time that the pudendal nerve can be blocked by kHz stimulation with a wire electrode without using a nerve cuff. When post-stimulation block by 1 kHz stimulation is combined with a 30 Hz pudendal nerve stimulation, a low-pressure and highly efficient voiding can be induced. These results indicate that neuromodulation to restore voiding function after SCI might be achieved by insertion of lead electrodes close to the pudendal nerves using a minimally invasive surgical approach instead of implanting nerve cuff electrodes on the pudendal nerves which requires major surgery.

Declaration of Competing Interest

Dr. Tai is the inventor of a patent related to this study. The other authors have no conflicts of interest.

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RESEARCH ARTICLE

Defecation induced by stimulation of sacral S2 spinal root in cats

Jicheng Wang,¹ Zhijun Shen,¹ Bing Shen,¹ Jianan Jian,¹ Travis Hannan,¹ Khari Goosby,¹ William Wang,¹ Jonathan Beckel,² William C. de Groat,² Christopher Chermansky,¹ and Changfeng Tai^{1,2,3}

¹Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania; ²Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania; and ³Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract

The aim of this study was to determine whether stimulation of sacral spinal nerve roots can induce defecation in cats. In anesthetized cats, bipolar hook electrodes were placed on the S1–S3 dorsal and/or ventral roots. Stimulus pulses (1–50 Hz, 0.2 ms) were applied to an individual S1–S3 root to induce proximal/distal colon contractions and defecation. Balloon catheters were inserted into the proximal and distal colon to measure contraction pressure. Glass marbles were inserted into the rectum to demonstrate defecation by videotaping the elimination of marbles. Stimulation of the S2 ventral root at 7 Hz induced significantly ($P < 0.05$) larger contractions (32 ± 9 cmH₂O) in both proximal and distal colon than stimulation of the S1 or S3 ventral root. Intermittent (5 times) stimulation (1 min on and 1 min off) of both dorsal and ventral S2 roots at 7 Hz produced reproducible colon contractions without fatigue, whereas continuous stimulation of 5-min duration caused significant fatigue in colon contractions. Stimulation (7 Hz) of both dorsal and ventral S2 roots together successfully induced defecation that eliminated 1 or 2 marbles from the rectum. This study indicates the possibility to develop a novel neuromodulation device to restore defecation function after spinal cord injury using a minimally invasive surgical approach to insert a lead electrode via the sacral foramen to stimulate a sacral spinal root.

NEW & NOTEWORTHY This study in cats determined the optimal stimulation parameters and the spinal segment for sacral spinal root stimulation to induce colon contraction. The results have significant implications for design of a novel neuromodulation device to restore defecation function after spinal cord injury (SCI) and for optimizing sacral neuromodulation parameters to treat non-SCI people with chronic constipation.

cat; colon; sacral root; spinal cord injury; stimulation

INTRODUCTION

After spinal cord injury (SCI) severe constipation occurs with slow transit of feces in the colon and/or dyssynergic contractions between the colorectum and anal sphincter (1). Clinical management of neurogenic bowel dysfunction, which usually involves digital evacuation, stool softeners, and suppositories, is very challenging (1). A fully implantable stimulator has also been used clinically to restore defecation function after SCI by stimulating sacral anterior roots, after surgically transecting the sacral posterior roots, i.e., sacral posterior rhizotomy (2, 3). Sacral anterior root stimulation requires a major invasive spinal surgery to expose the sacral spinal roots for implantation of stimulation electrodes. Sacral posterior rhizotomy prevents the dyssynergic contraction of the anal sphincter, but it also eliminates spinal reflex defecation and sexual function such as penile erection (4). Therefore, a minimal invasive neuromodulation approach that does not require invasive spinal surgery and sacral posterior rhizotomy will be desirable for management of bowel dysfunction after SCI.

A minimally invasive surgical approach to access the sacral spinal roots for electrical stimulation has been used successfully for many years to treat spinal intact patients with an overactive bladder (5). It uses a foramen needle to insert a tined lead electrode percutaneously in proximity to the S3 spinal root under the guide of fluoroscopic imaging. This minimally invasive sacral neuromodulation approach using S3/S4 spinal root stimulation has also been evaluated in recent years as a treatment for chronic constipation but was ineffective (6–8). However, the stimulation parameters/pattern used for sacral neuromodulation to treat overactive bladder may not be suitable for modulation of colorectal motility to improve chronic constipation caused by slow transit of feces in the colon. Therefore, optimization of sacral neuromodulation parameters/pattern for improving colorectal motility or inducing defecation is needed so that this minimally invasive surgical approach could be used as an effective therapy for SCI or non-SCI people with chronic constipation.

In this study using spinal cord intact cats under anesthesia, we investigated the effects of electrical stimulation of S1–S3 roots on colon activity to determine which spinal root and

which stimulation parameters are effective in inducing colon contraction and/or defecation. This preclinical study is important to better understand the potential of stimulating a sacral spinal root using the minimally invasive surgical approach as used in current clinical application (5) to restore defecation function in SCI people or for treating chronic constipation in non-SCI people.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical Procedures

A total of nine cats (4 females and 5 males, 4.0 ± 0.3 kg; Liberty Research, Waverly, NY) were anesthetized with isoflurane (2%–5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg iv and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847 V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, one balloon catheter (G15766, Cook Urological, Spencer, IN) 1.4 cm in diameter and 5 cm in length was inserted into the proximal colon via a small incision at the proximal end of the colon (Fig. 1). The second balloon catheter of the same size was inserted into the distal colon via the anus. The two balloons were filled with water (5–7 mL) to a resting pressure of 10–15 cmH₂O to measure proximal and distal colon contractions in the first group of seven cats. In the second group of two cats, a large balloon catheter made of a condom (3.5 cm in diameter and 15 cm in length) was inserted via the small incision at the proximal end of the colon to occupy the entire colon so that

both proximal and distal colon contractions were recorded. The condom was filled with water (60–70 mL) to a resting pressure of 10–15 cmH₂O. In these two cats, 1–4 glass marbles (diameter 1.5 cm) were also inserted into the rectum via the anus so that evacuation of these marbles could be videotaped simultaneously with the colon pressure recording.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1–S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together. The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35°C–37°C) mineral oil. A heating pad was used to maintain the animal body temperature between 35°C and 37°C. Bipolar stainless steel hook electrodes (2–3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Experimental Protocol

In the first group of seven cats, stimulation (7 Hz frequency, 0.2 ms pulse width, and 1 min duration) was delivered via the hook electrode to individual sacral ventral roots (S1, S2, or S3) at different intensity (1–16 V) to determine the intensity threshold (T) for inducing an observable pressure increase in the proximal colon. Then, an intensity (1.5–3 T) that induced the maximal colon contraction was used to test colon responses to different stimulation frequencies (1–50 Hz). If no colon contraction was induced by stimulation of an individual ventral root, the maximal stimulation intensity that did not produce movement of the animal's lower body was used to test the frequency response. It was our intention not to fix the hip and lower spine, so that sacral root stimulation by the hook electrode could produce local movement of the tail or hindlimb. However, the maximal stimulation intensity was limited by any movement of the lower spine,

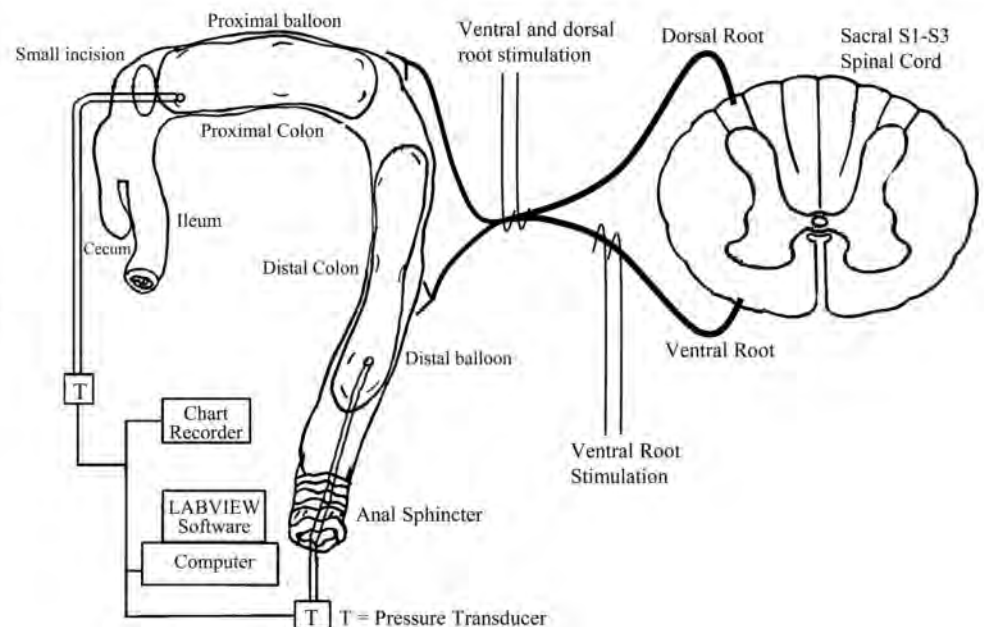


Figure 1. Experimental setup for electrical stimulation of sacral roots S1, S2, or S3 to induce colon contractions.

which would displace the hook electrode from the nerve. After stimulating each ventral root, the S2 ventral root, which elicited the largest colon contraction, was combined with the S2 dorsal root and the combination was stimulated with the most effective frequency (7 Hz.). The combined S2 root stimulation (1 min duration) was applied five times at 1-min intervals and then followed by a continuous 5-min stimulation to determine any fatigue in colon contraction.

In the second group of two cats, the combined S2 ventral and dorsal roots were stimulated (7 Hz) at an intensity that induced the largest colon contraction (distal or proximal colon) and for various durations (2.5–11 min) to determine if the stimulation could induce defecation, i.e., the evacuation of marbles that were inserted into the rectum. The defecation induced by S2 root stimulation was videotaped, and the time of evacuation of the marbles was marked on the colon pressure recording.

Data Analysis

To compare the colon contractions induced by stimulation of different ventral roots at different frequencies, the contraction amplitudes were always normalized to the maximal

contraction amplitude induced by stimulation in the same animal. The colon contractions induced by the repeated 1-min stimulations were normalized to the maximal contraction amplitude induced during the repeated stimulations to determine repeatability. The maximal contraction amplitude induced during the 5-min continuous stimulation, and the contraction amplitude at the end of the 5-min stimulation were compared to determine any fatigue during the continuous prolonged stimulation. The data from different animals were averaged and presented as means $\pm$ SE. Statistical significance ($P < 0.05$) was determined by paired t test or repeated-measures ANOVA followed by Dunnett multiple comparison (one-way) or Bonferroni multiple comparison (two-way).

RESULTS

Colon Contraction Induced by Sacral Ventral Root Stimulation is Dependent on Stimulation Frequency and the Spinal Segment Stimulated

Stimulation of the S2 ventral root induced larger colon contractions than stimulation of S1 or S3 ventral roots, and the most effective stimulation frequency was 7–10 Hz (Figs. 2 and

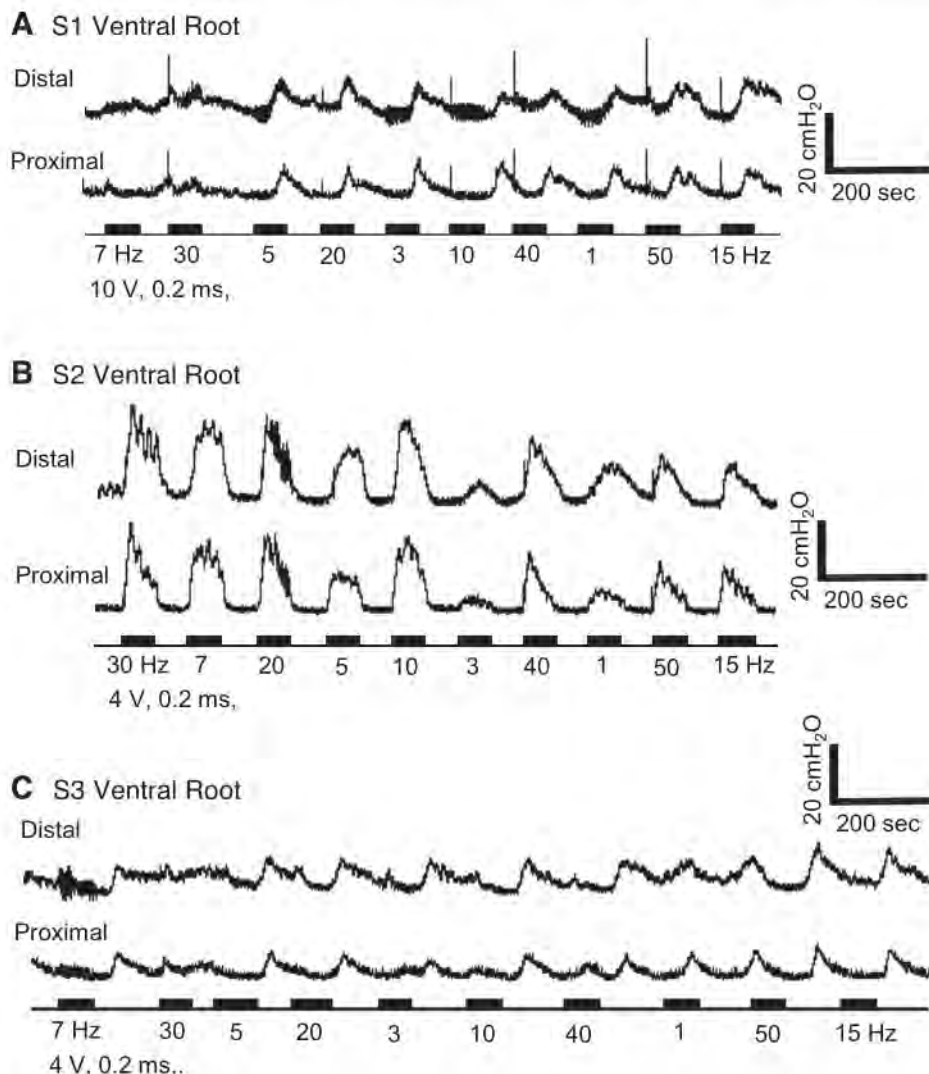


Figure 2. Distal and proximal colon responses to stimulation of sacral S1–S3 ventral roots at different frequencies (1–50 Hz). A: S1 ventral root. B: S2 ventral root. C: S3 ventral root. Data were obtained from the same animal.

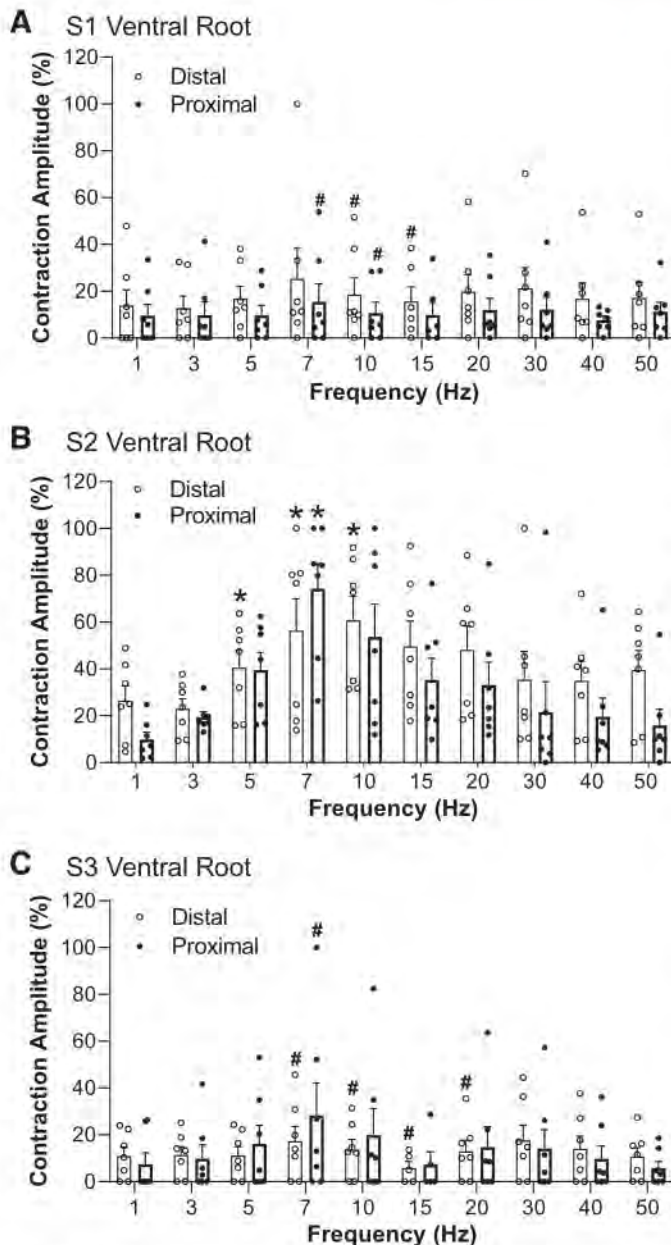


Figure 3. Distal and proximal colon responses to sacral ventral root stimulation are frequency and spinal segment dependent. A: S1 ventral root. B: S2 ventral root. C: S3 ventral root. The contraction amplitude induced by different stimulation frequencies in different sacral segments is normalized to the maximal response in the same animal. Stimulation intensity (4–16 V) for S2 ventral root was 1.5–3 times threshold intensity for 7 Hz stimulation to induce an observable contraction of proximal colon. When S1 and S3 ventral root stimulation did not induce a colon contraction, the maximal stimulation intensity (8–12 V) without causing lower body movement was used to test different frequencies. *Significantly different from 1 Hz (one-way ANOVA); #significantly different from S2 ventral root (two-way ANOVA). $n = 7$ cats.

3). Figure 2 shows the distal and proximal colon pressure traces recorded from the same cat. Stimulation of the S2 ventral root induced colon contractions >20 cmH₂O at frequencies 7–30 Hz with a similar contraction amplitude in the distal and proximal colon (Fig. 2B). Stimulation of the S1 or S3 ventral root did not induce colon contractions larger than the

small (<10 cmH₂O) rhythmic baseline colon contractions that occurred asynchronously during the periods of stimulation in a few experiments (Fig. 2, A and C). In this cat, the stimulation intensity used for S2 ventral root stimulation is two times the threshold intensity used during 7 Hz stimulation to induce an observable proximal colon contraction. Due to the weak colon response to S1 and S3 ventral root stimulation, the maximal stimulation intensity just below the threshold for inducing lower spine movement was applied to these roots. Hindlimb (mainly S1/S2 stimulation) and/or tail (mainly S2/S3 stimulation) movements were induced by stimulation of each of the three roots. Figure 3 summarizes the results from seven cats showing that S2 ventral root stimulation at 7–10 Hz was optimal for producing significantly ($P < 0.05$) larger colon contractions than those elicited by S1 or S3 ventral root stimulation. There was no significant difference between the contraction amplitudes of distal and proximal colon (Fig. 3).

Fatigue of Colon Contractions Induced by S2 Root Stimulation

Stimulation (7 Hz) of the S2 ventral and dorsal roots together induced large amplitude (>20 cmH₂O) contractions in both distal and proximal colon (Fig. 4). Figure 4A shows that intermittent 1-min stimulation at 1.5 times threshold intensity for inducing a proximal colon contraction produced a stronger contraction during the second period of stimulation and that the large amplitude contraction was maintained throughout the next three 1-min stimulations. However, the contraction amplitude gradually declined during a continuous 5-min stimulation, indicating a fatigue in the colon contractions (Fig. 4A). On average the contraction amplitude for both the distal and proximal colon was maintained during the five intermittent 1-min stimulations ($n = 4$ cats, Fig. 4B), but was significantly ($P < 0.05$) reduced from the mean maximal amplitude of 30 cmH₂O to ~ 18 cmH₂O at the end of the 5-min continuous stimulation ($n = 7$ cats, Fig. 4C).

Defecation Induced by S2 Root Stimulation

Stimulation of the S2 ventral and dorsal roots together induced a maximal amplitude colon contraction of 60 cmH₂O in cat 1 (Fig. 5A) and 40 cmH₂O in cat 2 (Fig. 5B). In cat 1 after three marbles were inserted into the rectum, S2 root stimulation (7 Hz, 6 V, 0.2 ms) induced a successful defecation that eliminated the first marble after 1 min of stimulation and the second marble after 2 min of stimulation (Fig. 5A). The elimination of the third marble, which started during the last 2 min of the 6-min stimulation, was incomplete (Fig. 5A). In cat 2 after one marble was inserted into the rectum, the S2 root stimulation (7 Hz, 6 V, 0.2 ms) eliminated the marble after 2.5 min of stimulation (Fig. 5B). However, after four marbles were inserted into the rectum, the stimulation eliminated only one of the four marbles after 4.5 min of stimulation with three marbles in the rectum at the end of 11 min of stimulation (Fig. 5B). Although stimulation of S2 ventral and dorsal roots together only eliminated 1 or 2 marbles in these two cats, stimulating the S2 ventral root alone without the dorsal root successfully eliminated all four marbles in both cats.

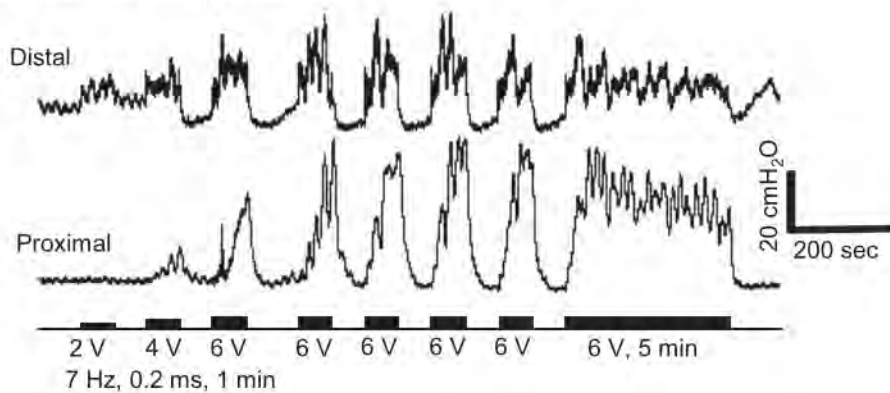
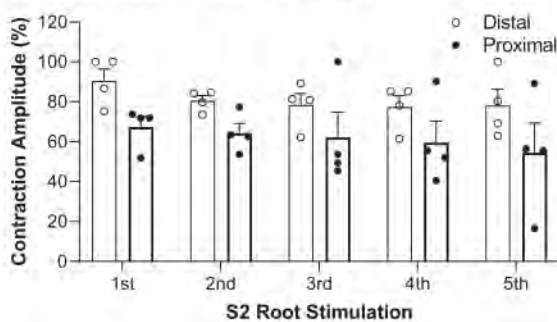
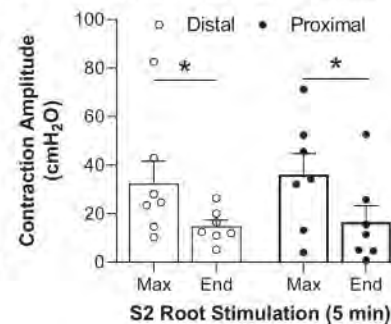
A S2 Ventral and Dorsal Roots

Figure 4. Distal and proximal colon responses to intermittent (5×1 min) or long duration (5 min) continuous stimulation of both S2 ventral and dorsal roots. **A:** pressure traces showing distal and proximal colon contractions. The intensity threshold (T) for proximal colon contraction was 4 V, and 6 V (1.5 T) was used to induce the intermittent or continuous contractions. **B:** summarized results ($n = 4$ cats) showing that the contraction amplitudes were maintained during intermittent stimulation (7 Hz, 0.2 ms, 1.5–4 T, T = 2–4 V). **C:** summarized results ($n = 7$ cats) showing that the maximal (Max) contraction amplitude was significantly reduced from mean 30 cmH₂O to about 18 cmH₂O at the end of the continuous 5-min stimulation (7 Hz, 0.2 ms, 1.5–3 T, T = 2–4 V). *Significantly different ($P < 0.05$, paired *t* test).

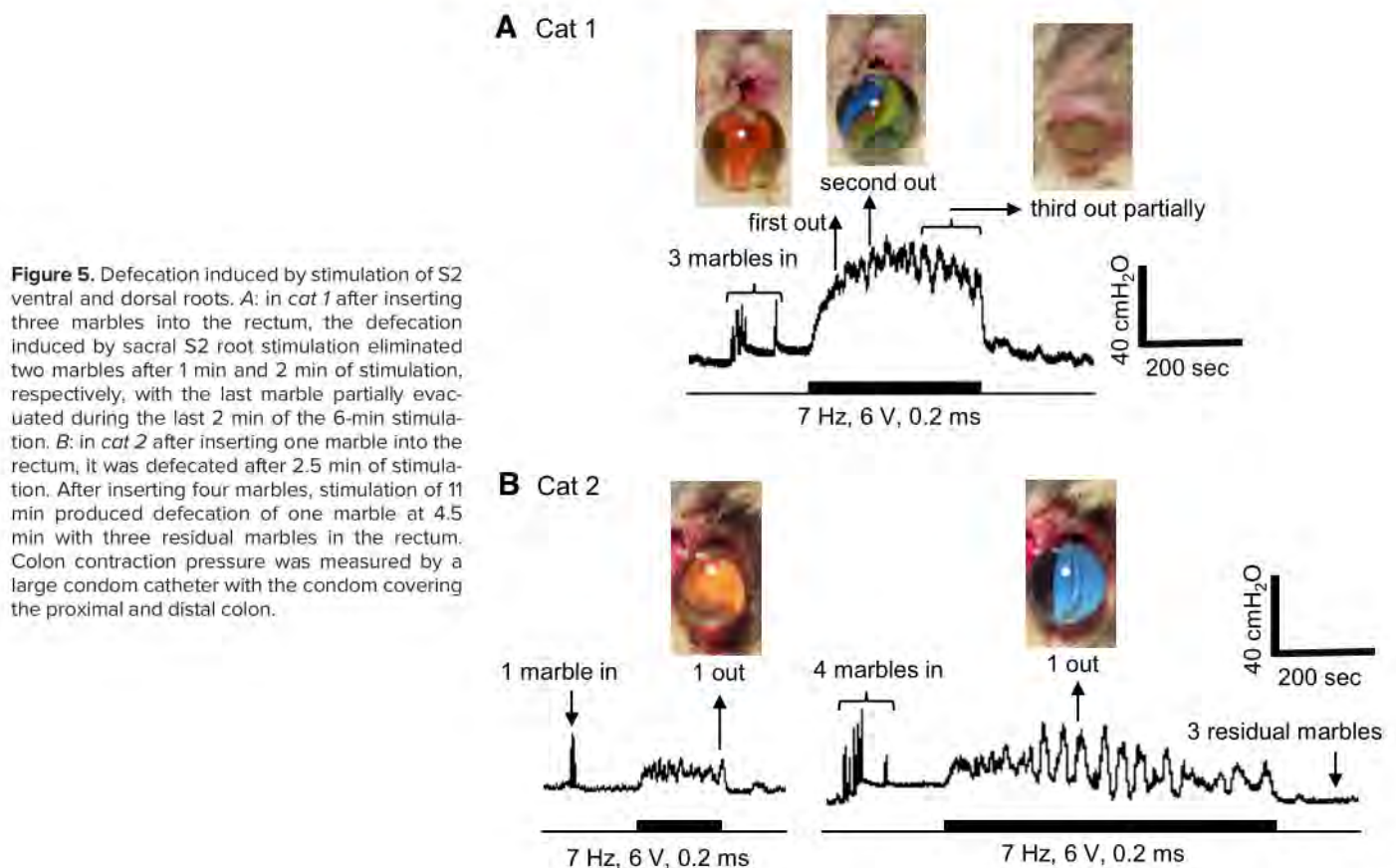
B Intermittent 1-min Stimulation**C** 5-min Continuous Stimulation**DISCUSSION**

This study in anesthetized cats shows that the colon response to sacral root stimulation is dependent on stimulation frequency and the spinal segment stimulated. S2 ventral root stimulation at 7 Hz is optimal to induce both proximal and distal colon contractions (Figs. 2 and 3). Stimulation of the S2 ventral and dorsal roots together is also effective in inducing colon contraction (Fig. 4A), and intermittent stimulation (1 min on and 1 min off) is more fatigue resistant than continuous stimulation (Fig. 4, B and C). More importantly, defecation can be induced by S2 spinal root stimulation (Fig. 5).

These results have significant implications for developing a new neuromodulation device to restore defecation function in humans after SCI. Since stimulation of the entire S2 spinal root (ventral and dorsal) is as effective as stimulation of the ventral root alone (Fig. 3) and successfully induces defecation (Fig. 4), sacral posterior rhizotomy may not be necessary, and stimulation of sacral spinal roots instead of ventral roots could be effective in restoring defecation function after SCI in human. The major reason to perform sacral posterior rhizotomy is to eliminate the detrusor sphincter dyssynergia (DSD) that can generate high pressure in the bladder and cause kidney damage (2, 9). However, a high pressure in colon and/or rectum might not be as harmful as the high bladder pressure that can cause kidney failure. If colorectal pressure is high enough to overcome the dyssynergic anal sphincter contraction, feces should be evacuated. In addition, our recent studies in cats (10, 11) have developed an

effective method to transiently suppress sphincter activity by blocking pudendal nerve conduction using kHz electrical stimulation. This method is effective in treat DSD in spinal cord injured cats (10, 11) and could also be applied to treat dyssynergic defecation if a strong enough colorectal contraction could not be generated to overcome the dyssynergic anal sphincter contraction. Therefore, a novel neuromodulation device may stimulate the sacral spinal root instead of the ventral root and at the same time prevent DSD and/or dyssynergic defecation by blocking pudendal nerve conduction to restore bladder function as well as defecation function after SCI. The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to use a minimally invasive surgical approach for stimulation. S1–S3 spinal roots are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder (5).

S2 ventral root stimulation eliminated all four marbles, but stimulation of S2 ventral and dorsal roots together only eliminated 1 or 2 marbles (Fig. 5). This difference suggests that stimulating sensory nerves in the dorsal root might have induced additional reflexes to the anal sphincter, which caused dyssynergic defecation and prevented complete emptying of the rectum. This possibility was shown clearly in *cat 1*, where the evacuation of the third marble was incomplete due to the anal sphincter contraction holding the marble in place (Fig. 5A). If the pudendal nerve were blocked in this experiment, the external anal sphincter would have been relaxed and the third marble would have been fully



evacuated. However, pudendal nerve block will be ineffective if the dyssynergic defecation is caused by the contractions of internal anal sphincter (smooth muscle) instead of the external anal sphincter (striated muscle). The internal anal sphincter and rectal contraction pressures were not recorded in this study to insert marbles into the rectum to test defecation. A small movable balloon in the distal bowel is needed to measure pressure simultaneously as the balloon is eliminated during defecation (12). Thus, additional studies are warranted to better understand the rectum and anal sphincter coordination during the defecation induced by sacral root stimulation. It is worth noting that in cats some sensory fibers travel in the ventral roots to the spinal cord (13, 14). However, our results show that defecation induced by stimulating the ventral root alone is more efficacious than that induced by stimulating ventral and dorsal roots together. This difference indicates that the small population of sensory fibers traveling in the ventral root did not affect the responses induced by stimulation of the motor fibers in the ventral root as much as activation of the sensory fibers in the dorsal root.

In this study, the colon contraction and defecation induced by S2 root stimulation always required a stimulation intensity higher than the intensity to induce hindlimb and/or tail movement. This result is expected because in cats the afferent and efferent nerve fibers in the S2 spinal root innervating the colon are small unmyelinated C-fibers that have higher excitation threshold than the large motor fibers (12,

15). Since small C-fibers in the dorsal root are also involved in transmission of nociception, the stimulation intensity required for defecation could also generate painful sensation at the same time. This should not be a critical issue for restoring defecation function in people with a complete SCI, since they have no sensation below the level of the injury. However, it will be a problem to induce defecation for non-SCI people with chronic constipation if the extrinsic innervation of the human colon is similar to that in cats. Previous studies in humans (16, 17) have shown that colon contractions can be induced by sacral root stimulation at a suprasensory intensity without causing painful sensation, indicating that the sacral nerve fibers innervating the colon in humans might be different from those in cats. Therefore, it might be possible to induce defecation for non-SCI people with chronic constipation by sacral root stimulation without producing painful sensation.

Recent tests of sacral neuromodulation for treating non-SCI people with chronic constipation failed (6–8), which could be partially due to the use of a low stimulation intensity that only produces somatic sensations. This low stimulation intensity is probably not directly stimulating the nerve fibers in the sacral spinal root innervating the colon. However, it may modulate colon function indirectly by activating the large somatosensory fibers in the sacral root that can trigger central mechanisms to facilitate colon motility via the enteric nervous system (18). This central modulation mechanism could be sensitive to stimulation frequency, but

only a single stimulation frequency of 14 Hz was used in recent clinical trials to treat non-SCI people with chronic constipation (6–8). Our study shows that a lower frequency 7 Hz is optimal for sacral root stimulation to induce colon contractions, whereas other studies in rats and dogs (19, 20) found an effective frequency of 5 Hz or 10 Hz. Maximal colon contraction could also be induced in cats by directly stimulating the pelvic nerve at a frequency as low as 8 Hz (21). Therefore, it seems that a frequency lower than 14 Hz should be tested in clinical studies to treat non-SCI people with chronic constipation.

Our study also shows that continuous stimulation can cause fatigue of colon contractions, whereas intermittent stimulation is more fatigue resistant (Fig. 4). This indicates that the continuous stimulation of a sacral spinal root used in recent clinical trials (6–8) to treat chronic constipation may not be optimal and intermittent stimulation may be more effective in modulating colon motility to improve colon transit time. Recent animal studies (20, 22) have begun to focus on developing optimal stimulation parameters to facilitate colon motility and improve sacral neuromodulation therapy for non-SCI people with chronic constipation.

In summary, this study in cats optimized the stimulation parameters for sacral spinal root stimulation to induce colon contraction. The results have significant implications for design of a novel neuromodulation device to restore defecation function after SCI, and for optimizing sacral neuromodulation parameters to treat non-SCI people with chronic constipation.

GRANTS

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DISCLAIMERS

The views, opinions, and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

DISCLOSURES

Dr. Tai is the inventor of a patent related to this study. No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. conceived and designed research; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. performed experiments; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. analyzed data; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. interpreted results of experiments; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. prepared figures; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. drafted manuscript; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. edited and revised manuscript; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. approved final version of manuscript.

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Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats

Zhijun Shen ¹, Jicheng Wang ¹, Bing Shen ¹, Jianan Jian ¹, Khari Goosby ¹, William Wang ¹,
Jonathan Beckel ², William C. de Groat ², Christopher Chermansky ¹, Changfeng Tai ^{1,2,3}

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

³ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Running Head: Penile Erection by Sacral Root Stimulation

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Correspondence to:

Changfeng Tai, Ph.D.
Department of Urology
University of Pittsburgh
700 Kaufmann Building
Pittsburgh, PA 15213
Phone: 412-692-4142
Fax: 412-692-4380
Email: cftai@pitt.edu

ABSTRACT

This study aimed at determining if stimulation of sacral spinal roots can induce penile erection in cats. In anesthetized cats, a 20-gauge catheter was inserted into the corpus cavernosum to measure the penile pressure. Stimulus pulses (5-80 Hz, 0.2 ms) were applied via bipolar hook electrodes to sacral ventral roots alone or to combined ventral and dorsal roots of a single S1-S3 segment to induce penile pressure increases and penile erection. Stimulation of the S1 or S2 ventral root at 30-40 Hz induced observable penile erection with rigidity and the largest increase (>150 cmH₂O) in penile pressure. Continuous stimulation (10 minutes) of afferent and efferent axons by simultaneous stimulation of the S1 or S2 dorsal and ventral roots at 30 Hz also produced a large (about 150 cmH₂O on average) increase in penile pressure that was sustainable during the entire stimulation period. After a complete spinal cord transection at the T9-T10 level, simultaneous stimulation of the S1 or S2 dorsal and ventral roots induced large (>100 cmH₂O) and sustainable increases in penile pressure. This study indicates the possibility to develop a novel neuromodulation device to restore penile erection after spinal cord injury using a minimally invasive surgical approach to insert a lead electrode via the sacral foramen to stimulate a sacral spinal root.

Keywords: stimulation, sacral spinal root, erection, spinal cord injury, cat

INTRODUCTION

After spinal cord injury (SCI) erectile dysfunction presents as a short duration or an absence of penile erection, or poor penile rigidity that is insufficient for sexual intercourse (1,2). Although drug treatment is effective in restoring erectile function after SCI, it is not suitable for every SCI person due to the drug side effects and the health conditions of the SCI person (2). In addition to drug treatment, a fully implantable stimulator has also been used successfully in humans to restore penile erection after SCI by stimulating sacral anterior roots (3-5). However, sacral anterior root stimulation requires a major invasive spinal surgery to expose the sacral spinal roots for implantation of stimulation electrodes. Therefore, a minimally invasive neuromodulation approach that does not require invasive spinal surgery will be desirable for treatment of erectile dysfunction after SCI.

A minimally invasive surgical approach for stimulation of sacral spinal roots has been available for many years to treat patients with an overactive bladder (6). It employs a foramen needle to insert a tined lead electrode percutaneously under the guide of fluoroscopic imaging in proximity to the S3 spinal root that consists of both anterior (motor) and posterior (sensory) spinal roots. This minimally invasive sacral neuromodulation approach has also been evaluated in recent years to treat erectile dysfunction with a significant improvement in quality of sexual life for 20-40% subjects (7,8). However, the sacral neuromodulation stimulation parameters used in these studies to treat erectile dysfunction may not be optimal for improving penile erection because they were the same as those used to treat overactive bladder. Therefore, optimization of sacral neuromodulation parameters for treatment of erectile dysfunction is needed so that this minimally invasive surgical approach could be used as an effective therapy for SCI or non-SCI people with erectile dysfunction.

In this study using anesthetized cats, we investigated the penile response to electrical stimulation of an individual S1-S3 ventral root to determine the optimal stimulation frequency and the most effective spinal root in inducing penile erection. Then, combined stimulation of ventral and dorsal roots (termed spinal root stimulation) of the most effective spinal segment (S1 or S2) was used to induce sustainable (>10 minutes) penile erection. The information obtained in this preclinical study is important to design a human clinical trial to further evaluate sacral spinal root stimulation using the minimally invasive surgical approach as used in current clinical application (6) to treat erectile dysfunction in both SCI and non-SCI people.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical procedures

A total of 8 male cats (4.6 ± 0.2 kg) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder

via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (see Fig.1). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Experimental protocol

At the beginning of the experiment, stimulation (30 Hz frequency, 0.2 ms pulse width, and 1-2 min duration) was delivered via the hook electrode to sacral S1 ventral root at a minimal intensity (0.5 V). If no erection was observed, the stimulation intensity was increased and then tested again until a penile erection was observed. If S1 ventral root stimulation was not effective, then the stimulation electrode was moved to the S2 ventral root, and the test was repeated. A penile erection, which was evident as a full protrusion of the penis with rigidity resistant to bending, was always observed by stimulation of the individual S1 and S2 ventral roots. After the penile erection was observed, a 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (see

Fig.1). Since electrically evoked penile protrusion is very well correlated with increased corpus cavernosum pressure (9,10), the pressure recording was used as a quantitative measurement of penile erection in this study.

After placement of the penile catheter (N=8 cats), stimulation (30 or 40 Hz frequency, 0.2 ms pulse width, and 1 min duration) was delivered via the hook electrode to individual left or right sacral ventral roots (S1, S2, or S3) at a range of intensities (0.5-15 V) to determine the intensity threshold (T) for inducing an observable increase in penile pressure. Then, an intensity (1.5-3T) that induced the maximal penile pressure (>100 cmH₂O) was used to test penile responses to different stimulation frequencies (5-80 Hz). If no penile pressure >100 cmH₂O was induced by stimulation of an individual ventral root, the maximal stimulation intensity that did not produce movement of the animal's lower body was used to test the frequency response. It was our intention not to fix the hip and lower spine, so that sacral root stimulation by the hook electrode could produce local movement of the tail or hindlimb. However, the maximal stimulation intensity was limited by any movement of the lower spine, which would displace the hook electrode from the nerve. After testing each ventral root, the ventral root (S1 or S2) that elicited the largest penile pressure was combined with the dorsal root and the combination was stimulated continuously for 10 mins with the most effective frequency (30 Hz) and intensity to determine if the induced penile erection was sustainable during the entire stimulation period. Finally, the spinal cord was completely transected at the T9-T10 level (N=6 cats). Then, the continuous 10-minute stimulation of the same spinal root (ventral and dorsal roots together) was tested again to induce penile erection.

Data analysis

To compare the penile erection responses induced by stimulation of different ventral roots at different frequencies, the maximal amplitudes of the penile pressure induced by stimulation were measured and averaged across different animals for the same stimulation conditions. To determine the sustainability of the induced penile erection, the maximal penile pressure induced by the continuous 10-minute stimulations was compared to the pressure at the end of the 10-minute stimulation. To determine the effect of the spinal cord transection, the maximal penile pressures induced by the continuous 10-minute stimulation were compared before and after the spinal cord transection. The data from different animals are averaged and presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined by paired t-test or repeated-measures one-way ANOVA followed by Dunnett multiple comparison.

RESULTS

Penile erection dependent on stimulation frequency and the spinal segment stimulated.

Penile erection was observed as a full protrusion of the penis with rigidity in 6 cats by stimulation (30 Hz, 0.2 ms, 4-8 V) of S1 ventral root (S2 and S3 ineffective) and in 2 cats by stimulation of S2 ventral root (S1 and S3 ineffective). These responses which were quantified by penile pressure recordings showed that S1 or S2 ventral root stimulation in 6 cats and 2 cats, respectively, induced large increases in pressure (about 150 cmH₂O on average, Fig.2 A-C). The optimal stimulation frequency to induce penile erection by S1 or S2 ventral root stimulation was 30-40 Hz on average (Fig.2D), although lower frequencies (10-20 Hz, see Fig.2A) or higher frequencies (60-80 Hz, see Fig.2 A-B) could also induce large increases in penile pressure in

some cats. The stimulation intensity (0.6-12 V, mean 3.5 ± 1.4 V) for 30-40 Hz S1 or S2 ventral root stimulation to induce the largest increase in penile pressure was always above the motor threshold to induce leg muscle contraction (S1) or anal sphincter contraction (S2).

Sustainable penile erection induced by S1/S2 spinal root stimulation

Continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (i.e., ventral and dorsal roots together) induced a large increase (>150 cmH₂O) in penile pressure (Fig.3). The maximal penile pressure was either sustained (Fig.3A) or gradually developed (Fig.3B) during the 10-minute stimulation. On average (N=8 cats), the penile pressure at the end of 10-minute stimulation was not significantly ($p>0.05$) different from the maximal pressure developed during the stimulation (Fig.3C). The maximal penile pressure induced by stimulation of ventral and dorsal root together was also not significantly ($p>0.05$) different from the maximal pressure induced by stimulation of ventral root alone (Fig.2D and Fig.3C).

After a complete spinal cord transection at the T9-T10 level, continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (ventral and dorsal roots together) also induced a large increase (>100 cmH₂O) in penile pressure (Fig.4). As seen prior to spinal transection, the maximal penile pressure was also sustained or gradually developed (Fig.4B) during the 10-minute stimulation. However, on average (N=6 cats) the induced penile pressure was significantly ($p<0.05$) reduced to 113 ± 19 cmH₂O after spinal cord transection when compared to the penile response (186 ± 9 cmH₂O) before the spinal cord transection (Fig.4C). The blood pressure was also significantly ($p<0.05$) reduced from 229 ± 11 cmH₂O to 186 ± 7 cmH₂O by spinal cord transection, but it was not changed by sacral root stimulation.

DISCUSSION

This study in anesthetized cats shows that the penile erection induced by sacral root stimulation is dependent on stimulation frequency and the spinal segment stimulated. S1/S2 ventral root stimulation at 30-40 Hz is optimal to induce the largest increase (>150 cmH₂O) in penile pressure (Fig.2). Stimulation of the S1/S2 spinal root (ventral and dorsal roots together) is also effective in inducing a large penile pressure increase that is sustainable for the entire 10-minute stimulation (Fig.3). More importantly, large sustainable penile pressure increase (>100 cmH₂O) can also be induced by S1/S2 spinal root stimulation after an acute complete transection of the spinal cord (Fig.4). These results are important for the design of a new neuromodulation device to treat erectile dysfunction.

Previous studies have shown that penile erection can be induced by electrical stimulation of pelvic nerve, cavernous nerve, or dorsal penile nerve in mice, rats, cats, dogs, monkeys, or humans (11-15). However, the pelvic and cavernous nerves require invasive surgery for implantation of stimulation electrodes while the dorsal penile nerve is not a convenient location to attach a stimulation electrode during sexual intercourse. Therefore, these stimulation methods are not adopted for clinical application to treat erectile dysfunction. Sacral ventral root stimulation was also shown to be effective in inducing penile erection in this study and in previous studies using cats (16) and dogs (17). In addition, sacral anterior root stimulation was used successfully to restore bladder function and erectile function for SCI people (3-5,18). However, invasive spinal surgery is required to implant the stimulation electrodes on the sacral anterior roots, preventing this effective treatment to be used in SCI or non-SCI people for the sole purpose of treating erectile dysfunction. Our study in cats indicates that penile erection can be induced by stimulation of the sacral spinal roots. A previous study in dogs also showed a

similar effect (17). The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to employ a minimally invasive surgical approach for stimulation. Sacral spinal roots in humans are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder (6). Therefore, our study has significant implications for developing a new sacral neuromodulation therapy using a minimally invasive surgical approach to restore erectile function for both SCI and non-SCI people.

Previous studies using sacral neuromodulation to treat overactive bladder also evaluated the effects on penile erection in both SCI and non-SCI people (7,8). Questionnaires indicate that subjects with moderate-mild erectile dysfunction significantly improved their quality of sexual life by sacral neuromodulation therapy. However, these studies used stimulation parameters optimized to treat overactive bladder, i.e., stimulation frequency 20 Hz at the sensory threshold intensity. These stimulation parameters may not be optimal for the treatment of erectile dysfunction because our study indicates that a higher stimulation frequency (30-40 Hz) at intensities above sensory and motor thresholds can induce penile erection quickly once the stimulation is turned on and the erection can be sustained during the entire 10-minute stimulation. This type of on-demand erection should be much more satisfactory for the patient than the quality-of-life improvement produced by continuous (24 hours x 7 days) sacral neuromodulation at a lower frequency and weaker stimulation intensity. Therefore, it seems that a higher frequency (30-40 Hz) and a stronger stimulation intensity (above sensory and motor thresholds) should be tested in clinical studies to treat erectile dysfunction. Although leg movement will be induced by a stronger stimulation, the leg muscle contraction will be tonic and the movement will only occur at the beginning of the stimulation, which should not be a problem for successful

sexual intercourse. When anterior root stimulation was used to induce penile erection in SCI people, it also induced leg movement but did not prevent successful sexual intercourse (18).

Stimulation of the sacral spinal root (ventral and dorsal roots together) induced a large increase in penile pressure similar to that produced by stimulation of the ventral root alone (Fig.3). This result indicates that activation of additional sensory nerves in the dorsal root did not disrupt the penile erection induced by direct stimulation of the efferent nerves in the ventral root. A previous study in dogs (17) also reported no difference in penile erection elicited by ventral root stimulation alone or by stimulation of ventral and dorsal roots together. In human application under awake conditions, activation of sensory nerves may cause uncomfortable or painful sensation that can limit the stimulation intensity and therefore limiting the efficacy of the stimulation in treating erectile dysfunction. This potential issue can only be resolved by a clinical study in non-SCI human subjects. However, it should not be an issue for subjects with a complete SCI who have no sensation below the injury.

Our study shows that the penile erection induced by sacral root stimulation is sustainable or gradually develops during the 10-minute stimulation (Fig.3 A-B). This indicates that sacral root stimulation can produce long duration erections when it is applied for a longer time. It also indicates that stimulation of afferent nerves in the dorsal root did not reflexively activate the inhibitory sympathetic pathway that can suppress penile erection (19). Sacral root stimulation probably activated both parasympathetic and somatic pathways to produce the erection and penile rigidity (19, 20). However, after spinal cord transection the amplitude of the induced penile pressure was significantly reduced, possibly due to the spinal shock effect and the decrease in blood pressure after an acute spinal cord transection. Nevertheless, sacral root stimulation still induced a large (>100 cmH₂O on average, Fig.4) sustained increase in penile

pressure after acute spinal cord transection, indicating that this stimulation could be useful in restoring erectile function after SCI (21). Whether sacral root stimulation will be less effective in SCI people than in non-SCI people to induce penile erection is a question to be answered by human clinical studies. However, it is known that sacral anterior root stimulation is effective in inducing penile erection strong enough for sexual intercourse in SCI people (3-5).

In summary, this study in cats optimized the stimulation parameters for sacral spinal root stimulation to induce penile erection. The results have significant implications for design of a novel neuromodulation device to restore erectile function after SCI, and for optimizing sacral neuromodulation parameters to treat non-SCI people with erectile dysfunction.

GRANTS

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DISCLOSURES

Dr. Tai is the inventor of a patent related to this study. The other authors have no conflicts of interest.

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FIGURE CAPTION

Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

Fig.2: Penile pressure in the corpus cavernosum induced by electrical stimulation of sacral ventral roots at different frequencies and different spinal segments. A. In a cat, S1 ventral root stimulation (10-80 Hz) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. B. In another cat, S2 ventral root stimulation (b)(4) was most effective in inducing large increases (150 cmH₂O) in penile pressure. C. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. * indicates significantly different from S1 ventral root ($p < 0.05$, ANOVA). Stimulation: 30 Hz, 0.6-12 V, 0.2 ms. D. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root. N = 8 cats. * indicates significantly different from 5 Hz ($p < 0.05$, ANOVA). Stimulation: 0.6-12 V, 0.2 ms.

Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually

increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

Fig.4: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the 10 minutes of stimulation. B. In the same cat, 10 minutes after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure during the first 3 minutes and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference ($p=0.0025$, paired t-test).

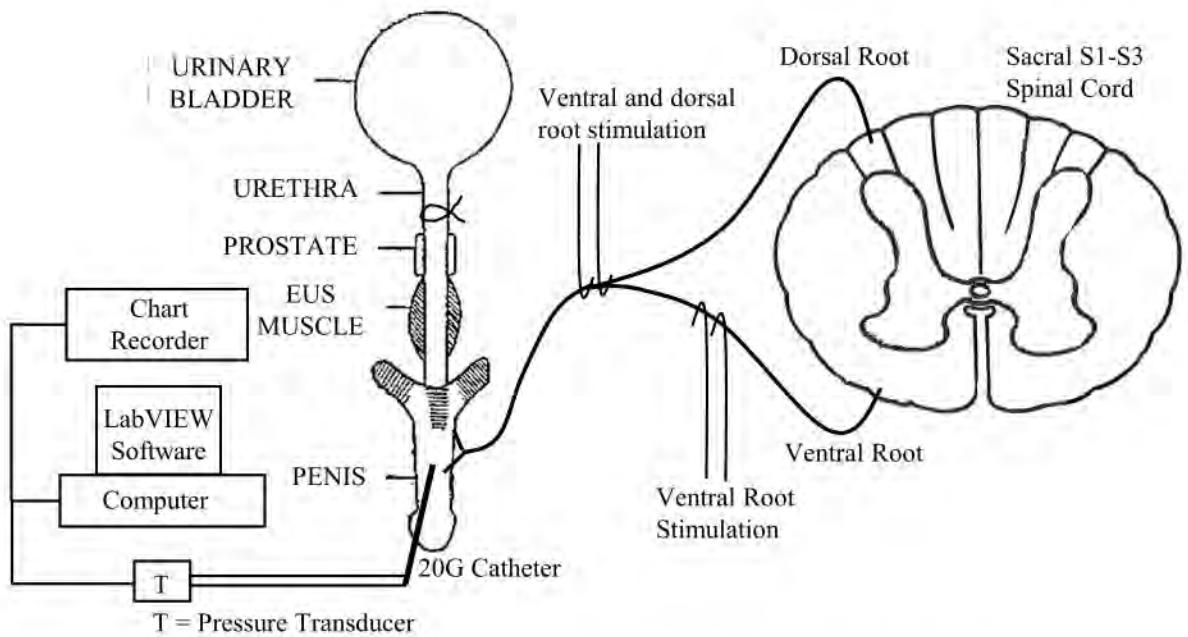
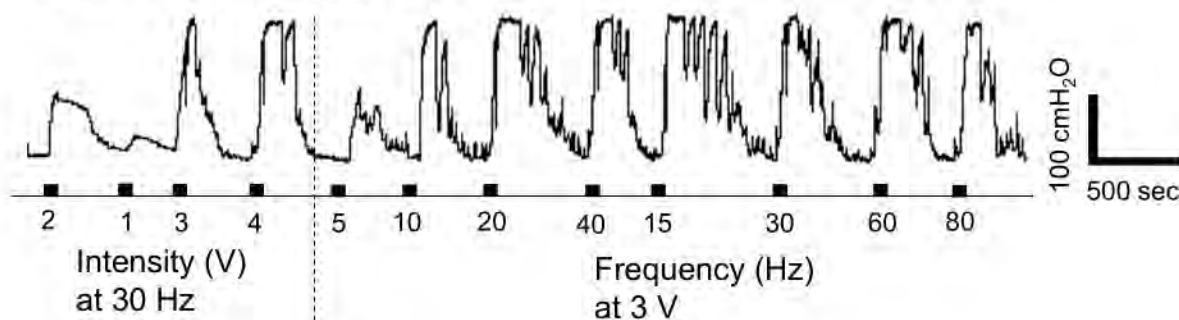
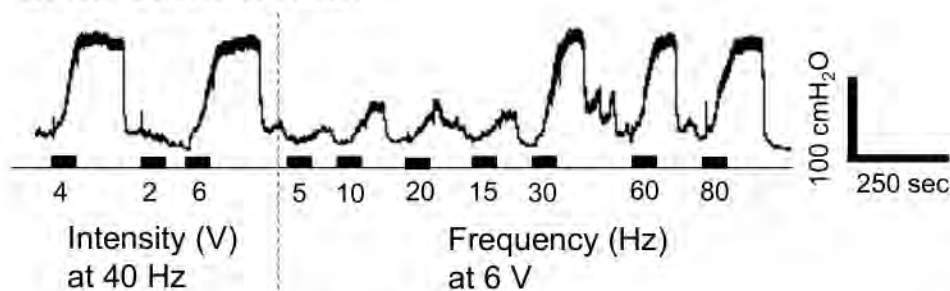


Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

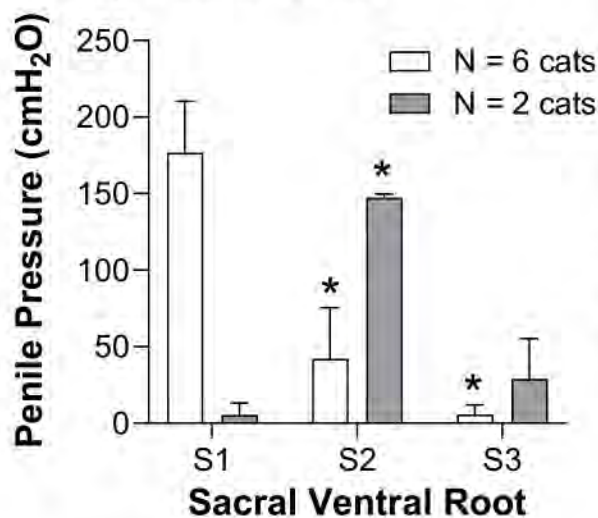
A. S1 Ventral Root



B. S2 Ventral Root



C. Segmental Effect



D. Frequency Effect

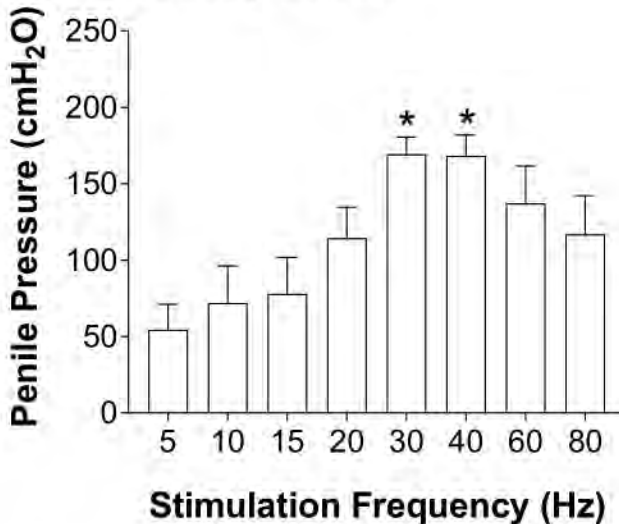
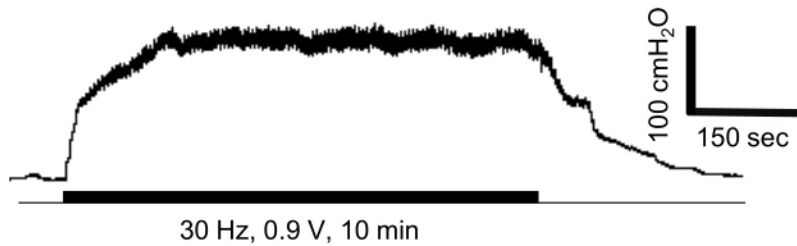
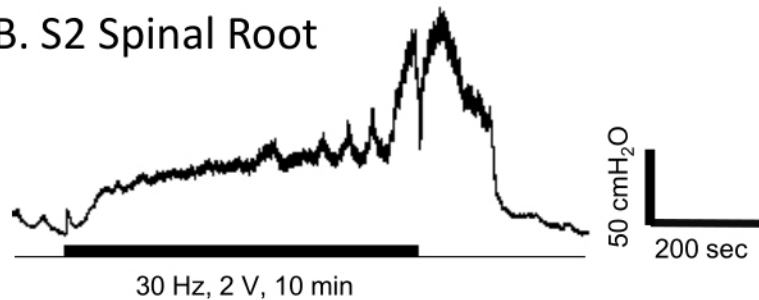


Fig.2: Penile pressure in the corpus cavernosum induced by electrical stimulation of sacral ventral roots at different frequencies and different spinal segments. A. In a cat, S1 ventral root stimulation (10-80 Hz) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. B. In another cat, S2 ventral root stimulation (30-80 Hz) of short duration (1 minute) was most effective in inducing large increases (150 cmH₂O) in penile pressure. C. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. * indicates significantly different from S1 ventral root ($p<0.05$, ANOVA). Stimulation: 30 Hz, 0.6-12 V, 0.2 ms. D. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root. N = 8 cats. * indicates significantly different from 5 Hz ($p<0.05$, ANOVA). Stimulation: 0.6-12 V, 0.2 ms.

A. S1 Spinal Root



B. S2 Spinal Root



C. Sustained Pressure

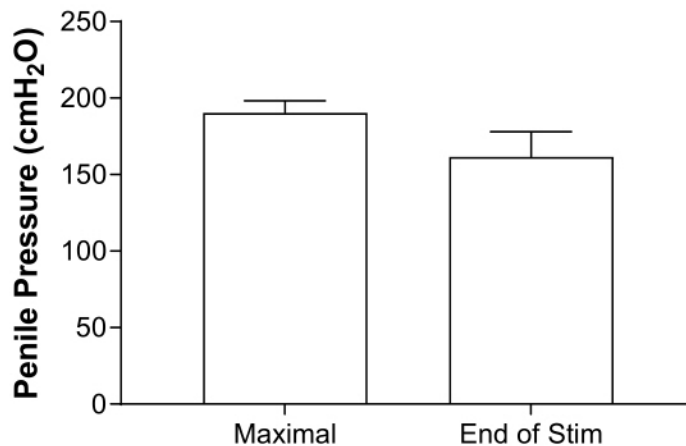
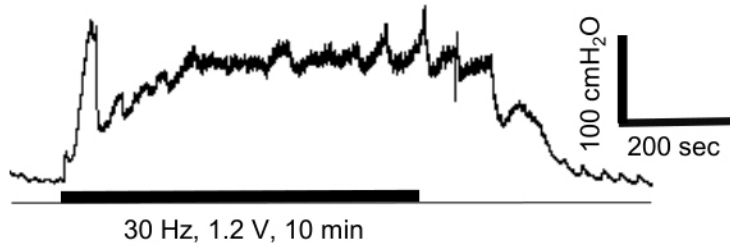
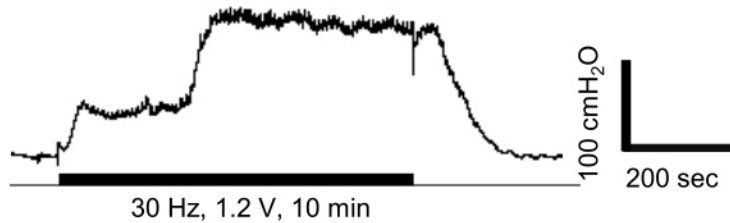


Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

A. Before Spinal Transection



B. After Spinal Transection



C. Maximal Pressure

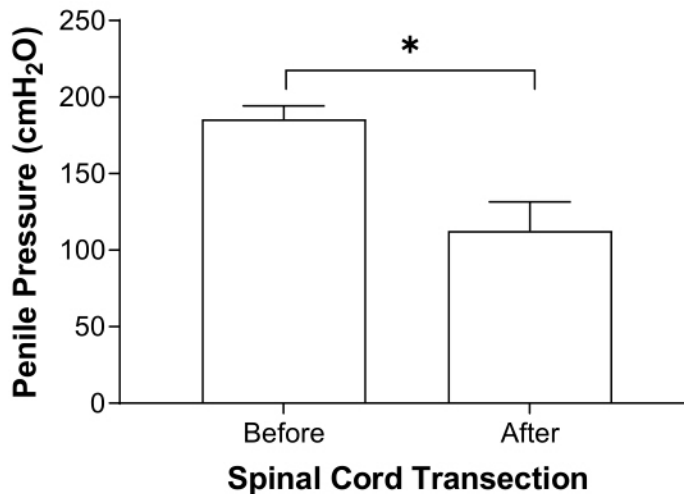


Fig.4: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the 10 minutes of stimulation. B. In the same cat, 10 minutes after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure during the first 3 minutes and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference (p=0.0025, paired t-test).

Withheld pursuant to exemption

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University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: September 30, 2020 through October 31, 2020 (b)(4)

Date of Report: November 30, 2020

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: \$10,451,439.00

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(6) DARPA/BTO

Submitted by:

(b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(6)

Email: (b)(6)@pitt.edu

1 High-Level Project Progress, including Big Wins

Since the contract started on (b)(4) this first quarterly report only covers (b)(4) period ending on (b)(4). During this (b)(4) period, we have made major progresses in the following areas:

1. Scientific progress:
 - Animal study protocol was approved by IACUC at University of Pittsburgh and DOD ACURO.
2. Engineering progress:
 - The engineering team was formed at (b)(4).
 - The (b)(4) and (b)(4) size and length were determined, and the design of the (b)(4) electrode was started.
 - The engineering development of the test bed environment was initiated.

3. Regulatory progress:

- The regulatory strategy was outlined by Thermaquil, Inc. in consultation with the FDA regulatory consulting company, (b)(4)
- FDA IDE (b)(4) was in preparation.
- Protocols for GLP animal study, (b)(4) and early feasibility clinical study were in preparation.

4. Administrative progress:

- Draft subcontracts were sent to Med-Ally and Thermaquil for review and negotiation.

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.1 Institutional IACUC approval and DOD ACURO review (b)(4)

We have obtained IACUC approval from University of Pittsburgh and DOD ACURO earlier than the original plan, which allowed us to initiate the animal study in the (b)(4) instead of (b)(4) as planned originally. Our next step is to initiate the animal study once our animal protocol is reviewed and approved by NAVY IACUC.

Task 1.3 Test Bed Environment (b)(4)

Sub-Task 1.3.1 Complete initial PCB schematic design with design review (b)(4)

The development of Test Bed Environment is based on (b)(4). Therefore, the initial PCB schematic design is mostly completed, but it still needs to be modified to meet this DARPA BG + project's specific requirements. In the next couple of months, (b)(4) will finish the modifications and send the Test Bed Environment to University of Pittsburgh for testing in animal studies.

Task 1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)

Sub-Task 1.5.1 Finalize on the lead/cuff size and length (b)(4)



(b)(4) Figure 1 below. The size and length of cuff

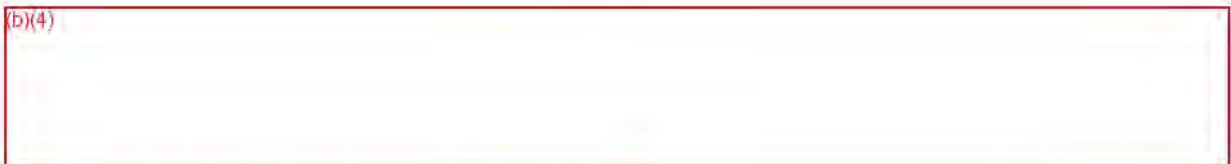
electrode for cat study have been finalized to follow those specifications as determined in our previous cat studies.



Sub-Task 1.5.2 Finalize design of cat lead/cuff (b)(4)

We are investigating if it is possible to further improve the (b)(4) design by reducing its diameter from (b)(4) so that the surgical procedure for lead insertion can be simplified. We will finalize this design in the next couple of months. (b)(4) electrode design has been finalized to follow those specifications as determined in our previous cat studies.

(b)(4)



Task 1.13 Early feasibility human clinical protocol (b)(4)

Thermaquil team has reviewed previous clinical protocol and FDA feedback for applicability to this DARPA BG+ project and are developing new clinical protocol for the (b)(4). This clinical protocol is needed for FDA IDE submission. We will continue develop this clinical protocol as our project progress.

Task 1.14 FDA IDE (b)(4)

Thermaquil started the preparation for FDA IDE (b)(4). This work will continue in the next couple of months. This FDA IDE (b)(4) is scheduled to be submitted to FDA in (b)(4). Figure 2 shows a part of the table of content for this planned (b)(4).

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

A weekly meeting with the team members from University of Pittsburgh, (b)(4) and Thermoquil was held regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermoquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermoquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

There is no publication/presentation (b)(4)

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(6)	(b)(6)@pitt.edu (b)(6)	(b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)

Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)

Subcontract Team Members and Contact Information: (b)(4)

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4);	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4)	(b)(6)

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.1 Institutional IACUC approval and DOD ACURO review	(b)(4)	(b)(4)					(b)(4)
1.1.1 IACUC approval	(b)(4)					Milestone 1.1.1 – Deliverable 1.1.1	
1.1.2 DOD ACURO approval						Milestone 1.1.2 – Deliverable 1.1.2	
1.3 Test Bed Environment	(b)(4)						
1.3.1 Initial PCB design	(b)(4)					Milestone 1.3.1	
1.5 Stimulation Leads and cuff electrodes for animal studies	(b)(4)						
1.5.1 Finalized on lead/cuff size and length	(b)(4)					Milestone 1.5.1	
1.5.2 Finalize design of cat lead/cuff						Milestone 1.5.2	
1.14 FDA IDE pre-submission #1	(b)(4)						
1.14.1 FDA IDE	(b)(4)					Milestone 1.14.1 – Deliverable 1.14.1	
1.12 Human cadaver study	(b)(4)						(b)(4)
1.12	(b)(4)					Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol	(b)(4)						(b)(4)
1.13 Early feasibility human clinical protocol for	(b)(4)					Milestone 1.13 – Deliverable 1.13	

University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: November 1, 2020 through January 31, 2021 (b)(4)

Date of Report: February 28, 2021

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: \$10,451,439.00

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(4), (b)(6)

Submitted by:

(b)(4), (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) quarter (b)(4) we have made major progresses in the following areas:

1. Scientific progress:

- Big Win – We have demonstrated in anesthetized cats that pudendal nerve can be successfully blocked by a (b)(4) electrode to achieve a low-pressure (≤ 50 cmH₂O) voiding. Currently blocking nerve conduction by a (b)(4) electrode is unknown to the field. Our study in the first time showed the nerve block by a (b)(4) electrode, which indicates a minimally invasive surgery approach by inserting (b)(4) electrode close to a peripheral nerve can be developed in clinical applications to block nerve conduction. One-page summary of the Big Win was submitted to DARDA team.
- Pudendal nerve block by (b)(4) electrode as well as the (b)(4) electrode provided by (b)(4) was confirmed in animal studies (b)(4)
- Low-pressure voiding was induced by (b)(4) stimulation combined with the pudendal nerve block using (b)(4) electrodes.
- Tested the (b)(4) electrode and the Test Bed provided by Med-Ally.

2. Engineering progress:

- Reviewed systems and technical requirements. Requirement and Risk documents completed.
- Reviewed the stimulator / test bed system along with applicable Graphical User Interface.
- Evaluated the schematics as compared to the system requirement document.
- Assembled stimulator and commenced power up and testing processes.
- Provided first (b)(4) electrode for evaluation by University of Pittsburgh.
- Market research performed on (b)(4)
- Human (b)(4) requirements input collected.
- Finalized animal IPG User/Customer Requirements
- Two independent (b)(4) test beds – Alpha version is finished and delivered for evaluation by University of Pittsburgh; Beta version is under further development.
- Initial PCB board layout, fabrication/assembly and evaluation has been completed.
- Finalized design of cat lead/cuff.
- (b)(4)
- (b)(4) system layout with different combinations of (b)(4) has been updated.
- (b)(4) designs have been updated to encompass various lengths and (b)(4) combinations.
- (b)(4)
- Authored, reviewed and released draft of system bench testing protocols for (b)(4)
- The industrial design and CAD drawings for the (b)(4) custom enclosure are in progress.
- The (b)(4) requires additional hardware modifications to (b)(4)
- (b)(4) prototypes have been designed in Solidworks. (b)(4)
- (b)(4)

3. Regulatory progress:

- FDA (b)(4) was submitted (b)(4) and informational meeting was requested and scheduled.
 - Proposed GLP swine safety studies, Non-GLP feline efficacy studies, and (b)(4) (b)(4) were submitted for FDA feedback.
 - Informational Meeting for (b)(4) is scheduled for (b)(4)
 - (b)(4)
- Early feasibility human clinical protocol was outlined.

- FDA IDE (b)(4) was drafted, which focuses on (b)(4)
- We continued to identify user needs and voice of customer to inform device requirements.

4. Administrative progress:

- The subcontracts were signed with (b)(4) and Thermaquil.
- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
- (b)(4) installment of funding was received by University of Pittsburgh. DARPA informed us the rest of phase 1 fund will be released (b)(4)
- Weekly meeting was hold between University of Pittsburgh, (b)(4) and Thermaquil to coordinate the project activity.
- Monthly meeting was hold with DARPA to report our progress.

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.1 Institutional IACUC approval and DOD ACURO review (b)(4)

We have obtained IACUC approval from University of Pittsburgh, DOD ACURO, and NAVY IACUC and started the animal studies during this quarterly report period.

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.1 Restore bladder function (b)(4)

We have successfully demonstrated in anesthetized cats that pudendal nerve can be blocked by (b)(4) using a lead electrode and a low-pressure voiding can be produced by a (b)(4) combined with the pudendal nerve block. These results indicate that a minimally invasive surgical approach by inserting the lead electrodes close to the pudendal nerves can be used to block and stimulate the pudendal nerves to restore bladder function after spinal cord injury (SCI). Our next step is to finish the animal studies for restoring bladder function and start investigations to restore bowel or sexual functions.

Nerve Block by a Lead Electrode

Our study in anesthetized cats has discovered that the pudendal nerve can be completely blocked by a lead electrode immediately following the termination of a (b)(4) a post-stimulation block (Fig.1). Blocking nerve conduction by a lead electrode is novel because all previous studies require a major invasive surgery to implant a cuff electrode to

block a peripheral nerve conduction. Our study demonstrates in the first time that a cuff electrode is not needed, and a nerve block can be induced by a lead electrode, which indicates that in clinical applications a lead electrode without a cuff could be used to block nerve conduction. This result is significant because a minimally invasive surgical approach could be developed to insert the lead electrode close to a peripheral nerve, instead of a major invasive surgery to implant a cuff electrode, to achieve a nerve block for clinical applications.

As shown in Fig.1, Stim.B (1 kHz, 0.5 mA, 2.5 min) successfully blocked the periodic EUS contractions induced by short bursts of Stim.C (30 Hz, 0.2 ms, 1.5 V, 5 secs on and 55 secs off) during a period after terminating the 1 kHz stimulation, i.e., a post-stimulation block. Since the EUS contractions induced by Stim.D (30 Hz, 0.2 ms, 1.2 V, 5 secs) was not changed while the contractions induced by Stim.C was blocked, it indicated that Stim.B blocked the pudendal nerve conduction instead of causing a EUS fatigue that should affect the responses induced by both Stim.C and Stim.D. For long-term bladder management after SCI, repeatedly fatiguing

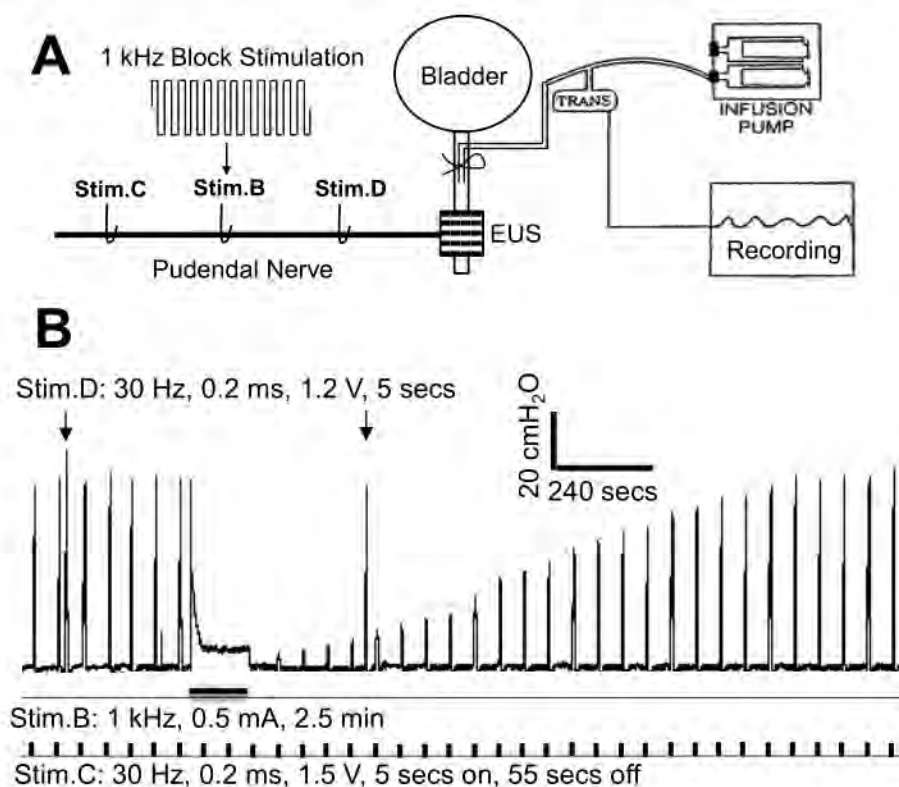


Fig.1: A. Experimental setup for pudendal nerve block by a hook electrode. B. Complete nerve block occurred after the end of the 1 kHz biphasic continuous stimulation. The arrows indicate the EUS contractions induced by the 30 Hz stimulation at the distal site (Stim.D). The thick bars under the EUS pressure trace indicate the stimulation durations for Stim.B and Stim.C. After ending the 1 kHz Stim.B, EUS contraction can still be induced by the distal Stim.D but the contractions induced by the central Stim.C are completely blocked initially and then gradually recover, indicating that nerve conduction block occurs locally at Stim.B and the EUS is not fatigued. EUS –External Urethral Sphincter.

the EUS by pudendal nerve stimulation will cause the EUS to become more and more fatigue resistant and not relax sufficiently for a low-pressure voiding.

The duration of 1 kHz post-stimulation block was dependent on the intensity and duration of the stimulation. As shown in Fig.2, the block duration was increased by increasing the intensity of 1 kHz stimulation (30 secs) from 1 mA to 4 mA. At the intensity of 3 mA, the duration for a complete post-stimulation block reached 2 mins with a recovery duration of 6 mins (see the third trace in Fig.2). When the intensity of 1 kHz stimulation increased to 4 mA, the complete block duration increased to 6 mins, but the recovery duration increased dramatically to 100

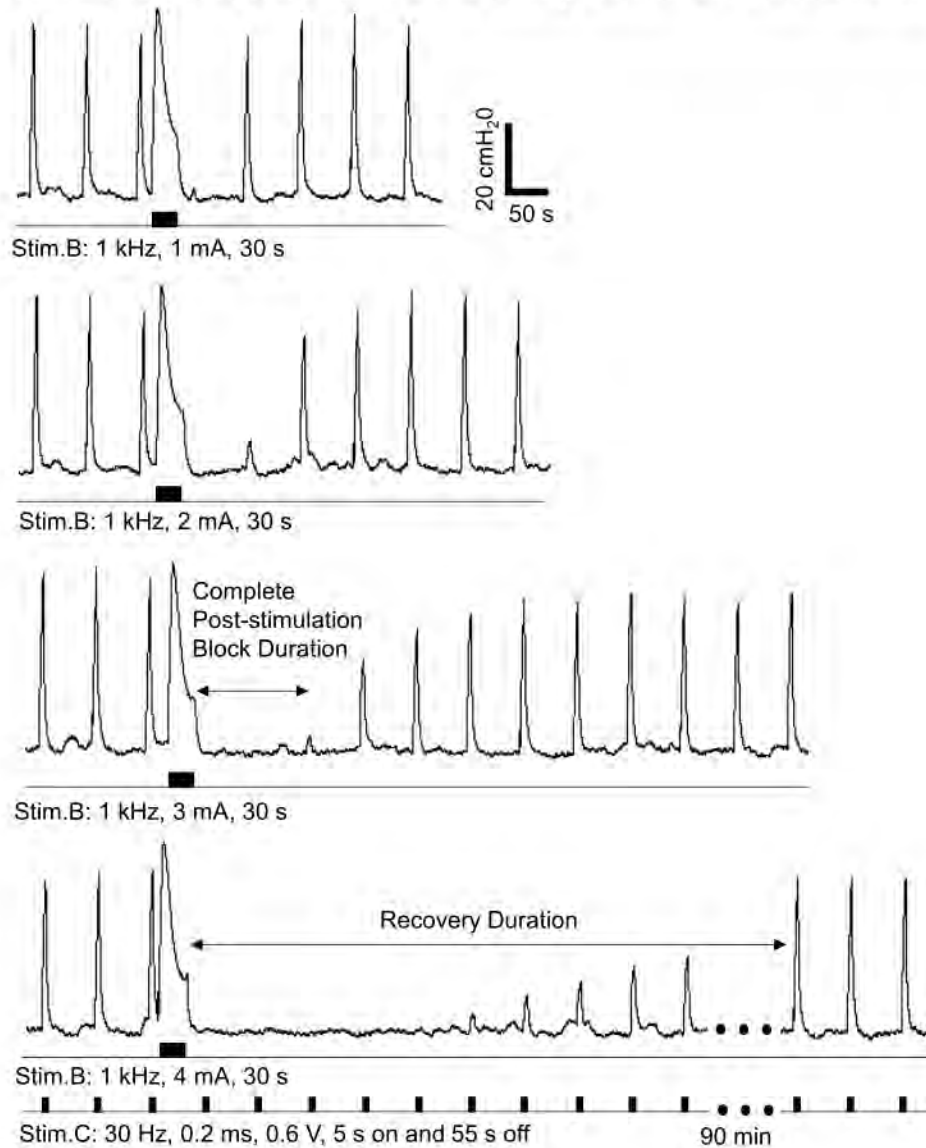


Fig.2: Block stimulation (Stim.B) produced a complete post-stimulation block of duration ≥ 2 minutes as the stimulation intensity gradually increased to 3 mA. At 4 mA intensity, Stim.B produced a prolonged post-stimulation block with a recovery period of 110 minutes. The short black bar under each pressure trace indicates the stimulation duration. The duration of Stim.C shown at the bottom applies to all pressure traces. The intensity for Stim.C is at 3 times of the threshold (T) intensity for inducing visible anal twitch to induce maximal contractions of the external urethral sphincter.

mins (see the fourth trace in Fig.2). In the other 2 cats, a prolonged recovery duration (58 mins or 60 mins) was induced by 1 kHz stimulation at the intensity/duration combination of 4 mA/30 secs or 2 mA/60 secs, respectively.

Fig.3 summarized the intensity and duration required for 1 kHz stimulation to induce a complete post-stimulation block of duration ≥ 2 mins and the corresponding duration for the block and recovery (N=6 nerves from 3 cats). In one cat, 1 kHz stimulation of 30 secs duration failed to induce a complete post-stimulation of duration ≥ 2 mins before its intensity increased to the level causing hindlimb movement. In this cat after increasing the stimulation duration to 60 secs, the 1 kHz stimulation required a much lower intensity (0.5-1 mA, see the black dots in Fig.3) than 30 secs duration to induce the complete block. These results indicate that there is a combination of intensity and duration for 1 kHz to induce a complete post-stimulation block of duration ≥ 2 mins that can recover relatively quick within 20 mins (Fig.3). However, if the intensity and/or the duration increased further, a prolonged recovery of 1-2 hours may occur (see the fourth trace in Fig.2). The complete block was defined as the EUS contraction pressure induced by Stim.C was reduced to <10 cmH₂O that would allow a low-pressure voiding to occur. The complete post-stimulation block of duration ≥ 2 mins would allow a duration long enough for voiding to complete.

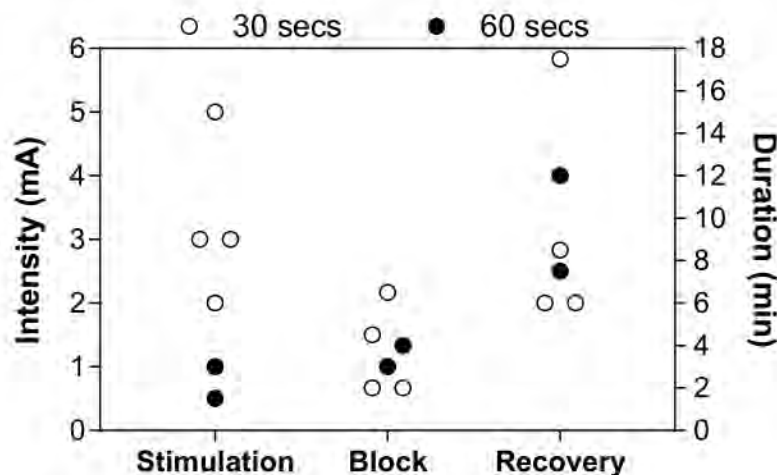


Fig.3: The intensity and duration (30 secs or 60 secs) required for 1 kHz stimulation to produce a complete post-stimulation block of duration ≥ 2 mins and the corresponding duration for the block and recovery. Stimulation intensity is shown on the left Y-axis. Duration for the block and recovery is shown on the right Y-axis. N = 6 nerves from 3 cats.

Low-Pressure Voiding by Lead Electrodes

More importantly, we further demonstrated that a low-pressure (≤ 50 cmH₂O) voiding can be successfully induced by a 30 Hz pudendal nerve stimulation during the post-stimulation block of bilateral pudendal nerves induced by lead electrodes (Fig.4). Low-pressure voiding induced by pudendal nerve stimulation and block is critical for long-term management of bladder

dysfunction after SCI, because high-pressure voiding can cause renal failure in a long-term clinical application. Therefore, the 1 kHz pudendal nerve block as shown in our study is very important because it can prevent detrusor sphincter dyssynergia (DSD) after SCI and reduce urethral outlet resistance for a low-pressure voiding to occur. The clinical significance of using lead electrodes to induce a low-pressure voiding is that a minimally invasive surgical approach by inserting the lead electrodes close to the pudendal nerves, instead of a major invasive surgery to implant the cuff electrodes, can be used to block and stimulate the pudendal nerves to restore bladder function after SCI.

Fig.4 shows that using monopolar hook electrodes the 30 Hz pudendal nerve stimulation combined with 1 kHz post-stimulation block successfully induced a low-pressure (≤ 50 cmH₂O) voiding. During the control CMG (Fig.4B), slow infusion of the bladder triggered a micturition

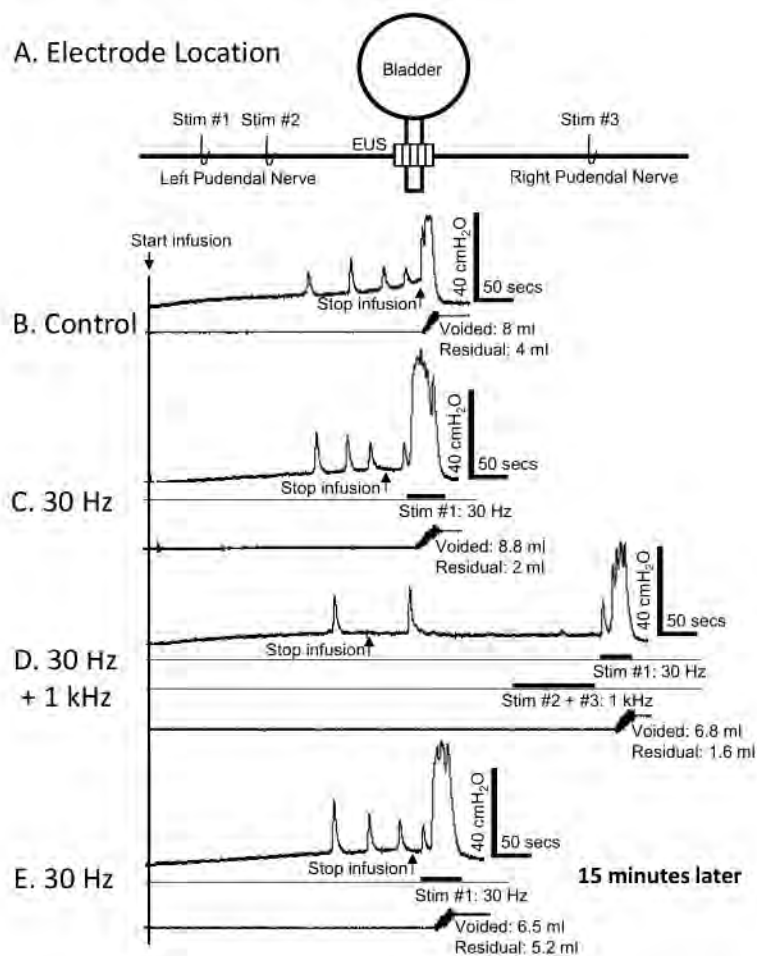


Fig.4. Low pressure voiding induced by 1 kHz post-stimulation block. A: Three monopolar hook electrodes were placed on bilateral pudendal nerves to deliver three stimulations (#1, #2, #3). Stim #1 (30 Hz, 0.2 ms) was always delivered to induce a reflex bladder contraction after Stim #2 and #3 delivered a short burst of 1 kHz stimulation to produce a post-stimulation block of the pudendal nerves. EUS - external urethral sphincter. B-E: Four cystometrograms were performed in sequence showing that Stim #1 (30 Hz, 0.2 ms, 9 V) induced a low pressure (≈ 40 cmH₂O) voiding (D) comparable to the control (B) immediately after Stim #2 and #3 delivered the 1 kHz stimulation (1.75 mA, 2 mins), while Stim #1 alone produced high pressure (≈ 60 cmH₂O) voiding before (C) and 15 mins after (E) the 1 kHz stimulation. The black bar under the pressure trace indicates the stimulation duration. Infusion rate = 2 ml/min.

reflex producing a low-pressure (≈ 40 cmH₂O) voiding. With the bladder partially full (Fig.4C), Stim #1 (30 Hz) alone induced a voiding of a high pressure (≈ 60 cmH₂O). However, the same Stim #1 successfully induced a low-pressure (≈ 40 cmH₂O) voiding when applied immediately after termination of the 1 kHz stimulation (1.75 mA, 2 mins) that was applied to both Stim #2 and #3 (see Fig.4D). The high-pressure (≈ 60 cmH₂O) voiding was returned in about 15 minutes after termination of the 1 kHz block stimulation (Fig.4E).

Fig.5 shows that a low-pressure voiding can also be induced by combination of 1 kHz block and 30 Hz stimulation using bipolar hook electrodes. However, the recovery from a low-pressure voiding to a high-pressure voiding was prolonged since the 1 kHz block stimulation was too strong. As shown in Fig.5A-B, micturition reflex induced a low-pressure (<50 cmH₂O)

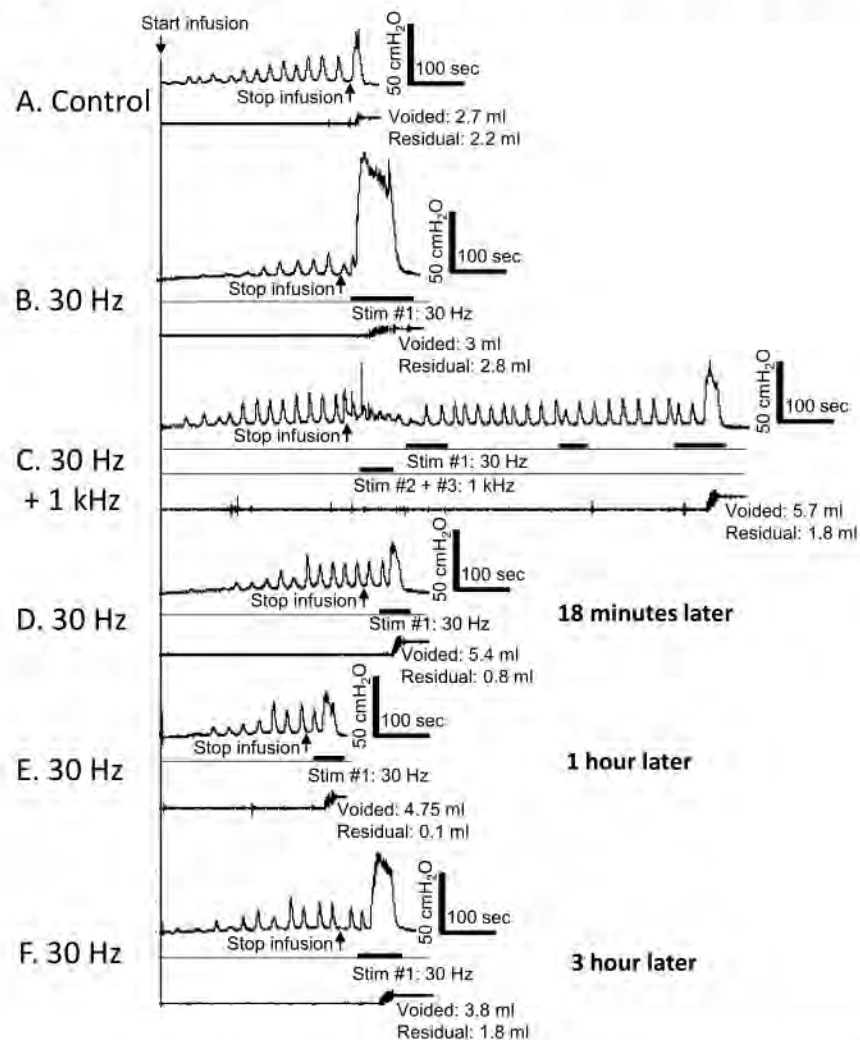


Fig.5. Long-lasting effect of 1 kHz post-stimulation block. A. Control cystometrogram showing a low-pressure (<50 cmH₂O) voiding produced by micturition reflex. B. Stim #1 (30 Hz, 0.2 ms, 0.48 V) alone produced a high-pressure (≈ 100 cmH₂O) voiding. C. After Stim #2 and #3 delivered the 1 kHz stimulation (2 mA, 1 min), Stim #1 lost the ability to induce a bladder contraction for about 10 minutes. Then, Stim #1 induced a low-pressure (≈ 50 cmH₂O) voiding. D-E. The low-pressure voiding by Stim #1 lasted for at least 1 hour. F. At 3 hours after the 1 kHz stimulation, the voiding pressure induced by Stim #1 increased to about 70 cmH₂O. The black bar under the pressure trace indicates the stimulation duration. Infusion rate = 1 ml/min.

voiding while Stim #1 (30 Hz) alone induced a high-pressure (≈ 100 cmH₂O) voiding. After applying 1 kHz stimulation (2 mA, 1 min) to both Stim #2 and #3, the same Stim #1 lost the ability to induce a bladder contraction in about 10 mins, and then it successfully induced a low-pressure (< 50 cmH₂O) voiding (see Fig.5C). The low-pressure voiding induced by Stim #1 alone lasted for at least an hour (Fig.5 D-E). In 3 hours, the voiding pressure returned to about 70 cmH₂O (Fig.5F). Please note that there was no leakage of fluid from the bladder during the period immediately following termination of the 1 kHz block stimulation although many non-voiding contractions occurred during this period (Fig.5C).

(b)(4)

Sub-Task 1.2.5 Test electrode and device prototypes (b)(4)

We have successfully tested the (b)(4) electrode provided by (b)(4) to block pudendal nerve conduction in an anesthetized cat. Fig.6 shows the experimental setup and Fig.7 shows the results. In addition, we also tested the first prototype of the Test Bed provided by (b)(4) which can generate (b)(4)

(b)(4)

(b)(4)

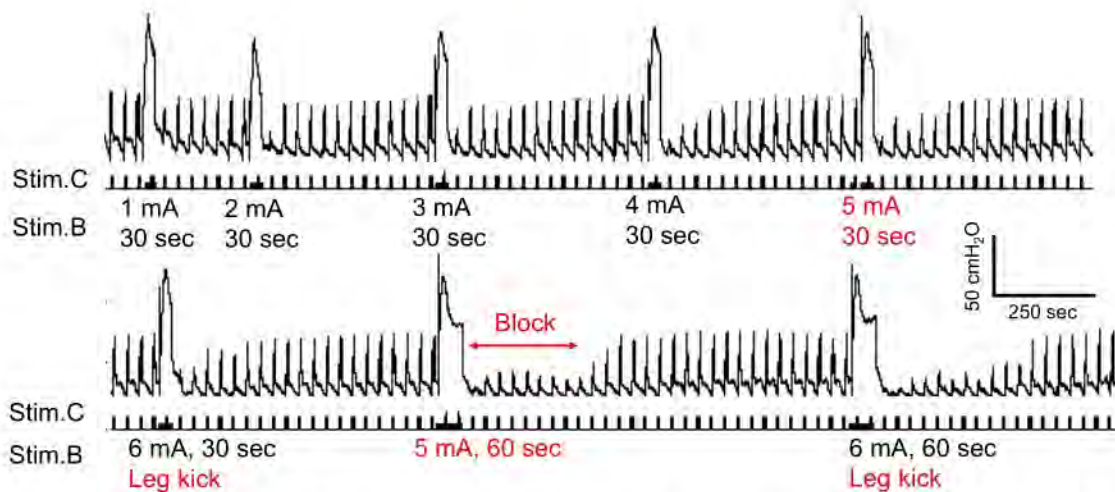


Fig7: Stim.B (1 kHz, 60 secs) reversibly blocked the increases in urethral pressure induced by Stim.C (30 Hz, 0.2 ms, 1 V, 5 secs on and 25 secs off). Stim.B is marked by the black bar of lower amplitude than Stim.C

Task 1.3 Test Bed Environment (b)(4)

Sub-Task 1.3.1 Complete initial PCB schematic design with design review (b)(4)

The development of the test bed environment is based on (b)(4). The (b)(4) had to be modified in order to achieve the desired design output requirements. Two test beds were chosen to be adapted to demonstrate specific attributes of the final (b)(4) system. The block diagram for the (b)(4) can be seen in Fig.8.

Sub-Task 1.3.2 Complete initial PCB board layout, fabrication/assembly and evaluation (b)(4)

The two adapted test beds were already fabricated and assembled. These are referred to as Alpha and Beta prototypes, whereby the Alpha is a simple proof of technical feasibility. During evaluation hardware and software limitations were identified. The Alpha was successfully modified to meet the design requirements subject to inherent hardware limitations. The second test bed is a more in-depth modification. The block diagram for visualizing the modified (b)(4) can be seen in Fig.8.

Sub-Task 1.3.3 PC test application with board connectivity (potentially (b)(4) depending on final requirements) (b)(4)

(b)(4) for graphical user interface of the Beta prototype is re-programmed to interface with the test bed system. (b)(4) communications are successful. Firmware has been updated and is functioning as desired.

(b)(4)

Sub-Task 1.3.4 Integration of (b)(4) in enclosure with firmware and (b)(4) connectivity

(b)(4)

The Alpha prototype test bed has been delivered for evaluation to UPitt. The Alpha test bed was modified to include (b)(4)

(b)(4)

The Alpha test bed was successful in demonstrating a (b)(4) with (b)(4)

(b)(4)

(b)(4) The Alpha test bed only has (b)(4) The knowledge gained from the Alpha pathway provides valuable inputs for the Beta prototype and ultimately the final (b)(4) design.

The Beta prototype is a modified (b)(4) configured for (b)(4)
(b)(4) The delivery of the Beta test bed (b)(4)
(b)(4)

(b)(4) The hardware (b)(4) The new block diagram for the Beta test bed can be seen in Fig.8. The (b)(4) addresses the
(b)(4)
(b)(4) The driver section (b)(4)
(b)(4) With a limit of (b)(4) and a source (b)(4)
(b)(4) There are (b)(4)
(b)(4)

(b)(4)

Task 1.4 Implantable pulse generator (b)(4)

(b)(4)

Sub-Task 1.4.1 Can/Header design with design review complete (b)(4)

The can and header designs are completed awaiting further inputs for possible design changes (Fig.11). The current design encloses the anticipated battery size and accompanying circuitry. After all inputs and requirements are finalized, the first prototypes of the can/header will be developed and evaluated.

(b)(4)

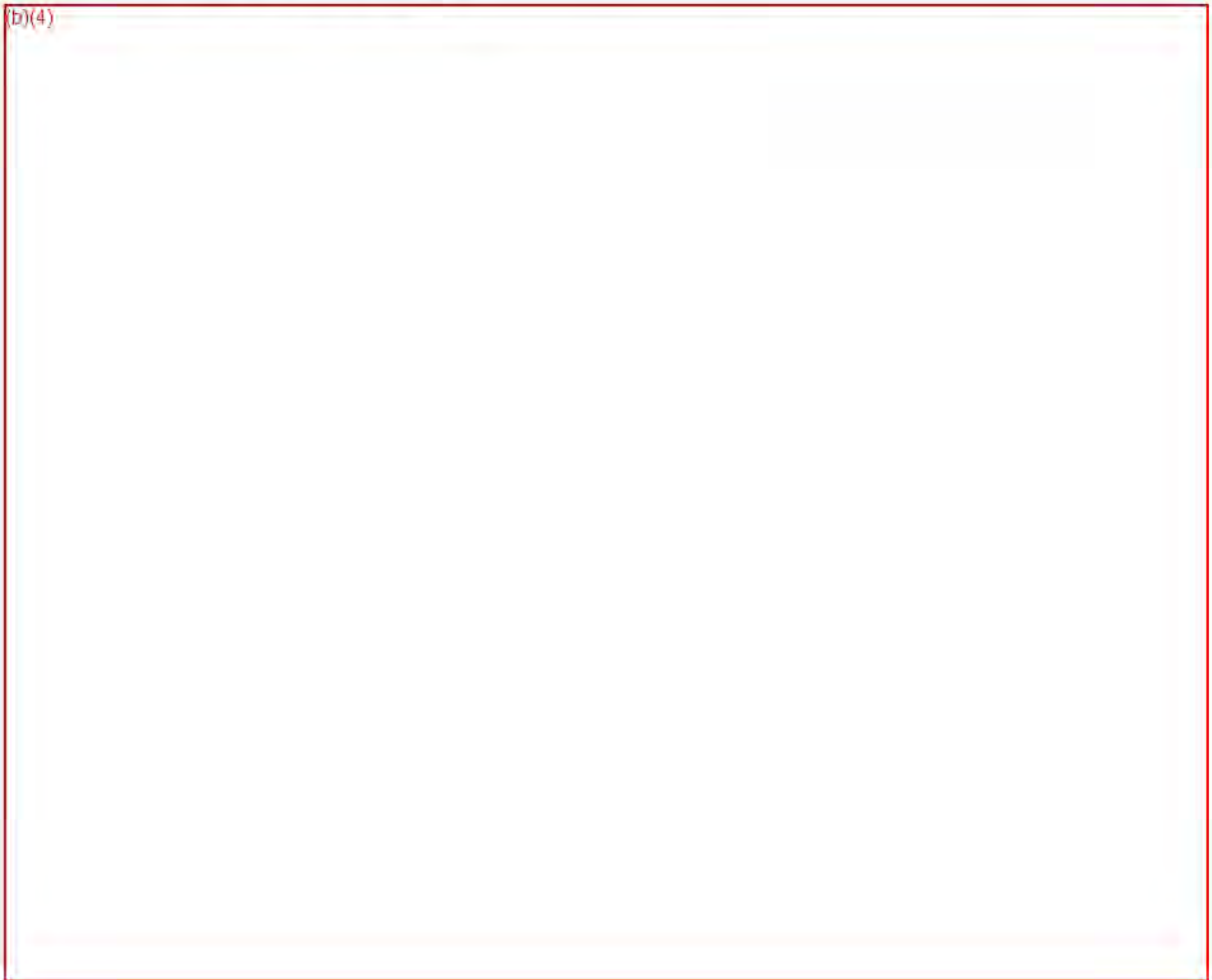
(b)(4)

(b)(4)



Sub-Task 1.5.2 Finalize design of cat lead/cuff (b)(4)

The cuff electrode design has been finalized to follow those specifications as determined in our previous cat studies. An example of the desired cuff electrode sizes for cat (b)(4) (b)(4) are shown in Figs.13-14. Examples of desired surgical accessories used during cuff implantation can be seen in Fig.15.



(b)(4)

Sub-Task 1.5.3 First prototypes of leads/cuffs delivered for test (b)(4)

The first percutaneous lead prototype was delivered to UPitt for evaluation. The pudendal nerve block was achieved using this prototype lead (Figs.6-7). Additional leads are estimated to be delivered in (b)(4). A cuff lead prototype to be used in animal studies (cats) has been conceived and a prototype will be provided - multiple sizes are identified and possible.

Sub-Task 1.5.4 Finalize design and lead/cuff electrode production (b)(4)

Suppliers have been contacted and are motivated to produce our finalized lead designs. A lead wire coiling machine has been purchased to bring the lead production in house. Bringing this process in house will expedite prototyping and production time. The coiling machine is in the process of being delivered during (b)(4). It will first need to pass our in-house validation process and quality standards. Second, a protocol will be developed for operation. Third, the final lead product will need to meet quality and performance standards and testing. The goal is to provide market available leads from approved suppliers and in-house developed leads to UPitt for animal and human testing evaluation.

Task 1.6 External pulse generator (b)(4)

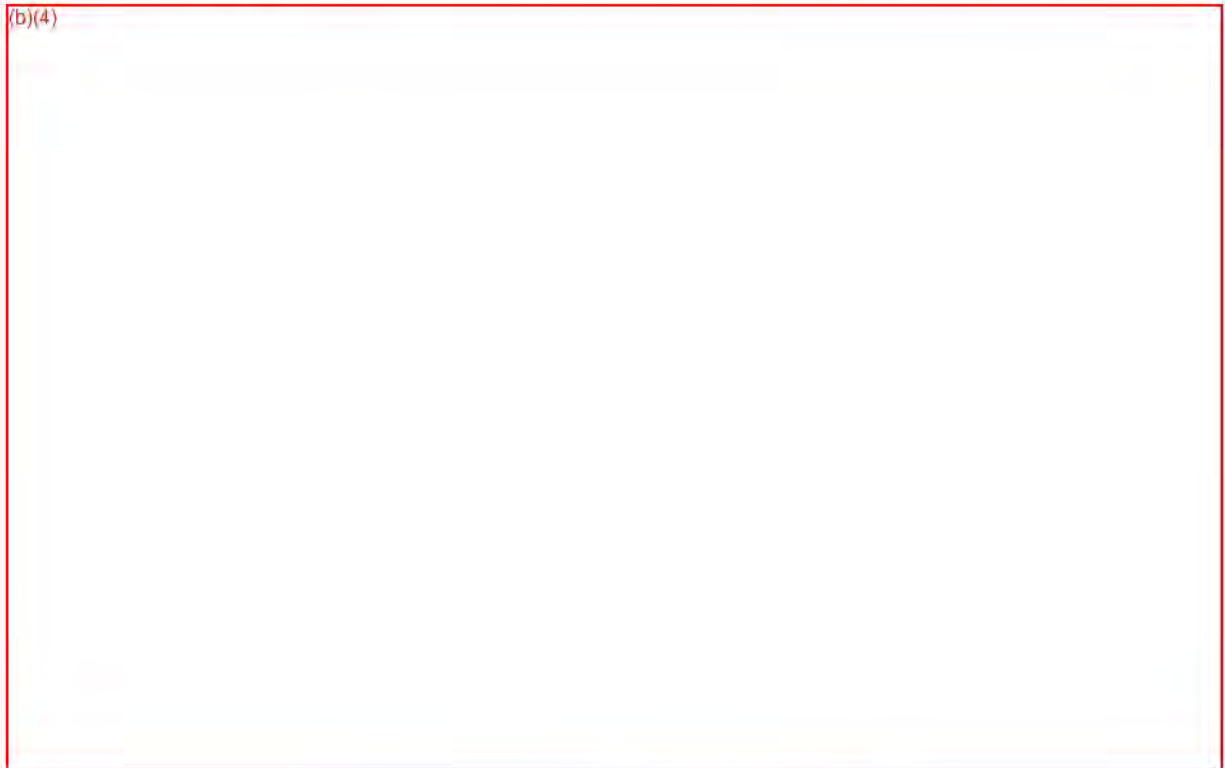
Sub-Task 1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)

A general (b)(4) enclosure has been designed including the materials and processes to be used during manufacturing. The dimensions may need to be adjusted (b)(4). (b)(4) The materials and manufacturing processes required to develop the enclosure may not change. Fig.16 is the current design of the (b)(4) enclosure. The final human (b)(4)

(b)(4)

Sub-Task 1.6.2 Integration of (b)(4) components with firmware and the enclosure (b)(4)

(b)(4) The firmware developed for the Beta test bed is expected to be adaptable to (b)(4) architecture. The enclosure will be incorporated once the (b)(4) dimensions and nuances are finalized. After the (b)(4) design is final, the enclosure can be manufactured.



Task 1.7 (b)(4) electrodes for human use (b)(4)

Sub-Task 1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)

The various (b)(4) configurations have been identified (b)(4) There is a combination (b)(4)

(b)(4)
(b)(4) will support stimulation and (b)(4)

The (b)(4) The stimulation (b)(4) will be used to (b)(4)

(b)(4)

Task 1.8 Physician/Investigator controller (b)(4)

Sub-Task 1.8.1 Schematic design complete (b)(4)

A (b)(4) has been specified and acquired (b)(4)

(b)(4)

(b)(4)

to connect to either the

(b)(4)

Sub-Task 1.8.2 Evaluation board fabricated/assembled and initial evaluation complete

(b)(4)

The (b)(4)

graphical user interface developed for the (b)(4)

(b)(4)

(b)(4) is under development. (b)(4) is an example of a particular (b)(4) being developed for the physician controller. The user interface will change as new design inputs are identified.

Sub-Task 1.8.3 Integration with a simple enclosure with display and firmware (b)(4)

The physician controller has functioning firmware and a working LCD display for graphical user interface interactions with the test bed. The firmware and user interface will be updated as new design inputs are identified. The enclosure has not begun development at this time. We expect the enclosure design to be a quick process to be implemented over the next coming months.

Task 1.10 Software Development Kit (SDK) (b)(4)

Sub-Task 1.10.1 Requirements understood for SDK (b)(4)

The SDK will be developed as part of the firmware development for the EPG and IPG. The requirements for the SDK are in the process of being established.

Task 1.11 GLP animal study (b)(4)

The purpose of the GLP safety studies is to provide support for the safe initiation of an Early Feasibility IDE Study (b)(4) in humans. We drafted our GLP animal study protocol and submitted it for FDA feedback in (b)(4). Fig.21 shows a part of (b)(4) (b)(4) that contains the proposed GLP animal study protocol. Our next step is to schedule our GLP study with the subcontractor.

(b)(4)

(b)(4)

(b)(4)

Task 1.13 Early feasibility human clinical protocol (b)(4)

Thermaquil team has outlined an early feasibility clinical study for the (b)(4) which they will gain feedback from clinicians during (b)(4) and (b)(4). This clinical protocol is needed for FDA IDE submission. Our next step is to acquire clinician feedback on our proposed human clinical protocol. We will continue to develop this clinical protocol as our project progresses. Fig.23 shows a flow chart of the early feasibility human clinical study.

(b)(4)

Task 1.14 FDA IDE (b)(4)

Task 1.14.1 (b)(4)

Task 1.14.2 (b)(4)

Thermaquil submitted FDA IDE (b)(4) and requested an information meeting with the FDA for the (b)(4) system in (b)(4). The Informational Meeting with the FDA is scheduled for (b)(4).

(b)(4)

Fig.24 shows a part of the table of content for this submitted pre-submission, Fig.25 shows the FDA email confirming the informational meeting, and Fig.26 shows the FDA email showing the status of scheduling our second meeting.

(b)(4)

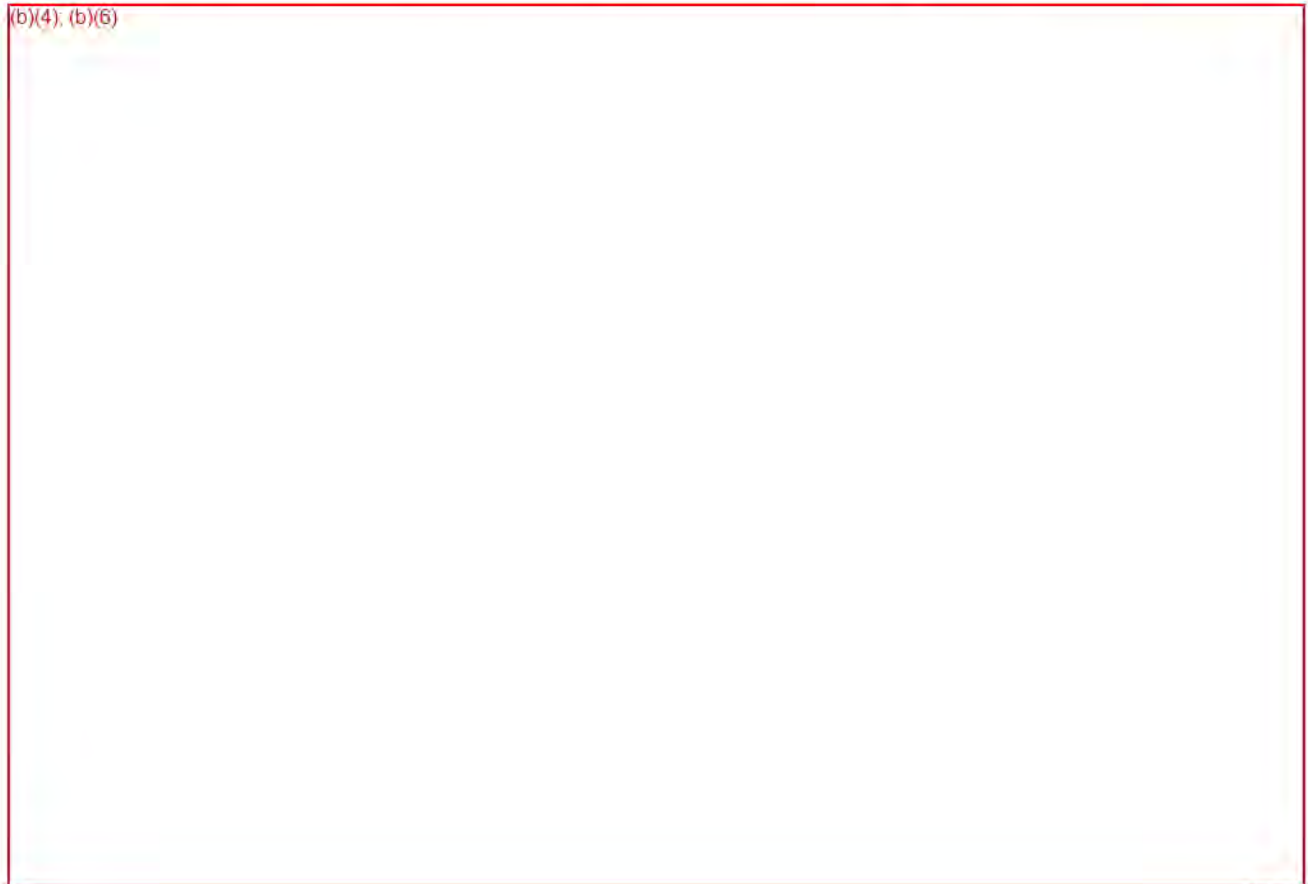
(b)(4); (b)(6)



More ways to join:

Fig.25: Email from the FDA confirming the informational meeting on (b)(4)

(b)(4); (b)(6)



Task 1.15 FDA IDE (b)(4)

Task 1.15.1 FDA IDE (b)(4)

Thermaquil has drafted FDA IDE (b)(4) which focuses on (b)(4)

(b)(4)

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

A weekly meeting with the team members from University of Pittsburgh, (b)(4) and Thermaquil was hold regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermaquil

provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

There is no publication/presentation during this quarterly report period.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6)	
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
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PI	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4);	

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system	(b)(4)						(b)(4)
1.2.1 Restore bladder function	(b)(4)					Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.2.5 To test the first prototype of .1 animal lead	(b)(4)					Milestone 1.2.5.1	
1.2.5 To test the first prototype of the .2 Test Bed Environment	(b)(4)					Milestone 1.2.5.2	
1.3 Test Bed Environment	(b)(4)						
1.3.1 Complete initial PCB schematic design with design review	(b)(4)					Milestone 1.3.1 – Deliverable 1.3	
1.3.2 Complete initial PCB board layout, fabrication/assembly and evaluation	(b)(4)					Milestone 1.3.2 - Deliverable 1.3	
1.3.3 test application with board connectivity	(b)(4)					Milestone 1.3.3 - Deliverable 1.3	
1.3.4 Integration of in enclosure with firmware and application connectivity	(b)(4)					Milestone 1.3.4 - Deliverable 1.3	
1.4 Implantable pulse generator with	(b)(4)						
1.4.1 Can/Header design with design review complete	(b)(4)					Milestone 1.4.1 – Deliverable 1.4	
1.5 Stimulation Leads and cuff electrodes for animal studies	(b)(4)						
1.5.1 Finalized on lead/cuff size and length	(b)(4)					Milestone 1.5.1 – Deliverable 1.5	
1.5.2 Finalize design of cat lead/cuff	(b)(4)					Milestone 1.5.2 – Deliverable 1.5	
1.5.3 First prototypes of leads/cuffs delivered for test	(b)(4)					Milestone 1.5.3 – Deliverable 1.5	
1.5.4 Finalize design and lead/cuff electrode production	(b)(4)					Milestone 1.5.4 – Deliverable 1.5	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.6 External pulse generator for (b)(4)	(b)(4)						(b)(4)
Industrial design and CAD	(b)(4)					Milestone 1.6.1 – Deliverable 1.6.1	
1.6.1 drawings for the custom enclosure (b)(4)	(b)(4)					– Deliverable 1.6.2	
Integration of the external pulse generator components with	(b)(4)					Milestone 1.6.2 – Deliverable 1.6.2	
1.6.2 firmware and the enclosure (b)(4)	(b)(4)						
1.7 (b)(4) electrodes for human use (b)(4)	(b)(4)						
1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)	(b)(4)					Milestone 1.7.1 – Deliverable 1.7	
1.8 Physician/Investigator controller (b)(4)	(b)(4)						
1.8.1 Schematic design complete (b)(4)	(b)(4)					Milestone 1.8.1 – Deliverable 1.8	
Evaluation board	(b)(4)					Milestone 1.8.1 – Deliverable 1.8	
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)	(b)(4)					Milestone 1.8.1 – Deliverable 1.8	
Integration with a simple	(b)(4)						
1.8.3 enclosure with display and firmware (b)(4)	(b)(4)						
1.10 Software Development Kit (SDK) (b)(4)	(b)(4)						(b)(4)
1.10. Requirements understood for	(b)(4)					Milestone 1.10.1 – Deliverable 1.10	
1 SDK (b)(4)	(b)(4)						
1.14 FDA IDE (b)(4)	(b)(4)						
1.14. FDA (b)(4) for (b)(4)	(b)(4)					Milestone 1.14.1 – Deliverable 1.14.1	
1 the (b)(4)	(b)(4)					Milestone 1.14.2 – Deliverable 1.14.2	
1.14. FDA (b)(4)	(b)(4)						
2 meeting	(b)(4)						
1.15 FDA IDE (b)(4)	(b)(4)						
1.15. FDA IDE (b)(4) for (b)(4)	(b)(4)					Milestone 1.15.1 – Deliverable 1.15.1	
1 the (b)(4)	(b)(4)						
1.12 (b)(4)	(b)(4)						(b)(4)
1.12 (b)(4)	(b)(4)					Milestone 1.12 – Deliverable 1.12	

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 0760



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.13 Early feasibility human clinical protocol	(b)(4)						
1.13 Early feasibility human clinical protocol	(b)(4)				10%	Milestone 1.13 – Deliverable 1.13	(b)(4)
1.11 GLP animal study	(b)(4)						
1.11 GLP animal study	(b)(4)				15%	Milestone 1.11 – Deliverable 1.11	

University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: February 1, 2021 through April 30, 2021

(b)(4)

Date of Report: May 31, 2021

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: \$10,451,439.00

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(4); (b)(6) DARPA/BTO

Submitted by:

(b)(4); (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4); (b)(6)

Email: (b)(4); (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) we have made major progresses in the following areas:

1. Scientific progress:

- Big Win – We have successfully demonstrated in anesthetized cats that pudendal nerve stimulation and block by lead electrodes can achieve a low-pressure (≤ 50 cmH₂O) voiding. This success indicates a minimally invasive surgical approach by inserting lead electrodes close to the pudendal nerves can be developed to restore bladder function after spinal cord injury. The data/results from this animal study have been processed, analyzed, and summarized. An abstract was accepted for presentation at American Urological Association (AUA) Annual Meeting, September 10-13, 2021, Las Vegas, Nevada. Another abstract was submitted to International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online. A manuscript was submitted to Experimental Neurology for review and publication.
- Animal study to restore defecation function after spinal cord injury has been initiated. Results from the first 2 cats show that stimulation of sacral S2 spinal root is effective in inducing colon contractions, while stimulation of S1/S3 spinal roots or pudendal nerve is ineffective. This study will continue into the next quarterly report period.

2. Engineering progress:

- Possible human (b)(4) have been identified. The final (b)(4) and (b)(4) will be confirmed with (b)(4)
- (b)(4)
- (b)(4) prototypes have been designed in Solidworks.
- (b)(4)
- We received sample elastomer 3D prints of our (b)(4) (b)(4) which underwent quality inspection. This ultimately led to (b)(4) purchasing (b)(4) (b)(4)
- (b)(4) lead designs have been defined and example cuffs were sent to University of Pittsburgh for evaluation
- Finalized design lead/cuff electrode production. Implementing novel closure methods and adapting (b)(4)
- The (b)(4) is at the (b)(4) facility. The (b)(4) have been produced and have been (b)(4) The (b)(4) had the contacts and electrodes adhered. (b)(4)
- Multiple lengths of the (b)(4) (b)(4)
- (b)(4)
- Novel surgical approaches (b)(4) are being pursued (b)(4) (b)(4)
- (b)(4)
- (b)(4)
- Industrial design and CAD drawings for the (b)(4) have been updated to accommodate (b)(4) (b)(4)
- The (b)(4) and IPG schematic have been updated to accommodate the (b)(4) (b)(4)
- The (b)(4) schematic is currently being fabricated on to the boards.
- Integration of (b)(4) with firmware and the enclosure is pending the board to be fabricated. Board layout and component choices have been decided.

- The graphical user interface (GUI) has made great progress and is under ongoing review with clinicians and the internal team.
- MRI compatibility testing has been arranged.

3. Regulatory progress:

- Met with the FDA twice regarding (b)(4)
 - Received feedback on the (b)(4)
(b)(4)
 - Given FDA's positive feedback, we (b)(4)
(b)(4)
- Drafted early feasibility human clinical protocol (b)(4)
- (b)(4)
- Continued to identify user needs and voice of customer to inform device requirements, which led to (b)(4)
(b)(4)
- FDA (b)(4) was drafted, which focuses on (b)(4)
(b)(4)

4. Administrative progress:

- The full funding for phase I has been received by University of Pittsburgh.
- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
- Two weekly meetings were hold between University of Pittsburgh, (b)(4) and Thermoquil to coordinate the project activity.
- Monthly meeting was hold with DARPA to report our progress.
- (b)(4)

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.1 Institutional IACUC approval and DOD ACURO review (b)(4)

The third-year renewal of our IACUC protocol was approved by University of Pittsburgh, DOD ACURO, and NAVY IACUC.

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.1 Restore bladder function (b)(4)

We have finished the animal studies for restoring bladder function. The data/results were processed, analyzed, and summarized for publication. Fig.1 summarizes the data for low pressure voiding, which together with the previous report completes the data set for this sub-task 1.2.1.

Fig.1 summarizes the results from 5 cats. On average, in the control condition the micturition reflex induced by filling the bladder produced low-pressure (≈ 40 cmH₂O) voiding (Fig.1A) of a 50% voiding efficiency (Fig.1B) with a post-void residual volume of 50% (Fig.1C) of the control bladder capacity. A slightly higher voiding efficiency, but not significantly different from control, and a lower post-void residual volume were produced by (b)(4) alone (Fig.1 B-C) at a significantly ($p < 0.05$) higher voiding pressure (90 cmH₂O, Fig.1A). However, 30 Hz stimulation combined with (b)(4) significantly ($p < 0.05$) increased voiding efficiency (80%, Fig.1B) and reduced post-void residual volume (20%, Fig.1C) but at a significantly ($p < 0.05$) lower pressure (≈ 40 cmH₂O) that was same as in the control condition (Fig.1A). After a recovery period ranging from 15 mins to 3 hours (see Fig.4 and Fig.5 in last report), the voiding pressure induced by (b)(4) alone increased to about 70 cmH₂O (Fig.1A) while voiding efficiency declined (50%, Fig.1B) and post-void residual volume significantly ($p < 0.05$) increased (50%, Fig.1C) above that measured immediately after the (b)(4). On average a similar amount of saline was infused into the bladder under the different conditions (Fig.1D).

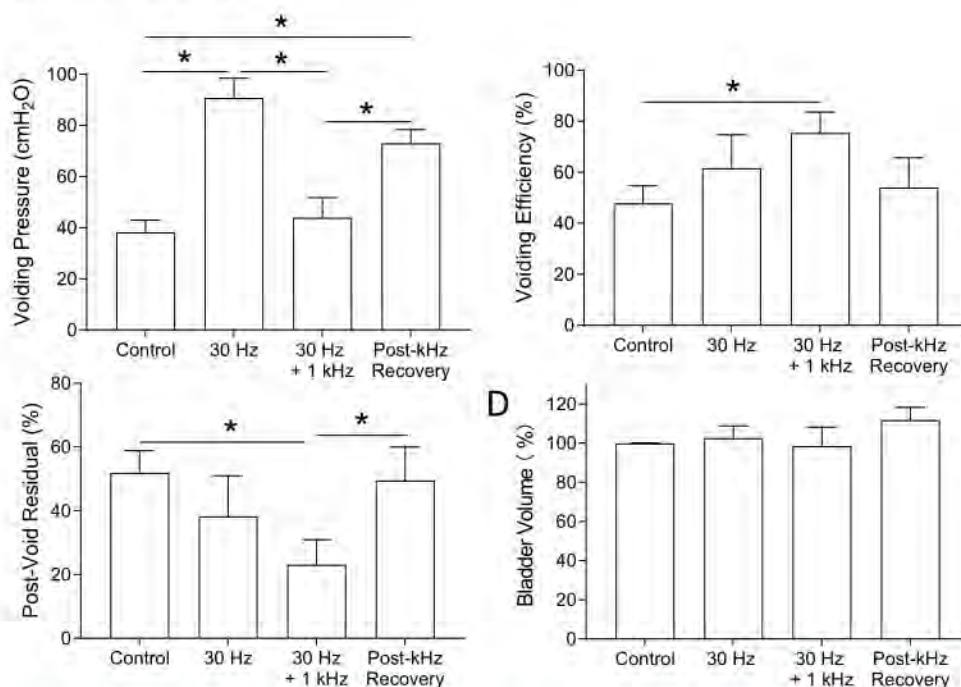


Fig.1. Summary of the urodynamic parameters for control condition. (b)(4)

(b)(4)

(b)(4)

A. Voiding pressure. B. Voiding efficiency. C. Post-void residual. D. Bladder volume. * indicates a significant difference $p < 0.05$, ANOVA). N = 5 cats.

Sub-Task 1.2.2 Restore bowel function (b)(4)

We have initiated the animal study in (b)(4) aimed at restoring bowel function. Fig.2 shows the experimental setup. In anesthetized cats, one balloon catheter was inserted into the proximal colon via a small incision at the most proximal end of the colon. Another balloon catheter was inserted into the distal colon via the anus. Laminectomy was performed to expose the sacral spinal cord and roots. Sacral S1-S3 dorsal and ventral root were isolated for electrical stimulation using a stainless-steel bipolar hook electrode. A cuff electrode was also implanted on the left pudendal nerve for stimulation.

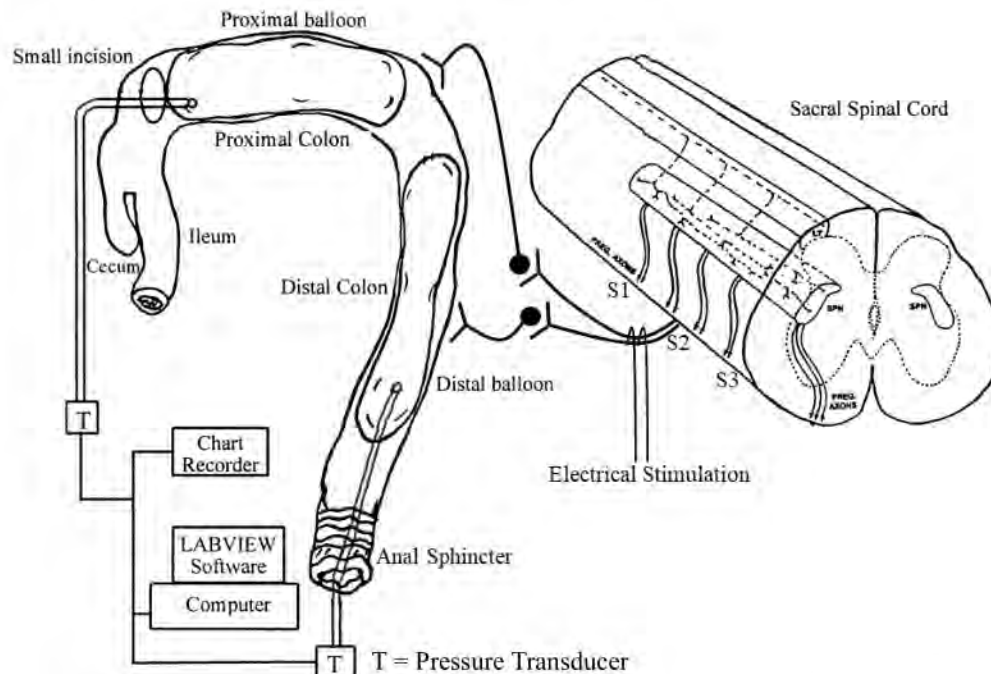


Fig.2. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce colon contractions.

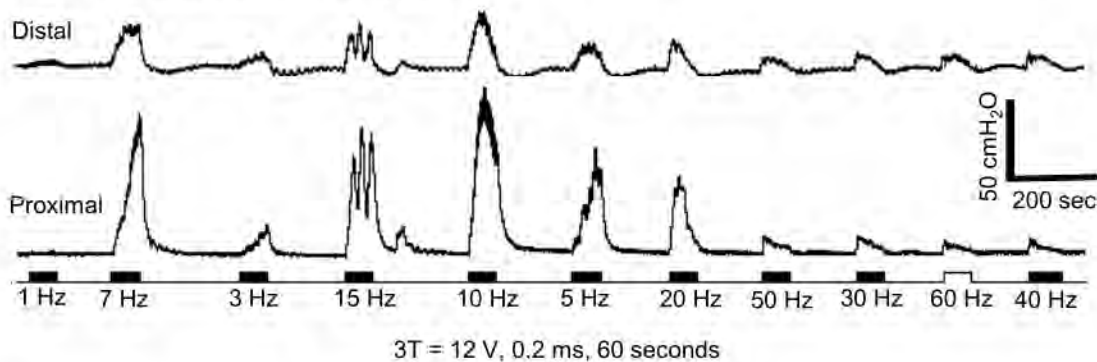
Fig.3A shows that S2 ventral root stimulation at a frequency of (b)(4) is most effective in inducing colon contractions. The proximal colon contraction is stronger than the distal colon contraction. Fig.3B shows that intensity of S2 ventral root stimulation must be higher than a minimal intensity threshold (T) to induce the colon contraction. The induced contraction can last at least 5 minutes as long the stimulation is continued (Fig.3B). Similar results can be achieved by stimulation of the entire S2 spinal root (ventral and dorsal roots) (Fig.3C).

Fig.4 shows that stimulation of S1 or S3 ventral root can only induce small (<10 cmH₂O) colon contractions, indicating that the S2 spinal root is the most effective site. In addition, Fig.4 also shows that the pudendal nerve stimulation is not effective in inducing colon contraction.

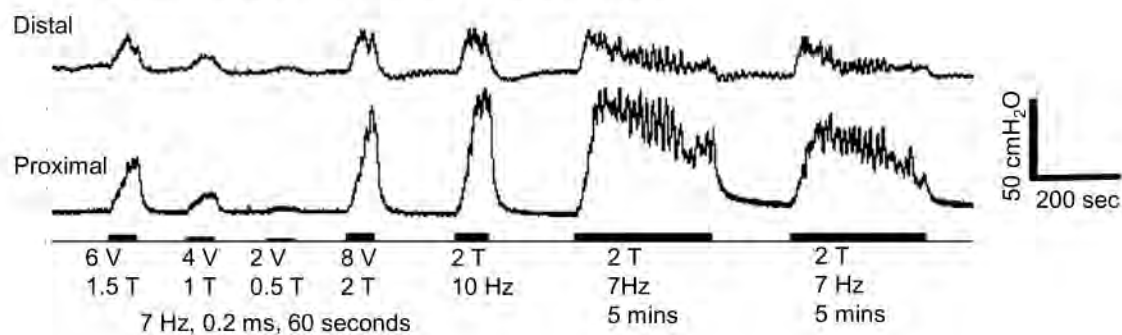
The similar results are obtained in the first 2 cats tested. In summary we may need to stimulate S2 spinal root to restore defecation function after spinal cord injury. S2 spinal root can be

accessed by a minimally invasive surgical approach by inserting a lead electrode percutaneously. This minimally invasive percutaneous approach is currently used routinely in urology clinics to implant Medtronic InterStim neuromodulation system. Therefore, this approach can be simply combined with our minimally invasive surgical approach for pudendal nerve stimulation to restore both bladder and defecation functions after spinal cord injury.

A. S2 Ventral Root - Frequency



B. S2 Ventral Root – Intensity and Duration



C. S2 Ventral + Dorsal Root

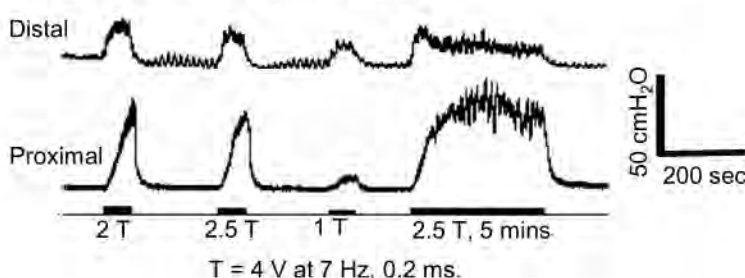
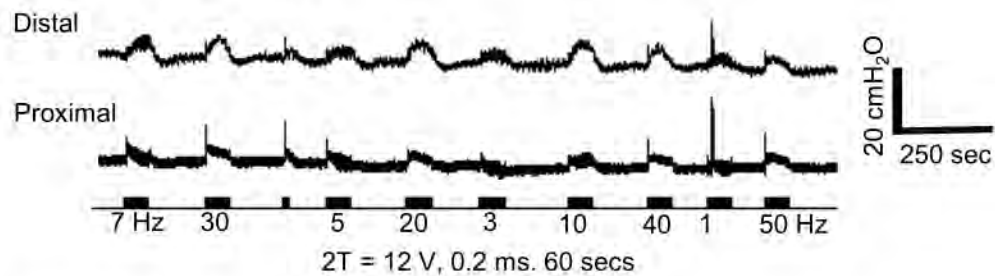
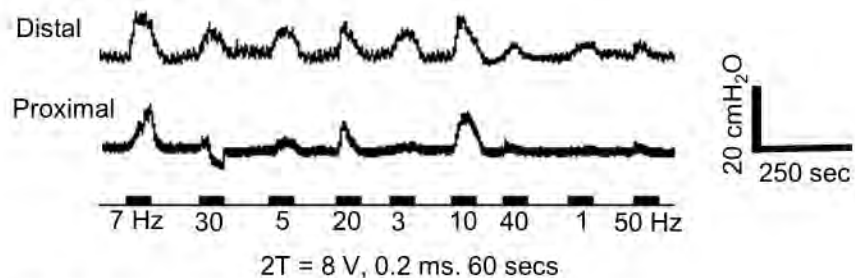


Fig.3. Proximal and distal colon responses to stimulation of S2 ventral and/or dorsal roots. A. Frequency responses showing that S2 ventral root stimulation at (b)(4) B. A minimal stimulation intensity threshold (T) is required for S2 ventral root stimulation to induce a colon contraction that can last for at least 5 minutes. C. Similar colon contraction responses can be induced by stimulation of the entire S2 spinal root including both ventral and dorsal roots.

A. S1 Ventral Root



B. S3 Ventral Root



C. Pudendal Nerve

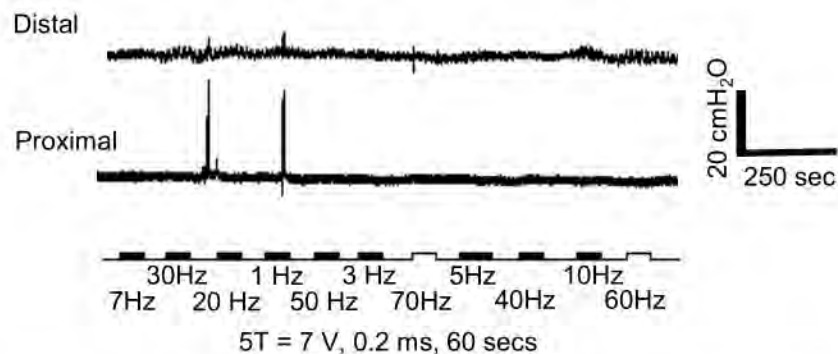


Fig.4. Proximal and distal colon responses to stimulation of S1/S3 ventral root or pudendal nerve. A. S1 ventral root stimulation induced small colon contractions. B. S3 ventral root stimulation also induced small colon contractions. C. Pudendal nerve stimulation failed to induce colon contractions at all tested frequencies (b)(4).

Task 1.3 Test Bed Environment (b)(4)

Sub-Task 1.3.4 Integration of (b)(4) in enclosure with firmware and (b)(4) connectivity

(b)(4)

University of Pittsburgh provided feedback on the capabilities of the (b)(4) test bed prototypes.

(b)(4)

(b)(4)

(b)(4) was determined to be (b)(4) Now the

electrical team is focusing on developing the (b)(4)

(b)(4)

Task 1.4 Implantable pulse generator with (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.4.1 Can/Header design with design review complete (b)(4)

The (b)(4) designs were updated to meet the new design requirements of (b)(4)

(b)(4)

Sub-Task 1.4.2 Can samples developed and fully evaluated (b)(4)

(b)(4)

It is a challenge to have a (b)(4)

(b)(4)

makes manufacturing more complex. The team is currently thinking through some solutions. One option is to use (b)(4)

(b)(4)

The next step is to finalize the header and feed through assembly processes. The first prototypes of the can/header can then be developed and evaluated. The laser welder and hermeticity testing equipment are ready to assemble and test the IPG, respectively.

(b)(4)

Task 1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)

Sub-Task 1.5.4 Finalize design and lead/cuff electrode production (b)(4)

(b)(4) configurations were identified as the desired cuff designs. Engineering drawings were created for version 1 of the cuffs (b)(4). Off the shelf example cuffs were provided to University of Pittsburg for evaluation.

(b)(4)

Task 1.6 External pulse generator (b)(4)

Sub-Task 1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)

A general (b)(4) was designed (b)(4)

(b)(4)

requirement slightly changed the shape of our design. (b)(4)

(b)(4)

(b)(4) is the current design of (b)(4)

Sub-Task 1.6.2 Integration of the (b)(4) with firmware and the enclosure (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.7 (b)(4) electrodes for human use (b)(4)

Sub-Task 1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)

The various (b)(4) configurations were updated to incorporate (b)(4)

(b)(4)

(b)(4)

(b)(4)

The various leads will be updated as further design changes are made. (b)(4)

(b)(4)

Sub-Task 1.7.2 Finalize design of human (b)(4)

The final (b)(4) design for humans has largely been decided. (b)(4)

(b)(4)

(b)(4)

The electrodes/contacts are then (b)(4) and (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.8 Physician/Investigator controller for (b)(4)

Sub-Task 1.8.1 Schematic design complete (b)(4)

(b)(4) is being customized for use as a researcher controller, physician controller and patient controller. The researcher will have unrestricted access to all the (b)(4) functionality, the clinician will interact with a refined version of the researcher interface (b)(4) and the patient will interact with a highly restricted, simple, user friendly interface (b)(4)

Sub-Task 1.8.2 Evaluation board fabricated/assembled and initial evaluation complete

(b)(4)
Once the (b)(4) is finished and being fabricated, the controller can be evaluated for connectivity and reliability bugs. This process is expected to (b)(4)
(b)(4) After the bugs have been worked out, the controller can be integrated into an enclosure with (b)(4) and firmware.

Sub-Task 1.8.3 Integration with a simple enclosure with (b)(4) and firmware (b)(4)

The (b)(4) has not begun development at this time. We expect the (b)(4) design to be a quick process to be implemented over the next coming months.

(b)(4)

(b)(4)

(b)(4)

Task 1.10 Software Development Kit (SDK) (b)(4)

Sub-Task 1.10.1 Requirements understood for SDK (b)(4)

The SDK will be developed as part of the firmware development for the (b)(4). The requirements for the SDK are defined.

Task 1.11 GLP animal study for (b)(4) system (b)(4)

The purpose of the GLP safety studies is to provide support for the safe initiation of an Early Feasibility IDE Study of our (b)(4) system in humans. (b)(4)

(b)(4)

(b)(4)

(b)(4)

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Task 1.13 Early feasibility human clinical protocol (b)(4)

Thermaquil team has drafted an early feasibility clinical study for (b)(4) with input from clinicians during (b)(4). This clinical protocol is needed for the FDA IDE submission. We will submit the clinical protocol for FDA feedback in (b)(4) (Fig. 25).

(b)(4)

(b)(4)

Task 1.14 FDA IDE (b)(4)

Task 1.14.1 FDA IDE (b)(4)

Task 1.14.2 FDA IDE (b)(4)

Thermaquil held an informational meeting with the FDA on (b)(4) (Fig.26) and also met with FDA (b)(4)

(b)(4) Given FDA's positive feedback, we amended (b)(4)

(b)(4)

(b)(4), (b)(6)

Task 1.15 FDA IDE (b)(4)

Task 1.15.1 FDA IDE (b)(4)

Thermaquil has drafted FDA IDE (b)(4) which focuses on the (b)(4) (b)(4). Our next step is to submit this (b)(4) (Fig.28), and if it is of interest to the FDA, we will request an informational meeting.

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Two weekly meetings with the team members from University of Pittsburgh, (b)(4) and Thermaquil were held regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermaquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"

Experimental Neurology, 2021, submitted.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6)	
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

		(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4)
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

			(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system	(b)(4)						(b)(4)
1.2.1 Restore bladder function (b)(4)	(b)(4)					Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.2.2 Restore bowel function (b)(4)	(b)(4)					Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment	(b)(4)						
1.3.4 Integration of (b)(4) in enclosure with firmware and PC application connectivity (b)(4)	(b)(4)					Milestone 1.3.4 - Deliverable 1.3	
1.4 Implantable pulse generator with	(b)(4)						
1.4.1 Can/Header design with design review complete (b)(4)	(b)(4)					Milestone 1.4.1 – Deliverable 1.4	
1.4.2 Can samples developed and fully evaluated (weld, header attach, feed throughs, etc.) (b)(4)	(b)(4)					Milestone 1.4.2 – Deliverable 1.4	
1.5 Stimulation Leads and cuff electrodes for animal studies	(b)(4)						
1.5.4 Finalize design and lead/cuff electrode production (b)(4)	(b)(4)					Milestone 1.5.4 – Deliverable 1.5	
1.6 External pulse generator for	(b)(4)						
1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)	(b)(4)					Milestone 1.6.1 – Deliverable 1.6.1 – Deliverable 1.6.2	
1.6.2 Integration of the external pulse generator components with firmware and the enclosure (b)(4)	(b)(4)					Milestone 1.6.2 – Deliverable 1.6.2	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.7 (b)(4) electrodes for human use (b)(4)	(b)(4)	(b)(4)					
1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.1 – Deliverable 1.7	(b)(4)
1.7.2 Finalize design of human (b)(4) (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.2 – Deliverable 1.7	
1.8 Physician/Investigator controller (b)(4)	(b)(4)	(b)(4)					
1.8.1 Schematic design complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1 – Deliverable 1.8	
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1 – Deliverable 1.8	
1.8.3 enclosure with display and firmware (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1 – Deliverable 1.8	
1.10 Software Development Kit (SDK) (b)(4)	(b)(4)	(b)(4)					
1.10. Requirements understood for 1 SDK (b)(4)	(b)(4)	(b)(4)				Milestone 1.10.1 – Deliverable 1.10	
1.14 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.14. FDA IDE (b)(4) 2 meeting (b)(4)	(b)(4)	(b)(4)				Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.15. FDA IDE (b)(4) 1 (b)(4)	(b)(4)	(b)(4)				Milestone 1.15.1 – Deliverable 1.15.1	
1.12 (b)(4)	(b)(4)	(b)(4)					
1.12 (b)(4)	(b)(4)	(b)(4)				Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)	(b)(4)					
1.13 Early feasibility human clinical protocol (b)(4)	(b)(4)	(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)	(b)(4)	(b)(4)					
1.11 GLP animal study (b)(4)	(b)(4)	(b)(4)				Milestone 1.11 – Deliverable 1.11	

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 0787

University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: May 1, 2021 through July 31, 2021

(b)(4)

Date of Report: August 31, 2021

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: \$10,451,439.00

Subcontractors: (b)(4) and Thermaquil, Inc.

Program Manager: (b)(4), (b)(6) DARPA/BTO

Submitted by:

(b)(4), (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) we have made major progresses in the following areas:

1. Scientific progress:

- We have successfully demonstrated in anesthetized cats that stimulation of sacral S2 spinal root at (b)(4) is most effective to induce colon contractions, while stimulation of S1/S3 root or pudendal nerve is not effective. In addition, defecation is successfully induced by stimulation of S2 spinal root using wire electrodes. This success indicates a minimally invasive surgical approach by inserting lead electrodes close to the S2 spinal root can be developed to restore bowel function after spinal cord injury. The data/results from this animal study have been processed, analyzed, and summarized. A manuscript is ready to be submitted for review and publication.

- (b)(4)

- (b)(4)

2. Engineering progress:

- (b)(4)

- Many (b)(4) iterations have been designed and printed for analysis and comparison.

- (b)(4)

- The (b)(4) has been eliminated from the surgical tool list (b)(4)

- (b)(4)

- (b)(4)

This resulted in a need for an IDE (b)(4)

- (b)(4)

- Completed the full electrical redesign schematics of a (b)(4)

- (b)(4)

- All safeties and electrical conditions suitable for human implantation have been reviewed, analyzed, and implemented in the new platform, thus making it suitable as (b)(4)

- Preliminary firmware has been written and completed to meet with the new schematics guided by BG+ schematics requirements.

- The new schematics design uses (b)(4) requiring software development. This software development has been completed.

- (b)(4)

- The PCB fabrication was successfully completed, (b)(4)

- (b)(4)

- A sample population of the PCB's were populated with acquired parts and tested, then brought up, loaded with firmware and tested for function and pulse generation.
- The enclosure design for the (b)(4) was completed, with specialty hardware located and acquired. (b)(4)
- (b)(4) enclosures were successfully fabricated as proof of concept, to be integrated with the (b)(4) then demonstrated to University of Pittsburgh.
- (b)(4) will be programed and controlled (b)(4) based Graphical User Interface (GUI). (b)(4) will communicate with (b)(4)
- Multiple sessions (b)(4), (b)(6) concluded in a complete GUI that meets with the BG+ therapy approach and requirements. The completed GUI is currently being interfaced with the EPG for full functionality.
- A new (additional) engineer, (b)(4), was welcomed to our team (b)(4) exclusively focused on (b)(4) design, manufacturing and testing.
- Progress on a (b)(4) was achieved. The newly acquired (b)(4) was successfully used to fabricate (b)(4). BG+ required materials were specified, identified, located then ordered, while (b)(4)
- Tooling was designed for (b)(4) manufacturing, completed then sent out for fabrication, anticipated (b)(4). Additionally, peripheral equipment was identified and purchased for (b)(4) manufacturing, currently in house awaiting final tooling. Lastly, needed raw materials and consumables were specified, located then purchased, currently in house to be enacted on shortly.
- The mechanical design of the BG+ implanted device (b)(4) progressed significantly in (b)(4) with a novel approach and design unique to the market, as far as we can ascertain. (b)(4)
- (b)(4)
- Also this quarter, we have compared and contrasted various communication schemes and resulting mechanical antennae designs for the IPG, deciding on optimal scenarios and resultant updates to the IPG, with two main considerations to test and simulate.
- (b)(4)
- (b)(4)

3. Regulatory progress:

- Met with the FDA regarding (b)(4)
 - Received feedback on the proposed GLP swine safety studies, Non-GLP feline efficacy studies (b)(4)
 - (b)(4)
- Drafted early feasibility human clinical protocol (b)(4)
- (b)(4), (b)(6)
- Continued to identify user needs and voice of customer to inform device requirements (b)(4)
- FDA (b)(4) was submitted, which focuses on (b)(4) (b)(4)

4. Administrative progress:

- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
- Two weekly meetings were hold between University of Pittsburgh, (b)(4) and Thermaquil to coordinate the project activity.
- Monthly meeting was hold with DARPA to report our progress.
- (b)(4)

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.2 Restore bowel function (b)(4)

A total of 9 cats (4 females and 5 male, 4.0 ± 0.3 kg; Liberty Research, Waverly, NY) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, one balloon catheter (G15766, Cook Urological, Spencer, IN) 1.4 cm in diameter and 5 cm in length was inserted into the proximal colon via a small incision at the proximal end of the colon (Fig.1). The second balloon catheter of the

same size was inserted into the distal colon via the anus. The two balloons were filled with water (5-7 ml) to a resting pressure of 10-15 cmH₂O to measure proximal and distal colon contractions in the first group of 7 cats. In the second group of 2 cats, a large balloon catheter made of a condom (3.5 cm in diameter and 15 cm in length) was inserted via the small incision at the proximal end of the colon to occupy the entire colon so that both proximal and distal colon contractions were recorded. The condom was filled with water (60-70 ml) to a resting pressure of 10-15 cmH₂O. In these 2 cats, 1-4 glass marbles (diameter 1.5 cm) were also inserted into the rectum via the anus so that evacuation of these marbles could be videotaped simultaneously with the colon pressure recording.

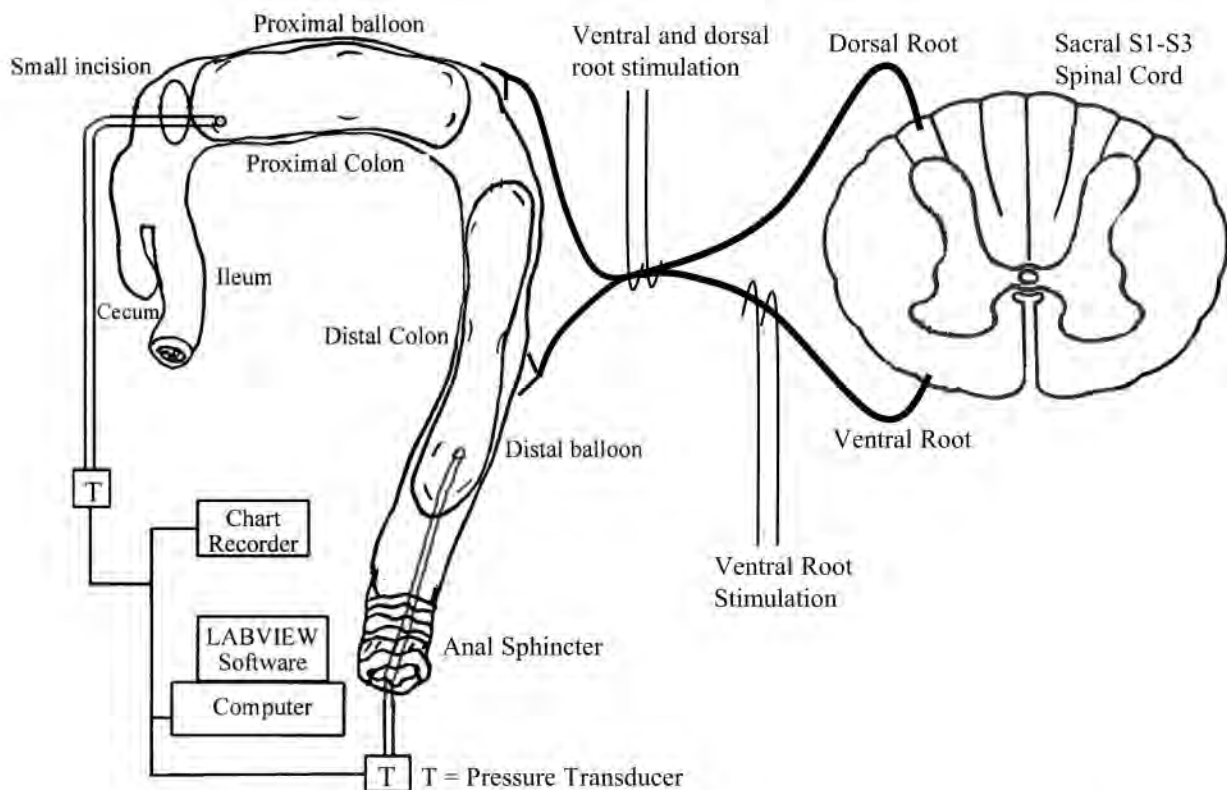


Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce colon contractions.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (Fig.1). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Colon contraction induced by sacral ventral root stimulation is dependent on stimulation frequency and the spinal segment stimulated.

Stimulation of S2 ventral root induced larger colon contractions than stimulation of S1 or S3 ventral roots, and the most effective stimulation frequency was (b)(4) (Figs.2-3). Fig.2

shows the distal and proximal colon pressure traces recorded from the same cat. Stimulation of S2 ventral root induced colon contractions >20 cmH₂O at frequencies (b)(4) with a similar contraction amplitude in the distal and proximal colon (Fig.2B). Stimulation of the S1 or S3 ventral root did not induce colon contractions larger than the small (<10 cmH₂O) rhythmic baseline colon contractions that were occurring asynchronously during the periods of stimulation (Fig.2 A and C). In this cat, the stimulation intensity used for S2 ventral root stimulation is 2 times the threshold intensity used during (b)(4) stimulation to induce an observable proximal



Fig 2. Distal and proximal colon responses to stimulation of sacral S1-S3 ventral roots at different frequencies (b)(4). A. S1 ventral root. B. S2 ventral root. C. S3 ventral root. Data were obtained from the same animal.

colon contraction. Due to the weak colon response to S1 and S3 ventral root stimulation, the maximal stimulation intensity just below the threshold for inducing lower spine movement was applied to these roots. Hindlimb (mainly S1/S2 stimulation) and/or tail (mainly S2/S3 stimulation) movements were induced by stimulation of each of the three roots. (b)(4) summarizes the results from 7 cats showing that S2 ventral root stimulation at (b)(4) was optimal for producing significantly ($p < 0.05$) larger colon contractions than those elicited by S1 or S3 ventral root stimulation. There was no significant difference between the contraction amplitudes of distal and proximal colon (b)(4).

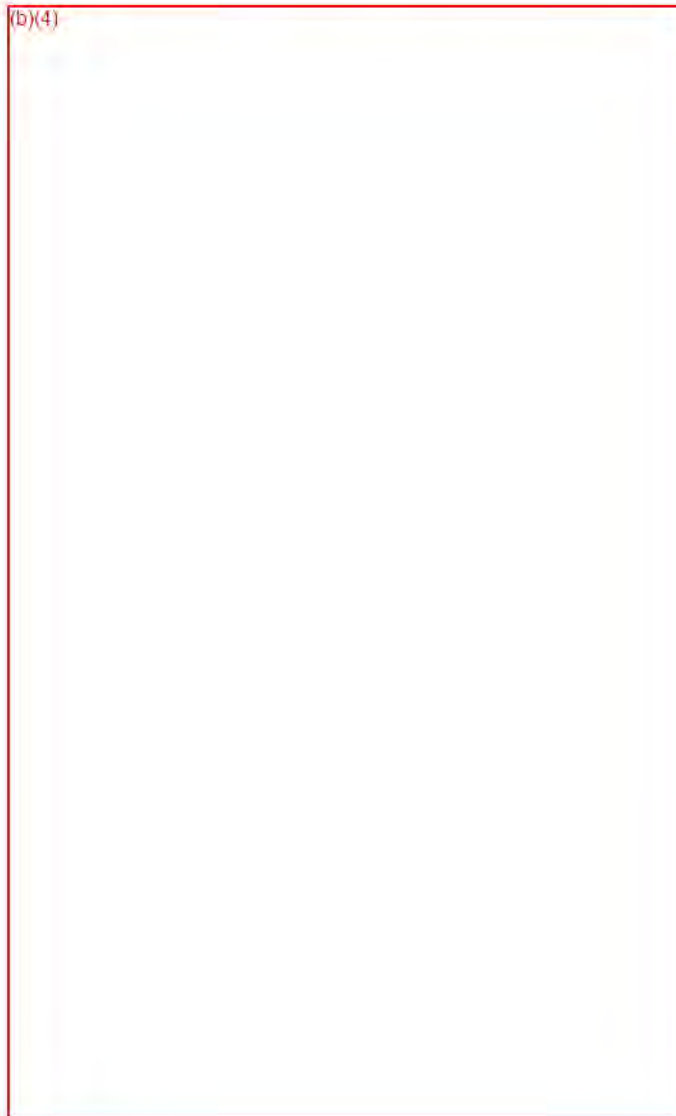


Fig.3. Distal and proximal colon responses to sacral ventral root stimulation are frequency and spinal segment dependent. A. S1 ventral root. B. S2 ventral root. C. S3 ventral root. The contraction amplitude induced by different stimulation frequencies in different sacral segments is normalized to the maximal response in the same animal. Stimulation intensity (4-16 V) for S2 ventral root was 1.5-3 times threshold intensity for (b)(4) to induce an observable contraction of proximal colon. When S1 and S3 ventral root stimulation did not induce a colon contraction, the maximal stimulation intensity (8-12 V) without causing lower body movement was used to test different frequencies. * significantly different from (b)(4) (one-way ANOVA); # significantly different from S2 ventral root (two-way ANOVA). N = 7 cats.

Fatigue of colon contractions induced by S2 root stimulation

Stimulation (b)(4) of the S2 ventral and dorsal roots together induced large amplitude (>20 cmH₂O) contractions in both distal and proximal colon (b)(4)

(b)(4)

However, the contraction amplitude gradually declined during a continuous 5-minute stimulation, indicating a fatigue in the colon contractions (b)(4). On average the contraction

amplitude for both the distal and proximal colon was maintained during the 5 intermittent 1-minute stimulations (N=4 cats, Fig.4B), but was significantly ($p<0.05$) reduced from the mean maximal amplitude of 30 cmH₂O to about 18 cmH₂O at the end of the 5-minute continuous stimulation (N=7 cats, Fig.4C).

A. S2 Ventral and Dorsal Roots

(b)(4)



(b)(4)

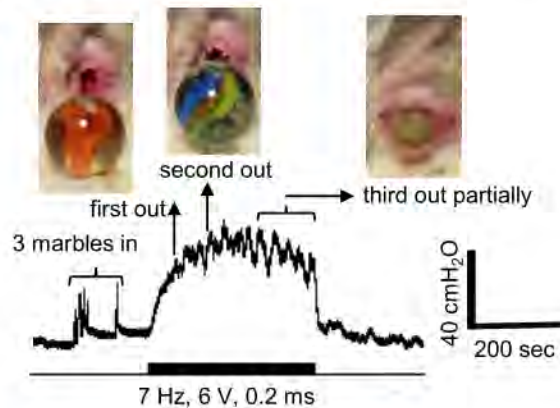


Defecation induced by S2 root stimulation

Stimulation of the S2 ventral and dorsal roots together induced a maximal amplitude colon contraction of 60 cmH₂O in cat #1 (Fig.5A) and 40 cmH₂O in cat #2 (Fig.5B). In cat #1 after 3 marbles were inserted into the rectum, S2 root stimulation (b)(4) induced a successful defecation that eliminated the first marble after 1 minute of stimulation and the second marble after 2 minutes of stimulation (Fig.5A). The elimination of the third marble which started during the last 2 minutes of the 6-minute stimulation was incomplete (Fig.5A). In cat #2 after 1 marble was inserted into the rectum, the S2 root stimulation (b)(4)

(b)(4) eliminated the marble after 2.5 minutes of stimulation (Fig.5B). However, after 4 marbles were inserted into the rectum, the stimulation eliminated only 1 of the 4 marbles after 4.5 minutes of stimulation with 3 marbles in the rectum at the end of 11 minutes of stimulation (Fig.5B). Although stimulation of S2 ventral and dorsal roots together only eliminated 1-2 marbles in these two cats, stimulating the S2 ventral root alone without the dorsal root successfully eliminated all 4 marbles in both cats.

A. Cat #1



B. Cat #2

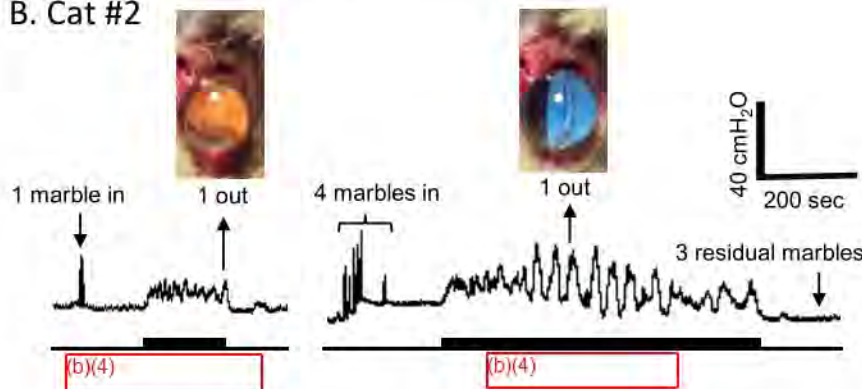


Fig.5. Defecation induced by stimulation of S2 ventral and dorsal roots. A. In cat #1 after inserting three marbles into the rectum, the defecation induced by sacral S2 root stimulation eliminated 2 marbles after 1 minute and 2 minutes of stimulation, respectively, with the last marble partially evacuated during the last 2 minutes of the 6-minute stimulation. B. In cat #2 after inserting 1 marble into the rectum, it was defecated after 2.5 minutes of stimulation. After inserting 4 marbles, stimulation of 11 minutes produced defecation of 1 marble at 4.5 minutes with 3 residual marbles in the rectum. Colon contraction pressure was measured by a large condom catheter with the condom covering the proximal and distal colon.

Task 1.4 Implantable pulse generator with (b)(4)

(b)(4)

Sub-Task 1.4.2 Can samples developed and fully evaluated (b)(4)

(b)(4)

The (b)(4) IPG design had (b)(4)

(Fig.6). (b)(4)

(b)(4). With this design, the radio communication antenna is (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

1.4.3 Integrate pulse generator circuit into can (b)(4)

The (b)(4) has been completed and received, currently under test and evaluation. Any modifications to the design will be identified and implemented for (b)(4)

(b)(4) has experience in miniaturizing the (b)(4). Steps have been taken by the (b)(4) electrical team to reduce the (b)(4). This task cannot be marked as completed until (b)(4) then (b)(4) then tested again.

1.4.4 Final (b)(4) with wireless board connectivity (b)(4)

A laptop based GUI has been completed and currently in (b)(4). The GUI was created this (b)(4) working in conjunction with (b)(4); (b)(4) thus guided on the clinical application and requirements therein. Laptop to communicate with (b)(4) (b)(4). This series of testing and communication activity tests will become the prelude of similarly configured (b)(4) to include (b)(4) (b)(4) and other tests as part and parcel of (b)(4) (b)(4)

1.4.5 Full integration and test completed for initial prototype (b)(4)

The (b)(4) requires the completion and successful (b)(4) given that (b)(4) (b)(4). These tasks are designed at the end to start to assure (b)(4)

Task 1.6 External pulse generator (b)(4)

The (b)(4) has progressed (b)(4) from an initial design and concept to (b)(4)

(b)(4)

Sub-Task 1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)

Sub-Task 1.6.2 Integration of the external pulse generator components with firmware and the enclosure (b)(4)

The (b)(4) enclosure was updated to (b)(4)

(b)(4) enclosure was (b)(4) using
(b)(4)

The Boards have been received fully populated (b)(4)

(b)(4)

Once received, firmware was loaded on the Boards and bring up was started immediately. To date, (b)(4) No effort is being spared, with the team working on this task continuously since PCBA receipt. As such,

(b)(4)

(b)(4)

(b)(4)

Task 1.7 (b)(4) electrodes for human use (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.8 Physician/Investigator controller for human (b)(4)

The first incarnation is the current GUI already in integration, with learnings and adjustments needed, if any, transposed to future controllers, example in Fig.19, below. This is a (b)(4) GUI written using (b)(4)

(b)(4)

Sub-Task 1.8.1 Schematic design complete (b)(4)

Control of the (b)(4) to be accomplished by using a (b)(4) so there is no (b)(4) (b)(4) The shown GUI (b)(4) is written using (b)(4)

Sub-Task 1.8.2 Evaluation board fabricated/assembled and initial evaluation complete

(b)(4)

The GUI is running on (b)(4)

Sub-Task 1.8.3 Integration with a simple enclosure with (b)(4) and firmware (b)(4)

Once more, the 'controller' is a software written for (b)(4)

(b)(4)

Task 1.9 Patient controller for (b)(4)

The current GUI will be used as a (b)(4)

(b)(4)

Sub-Task 1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)

The software is structured to (b)(4)

(b)(4)

Task 1.11 GLP animal study for (b)(4)

The purpose of the GLP safety studies is to provide support for the safe initiation of an Early Feasibility IDE Study of our (b)(4) in humans. To support the previously proposed (b)(4)

(b)(4)

(b)(4)

(b)(4), (b)(6)

(b)(4)

Task 1.13 Early feasibility human clinical protocol for (b)(4)

Thermaquil team has drafted an early feasibility clinical study for the (b)(4) with input from clinicians during (b)(4). This clinical protocol is needed for the FDA IDE submission. We submitted the clinical protocol for FDA feedback in (b)(4) (Fig. 24).

(b)(4)

Task 1.15 FDA IDE (b)(4)

Task 1.15.1 FDA IDE (b)(4)

Task 1.15.2 FDA IDE

(b)(4)

(b)(4), (b)(6)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Two weekly meetings with the team members from University of Pittsburgh, (b)(4) and Thermaquil were hold regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermaquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.
"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".

American Journal of Physiology - Gastrointestinal and Liver Physiology, submitted.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"

Experimental Neurology, 2021, in revision.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)		(b)(4); (b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6)	
		(b)(4); (b)(6)	

Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Clinical Consultant	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

			(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system	(b)(4)						(b)(4)
1.2.2 Restore bowel function (b)(4)	(b)(4)					Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment	(b)(4)						
Integration of (b)(4) in enclosure							
1.3.4 with firmware and (b)(4) connectivity (b)(4)	(b)(4)					Milestone 1.3.4 - Deliverable 1.3	
1.4 Implantable pulse generator with	(b)(4)						
Can samples developed and fully evaluated (b)(4)	(b)(4)					Milestone 1.4.2 – Deliverable 1.4	
1.4.3 Integrate pulse generator circuit into can (b)(4)						Milestone 1.4.3 – Deliverable 1.4	
1.4.4 Final PC test application with wireless board connectivity (b)(4)						Milestone 1.4.4 – Deliverable 1.4	
1.4.5 Full integration and test completed for initial prototype (b)(4)						Milestone 1.4.5 – Deliverable 1.4	
1.6 External pulse generator for	(b)(4)						
1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)	(b)(4)					Milestone 1.6.1 – Deliverable 1.6.1 – Deliverable 1.6.2	
1.6.2 Integration of the external pulse generator components with firmware and the enclosure (b)(4)						Milestone 1.6.2 – Deliverable 1.6.2	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.7 (b)(4) electrodes for human use (MAC4-MAC15)							
1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)	(b)(4)				(b)(4)	Milestone 1.7.1 – Deliverable 1.7	Delayed (Cadaver study required)
1.7.2 Finalize design of human (b)(4)	(b)(4)					Milestone 1.7.2 – Deliverable 1.7	None
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)					Milestone 1.7.3 – Deliverable 1.7	None
1.8 Physician/Investigator controller (b)(4)							
1.8.1 Schematic design complete (b)(4)	(b)(4)					Milestone 1.8.1 – Deliverable 1.8	None
1.8.2 Evaluation board fabricated/assembled and initial evaluation complete (b)(4)	(b)(4)					Milestone 1.8.1 – Deliverable 1.8	None
1.8.3 Integration with a simple enclosure with display and firmware (b)(4)	(b)(4)					Milestone 1.8.1 – Deliverable 1.8	None
1.9 Patient controller for (b)(4)							
1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)	(b)(4)					Milestone 1.9.1 – Deliverable 1.9	None
1.15 FDA IDE (b)(4)							
1.15.1 FDA IDE (b)(4)	(b)(4)					Milestone 1.15.1 – Deliverable 1.15.1	Delayed (cadaver study required)
1.15.2 FDA IDE (b)(4)	(b)(4)					Milestone 15.2.1 – Deliverable 15.1.2	Delayed – meeting scheduled on August 27, 2021
1.12 (b)(4)							
1.12 Human cadaver study	10/1/2020	10/1/2020	07/30/2021	5/22/2021	(b)(4)	Milestone 1.12 – Deliverable 1.12	None
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)					Milestone 1.13 – Deliverable 1.13	None
1.11 GLP animal study (b)(4)							
1.11 GLP animal study	(b)(4)					Milestone 1.11 – Deliverable 1.11	None

WCWP v DoD, No. 23-3264 DARPA 22-5-1285 0815

University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: August 1, 2021 through October 31, 2021 (b)(4)

Date of Report: November 30, 2021

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: (b)(4)

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(6)

Submitted by:

(b)(4), (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) quarter (b)(4), we have made major progresses in the following areas:

1. Scientific progress:

- We have successfully demonstrated in anesthetized cats that stimulation of sacral S1/S2 spinal root (b)(4) using wire electrodes is most effective to induce penile erection, while stimulation of S3 root or pudendal nerve is not effective. This success indicates a minimally invasive surgical approach by inserting lead electrodes close to the S1/S2 spinal root can be developed to restore penile erection after spinal cord injury. We are now processing and analyzing the data/results from this animal study and will summarize the results for a publication next month.
- Based on our studies of sacral spinal root stimulation to restore defecation and penile erection functions in cats. (b)(4)
(b)(4)
- Our first paper titled "Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats" has been published on Experimental Neurology.

- Our second paper titled "Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats" has been accepted for publication on American Journal of Physiology - Gastrointestinal and Liver Physiology.
- The (b)(4) has been successfully tested in 2 cats to inhibit bladder at (b)(4) excite bladder at (b)(4) and block pudendal nerve at (b)(4)

2. Engineering progress:

(b)(4)

- (b)(4) designs were conceived, down selected balancing (b)(4)
- Multiple (b)(4) were created for testing.

(b)(4)

- More fixtures and tools were created to aid in the construction of the (b)(4)
- New quotes were requested for (b)(4) from additional suppliers.

(b)(4)

- Updated firmware has been written and completed to meet the new schematics guided by requirements.
- With the drop test and water ingress test approaching, the (b)(4) design was updated to have (b)(4)
- The updated (b)(4) enclosures were printed for testing.
- Multiple sessions with UPitt and (b)(4) GUI and hardware developers concluded in a complete GUI and hardware system that meets with the BG+ therapy approach and requirements.
- The (b)(4) is programed and controlled by a (b)(4) Graphical User Interface (GUI).
- The (b)(4) GUI successfully communicates and controls the (b)(4) stimulation output wirelessly.
- All components for the IDE study are currently being compiled for FDA submission.

3. Regulatory progress:

- Submitted and met with the FDA regarding (b)(4) on the (b)(4)
 - Received specific feedback on (b)(4) for the proposed clinical trial protocol.
 - Received feedback on our proposed (b)(4) that is being incorporated into the (b)(4) of our IDE.
 - Given FDA's feedback, we (b)(4)
- Revised our draft early feasibility human clinical protocol for (b)(4)
- Began drafting (b)(4)

- Continued to refine user needs and voice of customer to continuously confirm device requirements to (b)(4)

4. Administrative progress:

- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
- Weekly meetings were held between University of Pittsburgh, (b)(4) and Thermoquil to coordinate the project activity.
- (b)(4)
- (b)(4)

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.3 Restore sexual function (b)(4)

During this quarter, a total of 6 male cats were used to study the penile erection by stimulation of sacral roots or pudendal nerve. The animals were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (see Fig.1). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

A 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (see Fig.1). Since electrically evoked penile erection is very well correlated with increased corpus cavernosum pressure, pressure recording from the corpus cavernosum was used as a quantitative measurement of penile erection in this study.

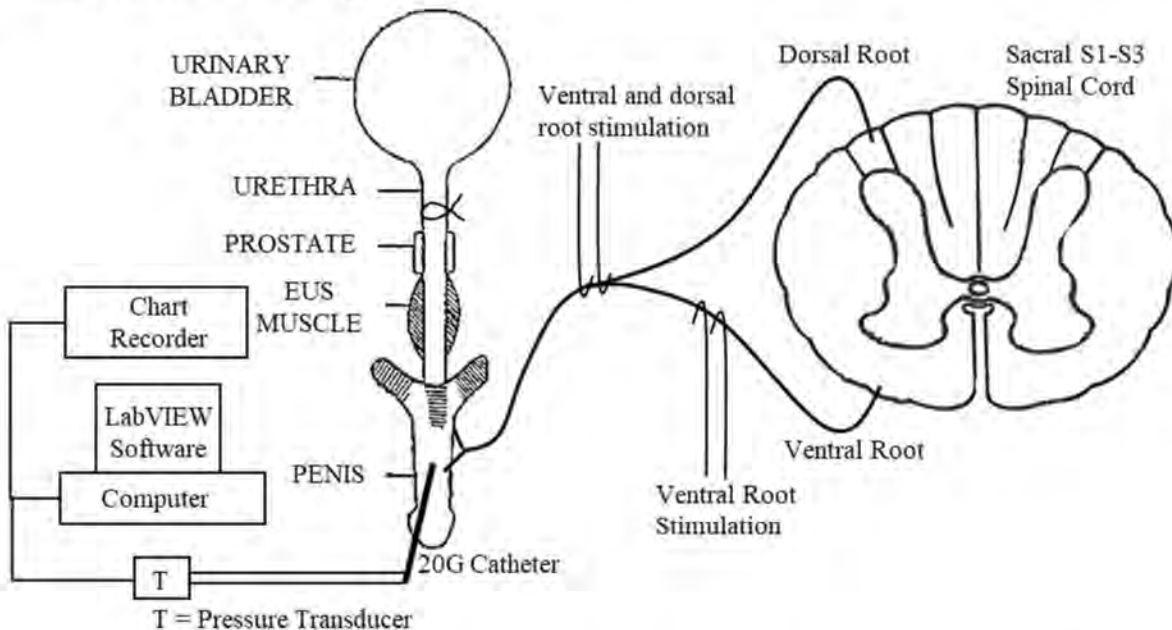


Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

Based on the results obtained from the 6 male cats, penile erection could be induced by stimulation of S1/S2 ventral root but stimulation of S3 ventral root or pudendal root was not effective. Fig.2 shows in a cat that S1 ventral root stimulation induced large (>200 cmH₂O) pressure increase in the penis at a frequency range of 30-60 Hz while S2 ventral root

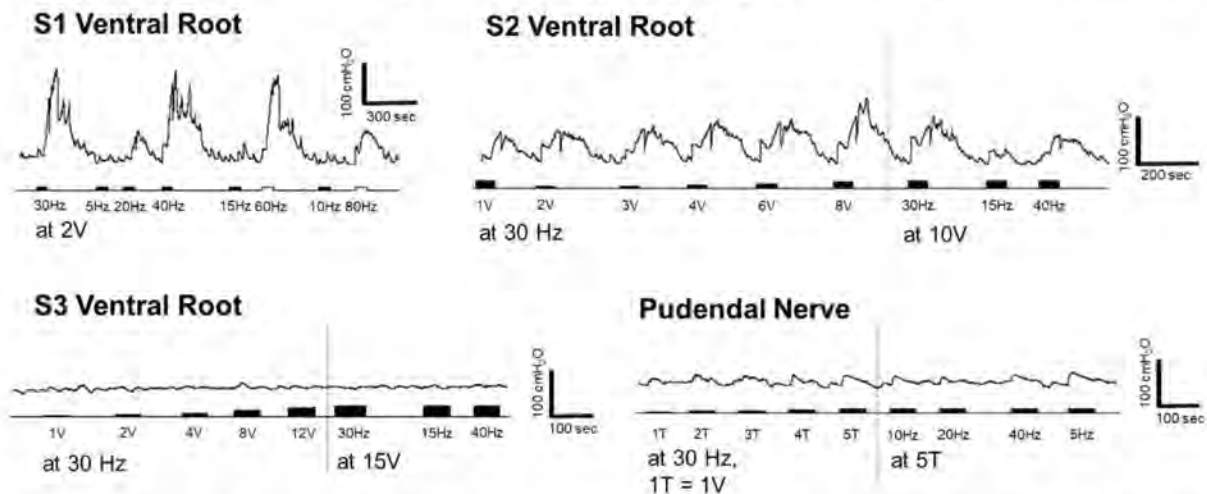


Figure 2. Penile pressure induced by stimulation of S1-S3 ventral root or pudendal nerve in the same cat.

stimulation only induce <100 cmH₂O penile pressure increase. Stimulation of S3 ventral root or pudendal nerve failed to induce increase in penile pressure (bottom traces in Fig.2). Fig.3 shows in another cat that the S2 ventral root stimulation was effective in inducing penile pressure increase but stimulation of S1/S3 ventral root or pudendal nerve was not effective. In the total 6 cats tested so far, S1 ventral root is most effective in 4 cats while S2 ventral root is most effective in 2 cats. On average, stimulation frequency of (b)(4) is the most effective frequency.

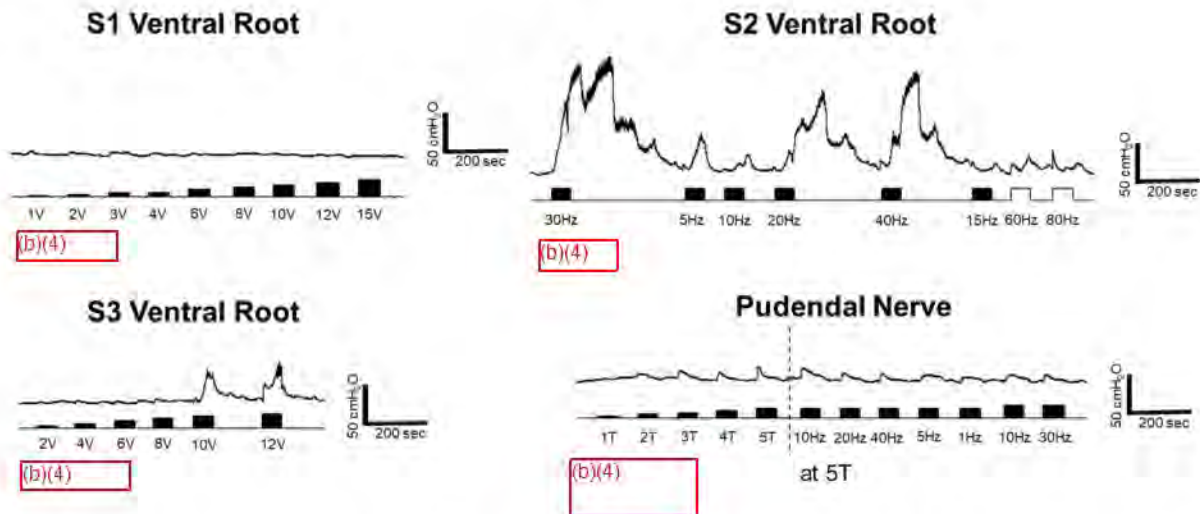


Figure 3. Penile pressure induced by stimulation of S1-S3 ventral root or pudendal nerve in the same cat.

Stimulation of the entire S1/S2 spinal root (ventral and dorsal roots together) is also effective in increasing penile pressure. Fig.4A shows in a cat that S1 spinal root stimulation induced large (>150 cmH₂O) penile pressure increase that sustained for the entire 10 minutes of stimulation. Fig.4B shows in another cat that S2 spinal root stimulation induced gradually increasing pressure that reached maximal (>100 cmH₂O) at the end of the 10 minutes of stimulation. Therefore, sacral spinal root stimulation could induce large sustained penile erection lasting as long as the stimulation continues. The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to employ a minimally invasive surgical approach for stimulation. Sacral spinal roots in humans are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder. Therefore, our study has significant implications for developing a new sacral neuromodulation therapy using a minimally invasive surgical approach to restore erectile function for SCI people.

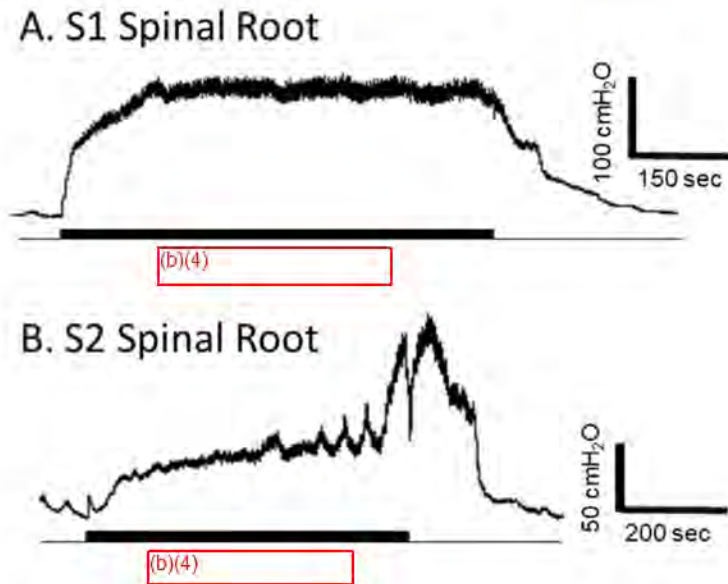


Fig.4: Stimulation of S1/S3 spinal root (ventral and dorsal roots together) can also induce large increase in penile pressure. A. S1 spinal root stimulation in a cat. B. S2 spinal stimulation in another cat.

Sub-Task 1.2.5 Test electrode and device prototypes (b)(4)

We also successfully tested the (b)(4) in 2 cats. The (b)(4) generated different types of stimulation signals to achieve the functions of inhibiting bladder, exciting bladder, or blocking the pudendal nerve. Fig. 5 shows that the (b)(4) inhibited bladder contractions by generating a (b)(4) stimulation of the pudendal nerve, excited the bladder by generating (b)(4) of the pudendal, and blocked the pudendal nerve conduction by generating (b)(4) waveform. We will continue testing the (b)(4) in more animal studies to fully evaluate its function and reliability.

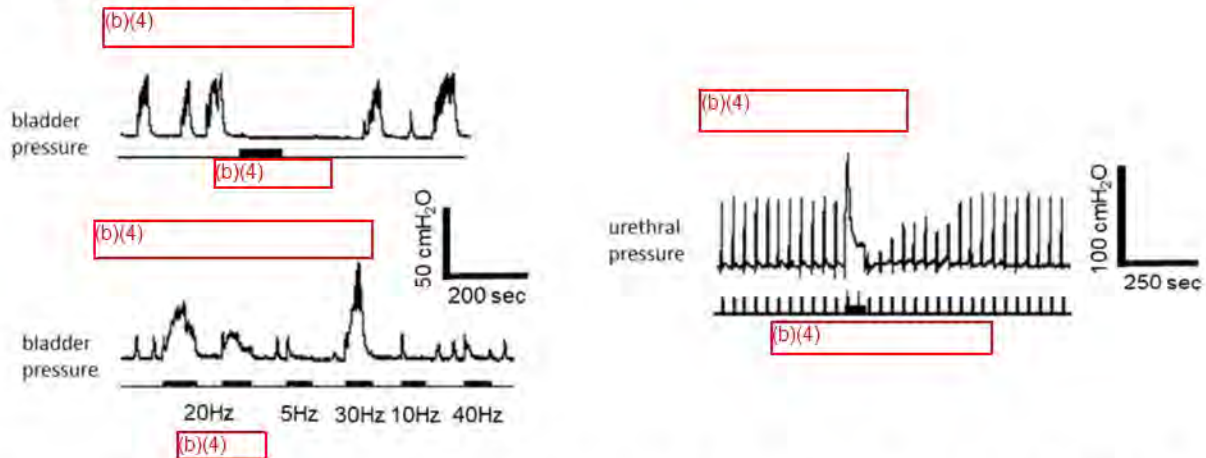


Figure 5. The EPG board successfully generated the required stimulation signals for bladder inhibition or excitation, and for pudendal nerve block.

Task 1.4 Implantable pulse generator with (b)(4)

(b)(4)

1.4.3 Integrate pulse generator circuit into can (b)(4)

The (b)(4) circuit miniaturization is underway. The current (b)(4) schematic has been reduced in size as much as possible with the current components. The smaller (b)(4) size is about the (b)(4). Smaller components have been identified and have been ordered. These smaller components will replace the current components and allow for the final miniaturization down to (b)(4) size (Figure 6, 7). New quotes were received for (b)(4) (b)(4) cans from a local supplier. This quote was reasonable and very encouraging with respect to (b)(4)

(b)(4)

1.4.4 Final (b)(4) test application with wireless board connectivity (b)(4)

The (b)(4) test application focused on use in an animal study has been working successfully over (b)(4) and now the GUI can wirelessly command the (b)(4) to stimulate (b)(4). Multiple cat experiments have been conducted while using the GUI to control the (b)(4). Additional testing will be completed upon the miniaturization of the (b)(4) board. Final communication testing will be completed upon schematic integration into the (b)(4)

1.4.5 Full integration and test completed for initial prototype (b)(4)

The complete integration of the (b)(4) circuit into an (b)(4) for animal implantation is currently underway. As mentioned before, (b)(4)

(b)(4) All parts have been ordered. (b)(4) has previously achieved the miniaturization and successful testing with other (b)(4) designs. All testing that can be done on previous (b)(4) enclosures and current (b)(4) boards, is being done. Testing design, setup and protocols are being prepared now so they are ready for when a complete (b)(4) can be assembled.

Task 1.6 External pulse generator for (b)(4)

Sub-Task 1.6.2 Integration of the (b)(4) with firmware and the enclosure (b)(4)

The (b)(4) enclosure was updated in preparation for the drop testing and transportation testing.

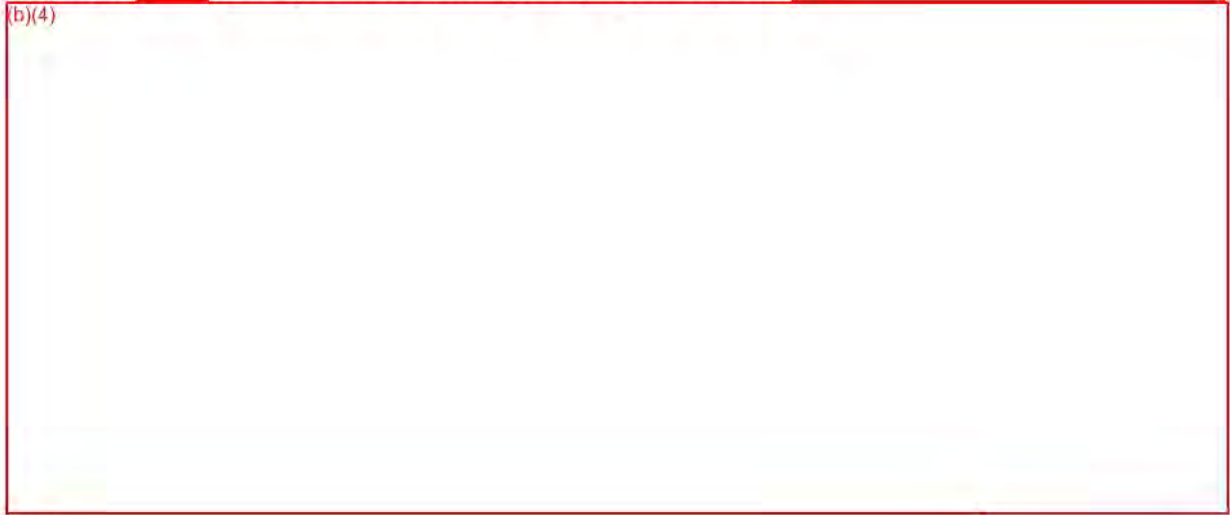
(b)(4)

(b)(4) have been installed into

(b)(4)

(Figures 8 and 9).

A simple (b)(4) overlay has been designed (Figure 10). The (b)(4)



(b)(4)

(b)(4)

(b)(4)

Task 1.7 (b)(4) electrodes for human use (b)(4)

Sub-Task 1.7.3 First prototypes of (b)(4) delivered for test (b)(4)

Both the installation qualification for the (b)(4) (b)(4) are completed and signed. The installation qualification is a protocol to ensure equipment used in production is installed and verified to be operational and maintainable for use in producing parts. Part of the installation qualification is doing a software validation. We must verify that when a value is added or changed in the program, the machine follows those parameters and gives the correct readout. All adjustable equipment parameters (b)(4) (b)(4) have to be verified. This was done by using a protractor to verify the (b)(4) was input by using a (b)(4)

The operational qualification is a protocol to verify that the designed process will produce acceptable results within established limits of a particular process's parameters. The operational qualification is more specifically for (b)(4) For this qualification, multiple (b)(4) were created at various (b)(4) to find (b)(4) (b)(4)

(b)(4)

(b)(4) Using statistical analysis of a 95% confidence level, we found that we were statistically in control (b)(4) The operational qualifications for the (b)(4)

(b)(4) will be completed soon.

(b)(4) and (b)(4) have been purchased in anticipation of the various upcoming tests. Biocompatibility testing for (b)(4) is underway. Material specifications and drawings are being created to be sent to suppliers for quotation.

Testing was done using the (b)(4)

(b)(4)

(b)(4)

(b)(4) This testing was completed using a (b)(4)

(b)(4) This testing was completed using a (b)(4)

(b)(4) These measurements gave a base-

(b)(4) These measurements gave a baseline to which (b)(4) should be comparable. (b)(4) were printed out of

(b)(4) is the leading material so far to be used to make (b)(4). The newly created (b)(4) were attached to a (b)(4). The

(b)(4) The newly created (b)(4) were attached to a (b)(4) The same tests that were completed on the (b)(4)

(b)(4) The result of the (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4) (b)(4)

(b)(4) (Figure 15). The (b)(4)

(b)(4) (Figure 15). The (b)(4)

The (b)(4) and the (b)(4)

The (b)(4) and the (b)(4)

(b)(4)

(b)(4) (Figure 16). Our bench tests corroborate our (b)(4) consultants, the (b)(4) (b)(4) are the most similar in (b)(4)

(b)(4) are the most similar in (b)(4)



Task 1.8 Physician / Investigator controller for (b)(4)

The first incarnation is the current Graphical User Interface (GUI) already in integration and is currently under test in a lab-bench environment. This is a (b)(4) GUI written using (b)(4) that replicates the (b)(4). The software has the ability to (b)(4).

(b)(4)

An updated and simplified GUI was also written and provided to UPitt for use in (b)(4). (b)(4) The system was also improved, and its function enhanced during this last reporting period (quarter) and successfully used, at least three times, in cat experiments. (b)(4), (b)(6) and staff were able to successfully block nerve and stimulate for bladder control. Since, a completely functioning system along with a newly acquired (b)(4) running the latest GUI was left behind (b)(4), (b)(6) for continued testing, evaluation and clinical assessment, while any latest updates are and will continue to be provided as 'software patches' for enhanced function and workflow adaptation. Lastly, the system (b)(4) was operating wirelessly (b)(4) very effectively managing stimulation and experimentation while controlling all function and parameter settings remotely.

Sub-Task 1.8.2 Evaluation board fabricated / assembled and initial evaluation complete

(b)(4)

(b)(4); (b)(6)

This task is complete (b)(4). A total of (b)(4) were created and continue to be tested, one complete system (b)(4) is currently residing at (b)(4), (b)(6) for continued testing, while any function amelioration is provided by a software patch either provided in person at (b)(4), (b)(6) or electronically transmitted for subsequent updates.

(b)(4)

Sub-Task 1.8.3 Integration with a simple enclosure with display and firmware

(b)(4)

We currently have created three distinct (b)(4) control GUI's. The first, perhaps most complex, is a (b)(4) that includes multi-access to Patients and Physician using password protected screens, has been operational and in testing for the last (b)(4). This multi-screen workflow was co-authored with (b)(4), and (b)(4) working together and includes a (b)(4). This complex ultimately clinical workflow is preceded by a simpler intuitive application that while also (b)(4) is more (b)(4) driven in a simple interface that, while it may lack in control and depth of function, is more tuned towards lab testing to focus on the hardware functionality over all else. The (b)(4) will also be used to torture the (b)(4) to assure its functionality and resilience while under test.

Task 1.9 Patient controller for

(b)(4)

The patient controller is a subset of the (b)(4) GUI that is password restricted with simplified function and interface. This has been in existence for the last (b)(4) while testing continues. The team is very encouraged with results obtained to date across the board, and especially on the testing that continues in (b)(4), (b)(6) on animals, and in two other (b)(4) to meet with electrical testing on safety and efficacy (also to pass all ISO standard testing for electrical safety) in anticipation of human testing to come.

Sub-Task 1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)

The software is structured (b)(4)

(b)(4)

Sub-Task 1.9.2 Verification testing completed (b)(4)

Verification testing continues as well as electrical safety and efficacy. While the systems are currently being used in an animal lab setting, we are exhausting every test in anticipation of upcoming clinical human work.

Task 1.11 GLP animal study for (b)(4)

The purpose of the GLP safety study is to provide support for the safe initiation of an Early Feasibility IDE Study of our (b)(4) in humans. This was originally intended to support

(b)(4)

(b)(4), (b)(6)

Task 1.13 Early feasibility human clinical protocol for (b)(4)

Thermaquil drafted an early feasibility clinical study for the (b)(4) with input from clinicians during (b)(4). This clinical protocol is needed for the FDA IDE submission and was submitted for (b)(4). We received specific feedback to improve clarity of our (b)(4). We have updated our protocol (b)(4).

(b)(4)

(b)(4)

(b)(4)

Task 1.15 FDA IDE (b)(4)

Task 1.15.1 FDA IDE (b)(4)

Task 1.15.2 FDA IDE

Thermaquil has submitted (b)(4) which focuses on the (b)(4) (b)(4) for the (b)(4) and held a meeting with (b)(4) on (b)(4)

Task 1.16 FDA IDE Submission (b)(4)

Task 1.16.1 Prepare and submit FDA IDE application for (b)(4)

(b)(4)

Thermaquil is preparing the IDE submission (b)(4). In response to FDA's feedback, we are (b)(4)

(b)(4)

(b)(4)

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Weekly meetings with the team members from University of Pittsburgh, (b)(4) and Thermaquil were held regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermaquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai
"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"
Experimental Neurology, 2021; 346:113860.

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.
"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".

American Journal of Physiology - Gastrointestinal and Liver Physiology, accepted for publication.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)		(b)(4); (b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
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Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Clinical Consultant	(b)(4); (b)(6)	(b)(4); (b)(6)	
Regulatory Consultant	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system	(b)(4)						(b)(4)
1.2.3 Restore sexual function	(b)(4)					Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment	(b)(4)						
1.3.4 Integration of (b)(4) in enclosure with firmware and (b)(4) connectivity	(b)(4)					Milestone 1.3.4 - Deliverable 1.3	
1.4 Implantable pulse generator with	(b)(4)						
1.4.3 Integrate pulse generator circuit into can (b)(4)	(b)(4)					Milestone 1.4.3 – Deliverable 1.4	
1.4.4 Final (b)(4) test application with wireless board connectivity	(b)(4)					Milestone 1.4.4 – Deliverable 1.4	
1.4.5 Full integration and test completed for initial prototype	(b)(4)					Milestone 1.4.5 – Deliverable 1.4	
1.6 External pulse generator for	(b)(4)						
1.6.2 Integration of the (b)(4) components with firmware and the enclosure	(b)(4)					Milestone 1.6.2 – Deliverable 1.6.2	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.7 (b)(4) electrodes for human use (b)(4)	(b)(4)	(b)(4)					(b)(4)
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.3 – Deliverable 1.7	
1.8 Physician/Investigator controller for (b)(4)	(b)(4)	(b)(4)					
1.8.2 Evaluation board fabricated/assembled and initial evaluation complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1 – Deliverable 1.8	
1.8.3 Integration with a simple enclosure with display and firmware (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1 – Deliverable 1.8	
1.9 Patient controller for (b)(4)	(b)(4)	(b)(4)					
1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)	(b)(4)	(b)(4)				Milestone 1.9.1 – Deliverable 1.9	
1.9.2 Verification testing completed (b)(4)	(b)(4)	(b)(4)				Milestone 1.9.2 – Deliverable 1.9	
1.15 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.15.2 FDA IDE meeting (b)(4)	(b)(4)	(b)(4)				Milestone 15.2.1 – Deliverable 15.1.2	
1.13 Early feasibility human clinical protocol (b)(4)	(b)(4)	(b)(4)					
1.13.1 Early feasibility human clinical protocol for (b)(4)	(b)(4)	(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)	(b)(4)	(b)(4)					
1.11.1 GLP animal study	(b)(4)	(b)(4)				Milestone 1.11 – Deliverable 1.11	
1.16 FDA IDE Submission (b)(4)	(b)(4)	(b)(4)					
1.16.1 FDA IDE application for the (b)(4) is submitted	(b)(4)	(b)(4)				Milestone 1.16 – Deliverable 1.16	

University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: November 1, 2021 through January 31, 2022 (b)(4)

Date of Report: (b)(4)

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: (b)(4)

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(4), (b)(6)

Submitted by:

(b)(4), (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) quarter (b)(4), we have made major progresses in the following areas:

1. Scientific progress:

- We have successfully demonstrated in 8 anesthetized male cats that stimulation of sacral S1/S2 spinal root at (b)(4) electrodes is most effective to induce penile erection, while stimulation of S3 root or pudendal nerve is not effective. This success indicates a minimally invasive surgical approach by inserting lead electrodes close to the S1/S2 spinal root can be developed to restore penile erection after spinal cord injury. We have finished processing and analyzing the data/results from this animal study and the manuscript has been submitted to Neuromodulation for publication.
- We have also successfully demonstrated in 3 anesthetized female cats that pudendal nerve stimulation at (b)(4) effective to induce vaginal lubrication and contractions. This success indicates that our pudendal stimulation/block device can also be used to restore female sexual function after SCI in addition to restoring bladder function. We are now processing and analyzing the data/results from this animal study and preparing a manuscript for publication.

- Our second paper titled "Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats" has been published on American Journal of Physiology - Gastrointestinal and Liver Physiology. **This is a Big Win for this DARPA BG+ project.**
- Our third paper titled "Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats" has been submitted to Neuromodulation for publication (see attachment).

2. Engineering progress:

- The GUI successfully controlled the (b)(4) stimulation output to each channel during a UPitt cat experiment wirelessly (b)(4)
- (b)(4)
- (b)(4)
- (b)(4)
- The complete (b)(4) manufacturing process continues to be improved upon, inspected, and tested.
- (b)(4) is better defined.
- (b)(4) and delivered to (b)(4)
- (b)(4) is being investigated to replace some (b)(4) processes.
 - (b)(4)
- (b)(4) was delivered to (b)(4) for lead construction.
- (b)(4)
- The local (b)(4) supplier delivered (b)(4) samples
 - (b)(4)
 - (b)(4)
- (b)(4) and Thermaquil team members compiled the documentation supporting the IDE submission.
- Firmware features to (b)(4) has been fully defined and created for (b)(4)
 - The next steps will be to (b)(4) onto a patient controller.
- The (b)(4) were tested for (b)(4) connections.

- Boxes and foam were purchased to create the cushioned packaging (b)(4) (b)(4)
- Protocols for the electrical and mechanical testing (b)(4) (b)(4) were written.
- (b)(4) released (b)(4) but all files were successfully completed and released.
- (b)(4) programs were developed for labeling the (b)(4) and (b)(4)
- (b)(4) was tested electrically and mechanically per the defined protocols and work instructions.
 - The electrical and mechanical testing of the (b)(4) was completed.
- The (b)(4) was moved to (b)(4) in order to (b)(4) (b)(4) (b)(4)
- Labels were created for the (b)(4)
- The IFU was written and completed by (b)(4) for Thermoquil's submission.
- The (b)(4) is ready for FDA IDE submission. All tests and documentation required for the IDE study was completed and submitted. **This is a Big Win for this DARPA BG+ project.**

3. Regulatory progress:

- Submitted and met with the FDA (b)(4)
 - Received specific feedback on (b)(4) (b)(4)
 - Received feedback on our proposed (b)(4) (b)(4)
 - Given FDA's feedback, we (b)(4) (b)(4)
- Revised our draft early feasibility human clinical protocol for the (b)(4)
- Fully drafted IDE submission
- Continued to refine user needs and voice of customer to continuously confirm device requirements (b)(4)

4. Administrative progress:

- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
- Weekly meetings were hold between University of Pittsburgh, (b)(4) and Thermoquil to coordinate the project activity.
- Monthly meeting was hold with DARPA to report our progress.

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.3 Restore sexual function (b)(4)

During this quarter and previous quarter, a total of 8 male cats were used to study the penile erection by stimulation of sacral roots or pudendal nerve. The animals were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (see Fig.1). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

A 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (see Fig.1). Since electrically evoked penile erection is very well correlated with increased corpus cavernosum pressure, pressure recording from the corpus cavernosum was used as a quantitative measurement of penile erection in this study.

Based on the results obtained from the 8 male cats, penile erection could be induced by stimulation of S1/S2 root but stimulation of S3 root or pudendal root was not effective. Penile erection was observed as a full protrusion of the penis with rigidity in 6 cats by stimulation (b)(4) (b)(4) of S1 ventral root (S2 and S3 ineffective) and in 2 cats by stimulation of S2 ventral root (S1 and S3 ineffective). These responses which were quantified by penile pressure recordings showed that S1 or S2 ventral root stimulation in 6 cats and 2 cats,

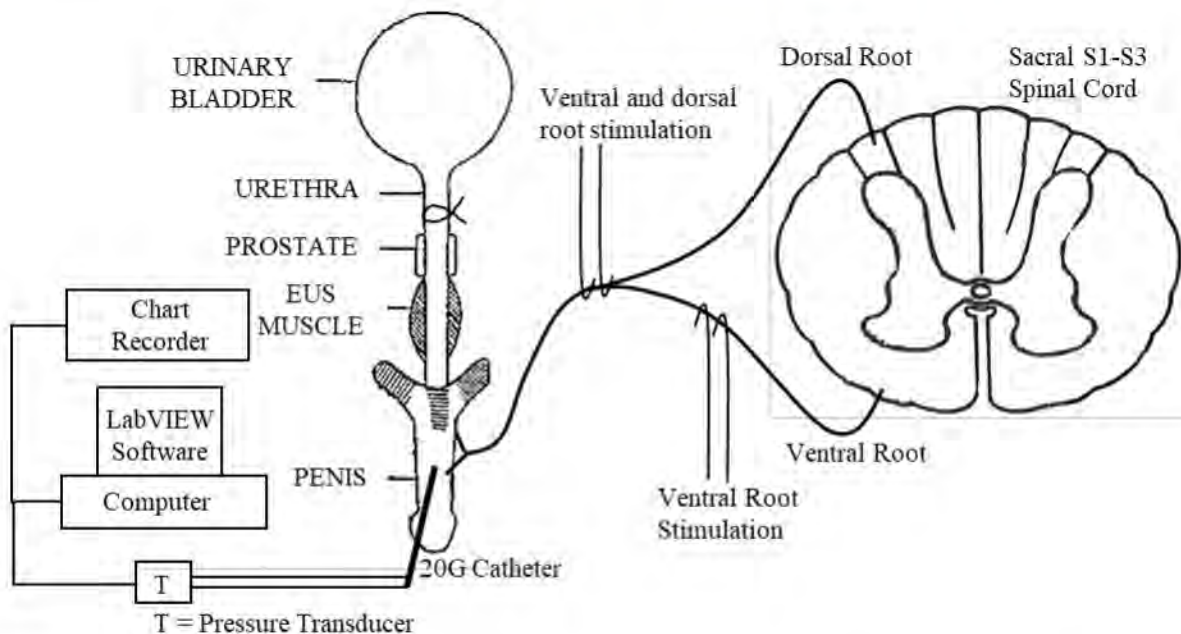


Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

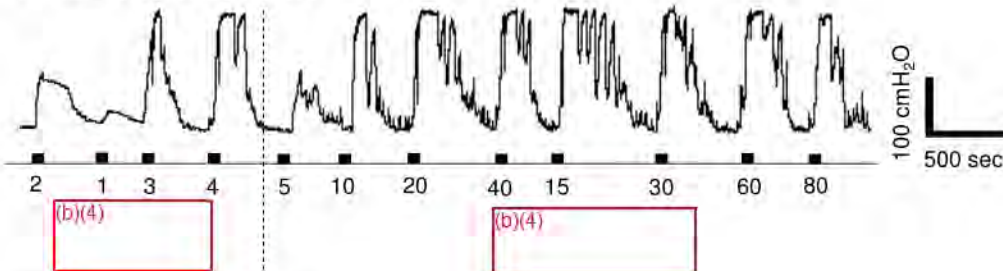
respectively, induced large increases in pressure (about 150 cmH₂O on average, Fig.2 A-C). The optimal stimulation frequency to induce penile erection by S1 or S2 ventral root stimulation was (b)(4) or higher frequencies (b)(4) could also induce large increases in penile pressure in some cats. The stimulation intensity (b)(4) S1 or S2 ventral root stimulation to induce the largest increase in penile pressure was always above the motor threshold to induce leg muscle contraction (S1) or anal sphincter contraction (S2).

Continuous 10-minute stimulation (b)(4) of the S1 or S2 spinal root (i.e., ventral and dorsal roots together) induced a large increase (>150 cmH₂O) in penile pressure (Fig.3). The maximal penile pressure was either sustained (Fig.3A) or gradually developed (Fig.3B) during the 10-minute stimulation. On average (N=8 cats), the penile pressure at the end of 10-minute stimulation was not significantly ($p>0.05$) different from the maximal pressure developed during the stimulation (Fig.3C). The maximal penile pressure induced by stimulation of ventral and dorsal root together was also not significantly ($p>0.05$) different from the maximal pressure induced by stimulation of ventral root alone (Fig.2D and Fig.3C).

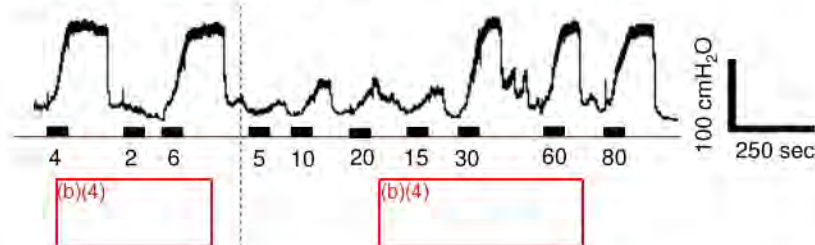
After a complete spinal cord transection at the T9-T10 level, continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (ventral and dorsal roots together) also induced a large increase (>100 cmH₂O) in penile pressure (Fig.4). As seen prior to spinal transection, the maximal penile pressure was also sustained or gradually developed (Fig.4B) during the 10-minute stimulation. However, on average (N=6 cats) the induced penile pressure was

significantly ($p < 0.05$) reduced to 113 ± 19 cmH₂O after spinal cord transection when compared to the penile response (186 ± 9 cmH₂O) before the spinal cord transection (Fig.4C). The blood pressure was also significantly ($p < 0.05$) reduced from 229 ± 11 cmH₂O to 186 ± 7 cmH₂O by spinal cord transection, but it was not changed by sacral root stimulation.

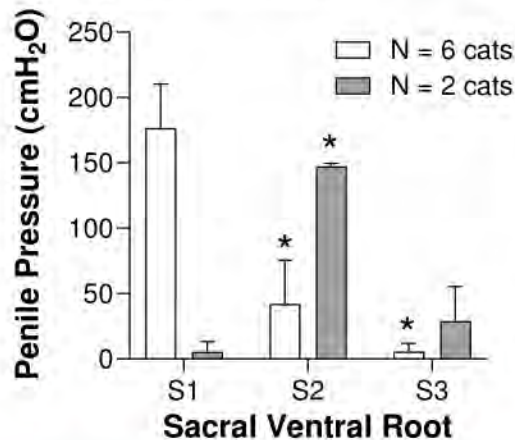
A. S1 Ventral Root



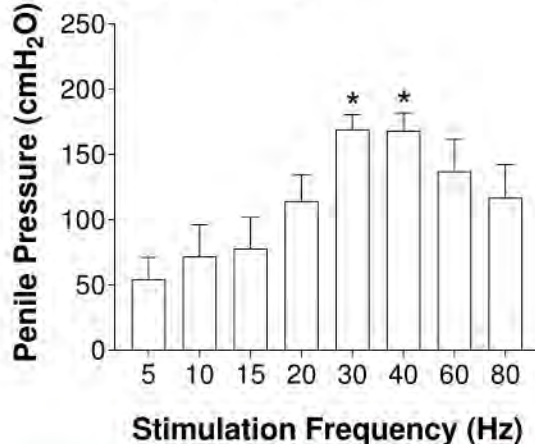
B. S2 Ventral Root



C. Segmental Effect



D. Frequency Effect

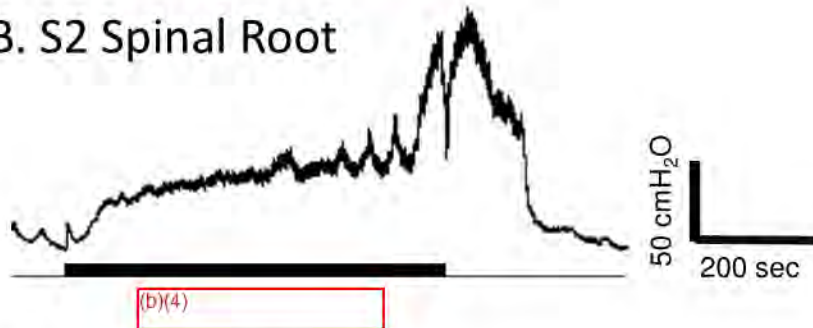


(b)(4)

A. S1 Spinal Root



B. S2 Spinal Root



C. Sustained Pressure

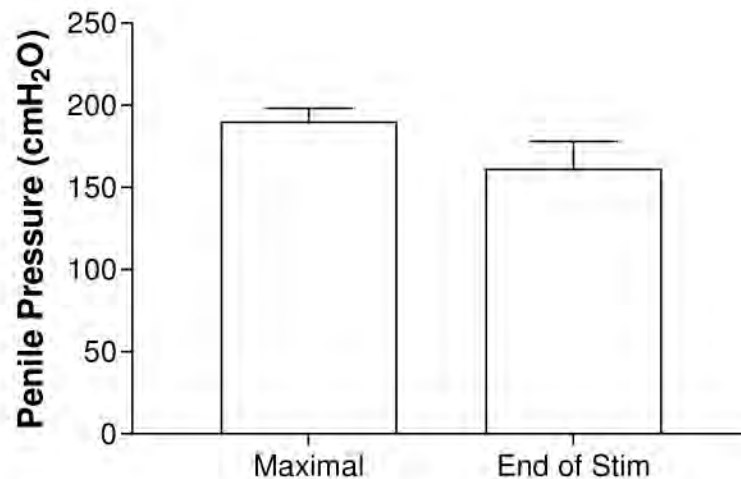
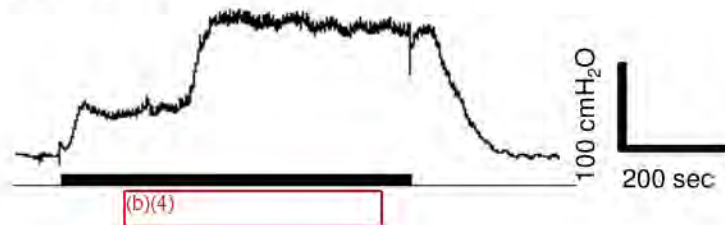


Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

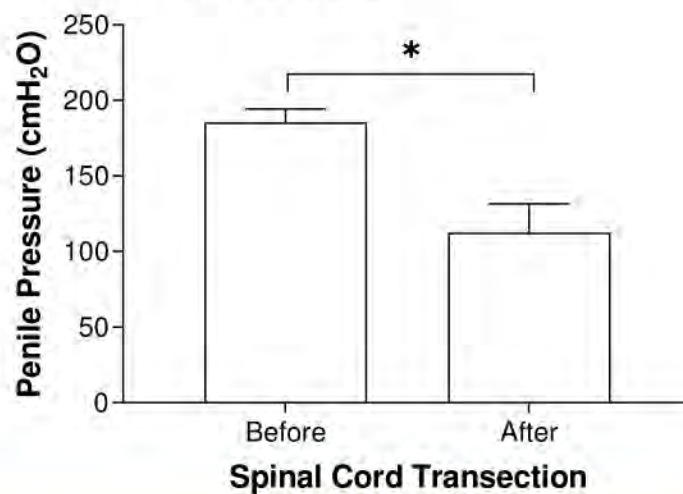
A. Before Spinal Transection



B. After Spinal Transection



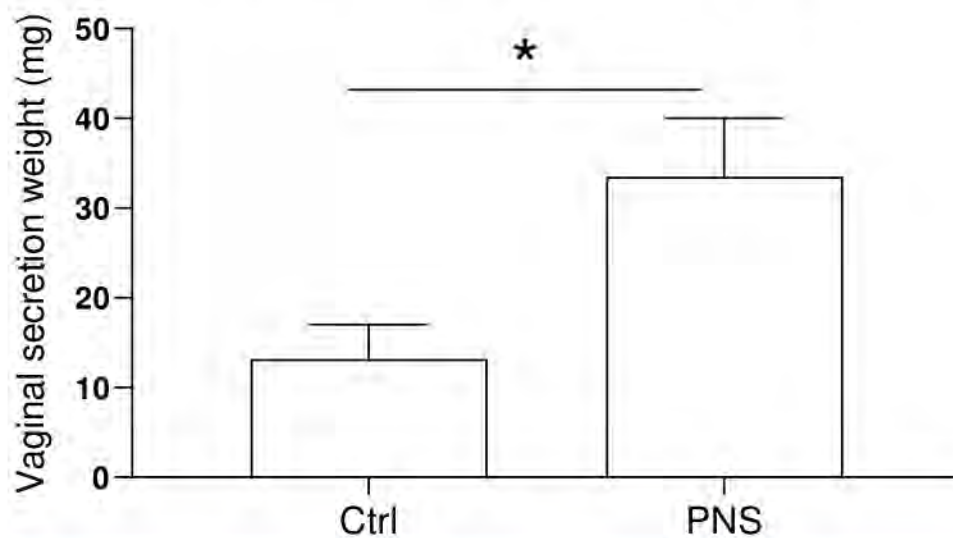
C. Maximal Pressure



(b)(4)

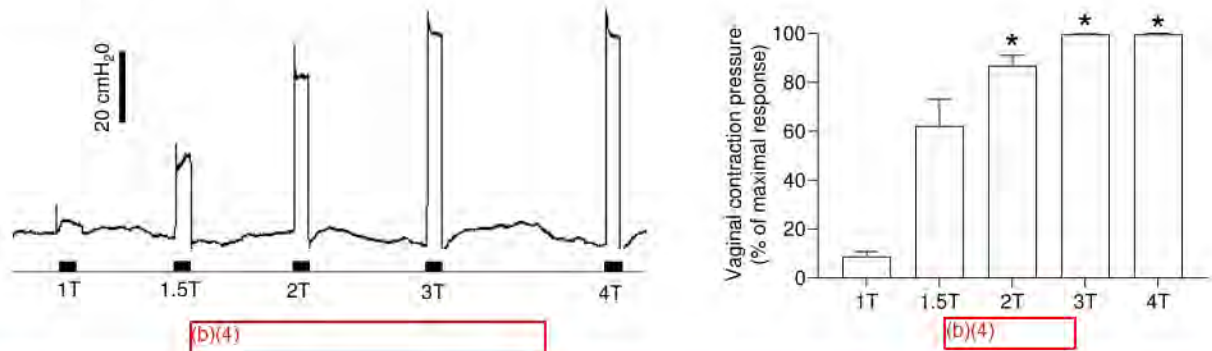
In addition to restore male sexual function, we also successfully restored female sexual functions in 3 anesthetized female cats by pudendal nerve stimulation to increase the vaginal lubrication and produce vaginal contractions. The surgical procedure is similar to the male cats as described above, but the experimental protocol is different. At the beginning of the experiment, a small piece of dry cotton was weighted and inserted into the vagina for 10 minutes without any stimulation. After the 10 minutes, the cotton was pulled out and weighted again to determine the increase in the cotton's weight that is used as the control value to indicate the vaginal wetness. Then, the same 10-minute cotton test was repeated using another piece of dry cotton while PNS (b)(4) at the 2 times intensity threshold for inducing anal twitching was also applied intermittently (b)(4) during the 10-minute period to increase the vaginal wetness.

After testing the vaginal wetness, a small catheter (7 French) with a balloon at the tip was inserted into the vagina with the balloon slightly distended by 0.2 ml air to measure vaginal contraction. (b)(4) was applied for 5 secs each time at different intensities to determine the intensity response. Then, PNS at an intensity of 2 times the threshold (T) for inducing a small observable vaginal contraction was applied at different frequencies (b)(4) to determine the frequency response. Finally, (b)(4) was applied for 4 minutes either continuously or intermittently (5 secs on and 5 secs off) in a random order to determine vaginal fatigue during the 4-minute-long contraction. A time interval of 5-10 minutes was inserted between the two 4-minute PNS to avoid any carry-over fatigue effect.



(b)(4)

PNS applied intermittently (5 secs on and 5 secs off) for 10 minutes significantly increased vaginal wetness (Fig.5). Without PNS, the vaginal wetness as indicated by the increase in the vaginal cotton weight during a 10-minute period is 13.2 ± 3.9 mg (control in Fig.5). After 10-minute PNS, the vaginal wetness was significantly ($p=0.02647$) increased to 33.5 ± 6.5 mg (PNS in Fig.5).



(b)(4)

* one-way ANOVA compared to 1T. N =3 cats.

Vaginal contraction was significantly ($p<0.05$) increased as the PNS intensity increased (Fig.6). (b)(4) at an intensity $\geq 2T$ induced vaginal contraction stronger than 90% of the maximal contraction (Fig.6). Meanwhile, at intensity of 2T the PNS induced significantly ($p<0.05$) large vaginal contraction at frequencies (b)(4) (Fig.7). At frequencies (b)(4) induced vaginal contraction larger than 80% of the maximal contraction (Fig.7). When PNS (b)(4) was applied for 4 minutes, it produced less fatigue when applied intermittently than continuously (Fig.8). On average ($N=3$), intermittent PNS induced fatigue that is significantly ($p=0.0142$) smaller than continuous PNS Fig.8).

In summary, our studies in anesthetized male and female cats have shown successfully that sexual functions could be restore after SCI by stimulation of the sacral roots or pudendal nerve.

(b)(4)

(b)(4)

Task 1.4 Implantable pulse generator with custom (b)(4)

(b)(4)

1.4.3 Integrate pulse generator circuit into (b)(4)

(b)(4) circuit miniaturization to (b)(4) size is complete (Fig.9-15). (b)(4)

(b)(4)

(b)(4) We were finally able to purchase a large amount of the (b)(4)

(b)(4) that replace (b)(4) This purchase was one of three

(b)(4)

(b)(4)

required for the (b)(4)

requires a (b)(4)

(b)(4) makes use of (b)(4)

(b)(4)

(b)(4) This decision increased the size of our (b)(4), which increased the (b)(4) charging throughput. It also enabled (b)(4)

(b)(4)

Finding a reliable supplier to make the (b)(4)

(b)(4)

1.4.4 Final (b)(4) test application with wireless (b)(4)

The (b)(4) application designed to control the (b)(4) wirelessly for the (b)(4). The graphical user interface (GUI) controls the various stimulation parameters to the (b)(4).

(b)(4)

(b)(4)

The GUI currently utilizes (b)(4).

(b)(4)

The final GUI requires (b)(4).

(b)(4)

but both applications can control the (b)(4) with wireless board connectivity.

1.4.5 Full integration and test completed for (b)(4)

The complete integration of the (b)(4) for animal implantation is currently underway. As mentioned before, there are (b)(4).

(b)(4)

All parts except the (b)(4) are ordered. The (b)(4) will get ordered (b)(4).

(b)(4)

now that the (b)(4).

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)



Task 1.6 (b)(4) for human study (b)(4)

Sub-Task 1.6.3 System verification completed (b)(4)

(b)(4)

Sub-Task 1.6.4 Documentation supporting the IDE submission (b)(4)

This task is complete. All documentation supporting the IDE was submitted. Please see the submitted IDE documents for the details.

The manufacturing process of (b)(4)

(b)(4) performed by (b)(4) and its supplier, (b)(4). The process starts with a signed contract between Thermaquil and (b)(4). (b)(4) is Thermaquil's main subcontractor responsible for the assembly process and supplier management. (b)(4) is certified to ISO13485:2016. (b)(4) orders components through (b)(4) supplier management procedures. Components are properly identified and documented during assembly in the device history files. (b)(4) assembles the (b)(4).

(b)(4). The assembly and tests are performed to (b)(4) work instructions and documented in the device history record forms.

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4) The final (b)(4) ready for FDA IDE submission and human clinical study are shown in (b)(4) including the package, the (b)(4) different components, and materials used to build the (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.7 (b)(4) electrodes for human use (b)(4)

Sub-Task 1.7.3 First prototypes of (b)(4) delivered for test (b)(4)

We have made and tested hundreds of (b)(4) The very specialized process of (b)(4)

(b)(4)

Sub-Task 1.7.4 Finalize design and (b)(4) production (b)(4)

(b)(4)

The tine design was reviewed by the internal team and by the molders. The molding feedback was incorporated into the design changes. We have three quotations ranging from (b)(4) (b)(4) The biocompatibility testing timeline is driving the tine developing decision.

Sub-Task 1.7.5 Biocompatibility test is completed. Documentation for FDA submission (b)(4)

Biocompatibility quotes from three test houses for the (b)(4) were received. The biocompatibility discussions with the test houses are ongoing while we prepare the materials and assemblies to be tested. The biological evaluation write up will be purchased during the (b)(4) The testing will begin as soon as all the end use components are delivered and assembled with final processes.

(b)(4)

Task 1.8 Physician / Investigator controller for human (b)(4)

Sub-Task 1.8.2 Evaluation board fabricated / assembled and initial evaluation complete

(b)(4)

The (b)(4) GUI for the (b)(4) is complete (Figs.27-28). The application runs on a (b)(4). At this time, there is no need for a (b)(4).

(b)(4) The (b)(4) IDE study can be completed (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.8.3 Integration with a simple enclosure with display and firmware (b)(4)

The firmware (b)(4) was created for the researcher, clinician and patient (Fig.29). The firmware is laptop based so there was no need to integrate a simple enclosure with display.

- The (b)(4) will consist of the (b)(4)

(b)(4)

Sub-Task 1.8.4 Final documentation to support the FDA IDE as an accessory device (MAC15)

All documentation required for the IDE was submitted. A (b)(4) is being used as the physician controller. The cybersecurity, system safety testing, and system bench test documents are all completed.

Task 1.9 Patient controller (b)(4)

Sub-Task 1.9.2 Verification testing completed (b)(4)

Firmware features to (b)(4) has been fully defined and created for (b)(4) use. The next steps will be to load the GUI and firmware onto (b)(4) The (b)(4) firmware has been verified. (b)(4)

(b)(4)

Task 1.10 Software development kit (SDK) (b)(4)

Sub-Task 1.10.2 At the completion of the development, the SDK is complete with the documentation on how to access the devices via the software API (b)(4)

(b)(4) A detailed instruction for use (IFU) was written to document how to use the software application prepared for the IDE study with the (b)(4)

Task 1.11 GLP animal study for (b)(4)

The purpose of the GLP safety study is to provide support for the safe initiation of an Early Feasibility IDE Study of (b)(4) in humans. This was originally intended to support (b)(4) in which case a (b)(4) GLP animal study protocol would be required, and this was submitted to FDA (b)(4)

(b)(4)

(b)(4), (b)(6)

(b)(4), (b)(6)

Task 1.13 Early feasibility human clinical protocol for (b)(4)

Thermaquil drafted an early feasibility clinical study for the (b)(4) with input from clinicians during (b)(4). This clinical protocol is needed for the FDA IDE submission and was submitted for FDA feedback in (b)(4). We received specific feedback to (b)(4). We have updated our protocol (Fig.31) by changing

(b)(4)

(b)(4)

Task 1.16 FDA IDE Submission (b)(4)

Task 1.16.1 Prepare and submit FDA IDE application for the (b)(4)

(b)(4)

Thermaquil has fully drafted our IDE submission (Fig.33). We fully drafted the monitoring procedures to comply with good clinical practice guidelines (Fig.34). In response to FDA's feedback, we have updated the informed consent (Fig.35).

(b)(4)

(b)(4)

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Weekly meetings with the team members from University of Pittsburgh, (b)(4) and Thermoquil were held regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermoquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermoquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"

Experimental Neurology, 2021; 346:113860.

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".

American Journal of Physiology - Gastrointestinal and Liver Physiology, 2021; 321: G735-G742

Zhijun Shen, Jicheng Wang, Bing Shen, Jianan Jian, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

"Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats"

Neuromodulation, 2022. Submitted for review and publication.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4), (b)(6)		

			Bioengineering, and Pharmacology
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Urologist	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
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7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
(b)(4) 1.2.3 Restore sexual function (b)(4)	(b)(4)					Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment (b)(4)							
Integration of (b)(4) in enclosure	(b)(4)					Milestone 1.3.4 - Deliverable 1.3	
1.3.4 with firmware and application connectivity (b)(4)	(b)(4)						
1.4 Implantable pulse generator with (b)(4)							
1.4.3 Integrate pulse generator circuit into can (b)(4)	(b)(4)					Milestone 1.4.3 – Deliverable 1.4	
Final test application with							
1.4.4 wireless board connectivity (b)(4)	(b)(4)					Milestone 1.4.4 – Deliverable 1.4	
Full integration and test							
1.4.5 completed for initial prototype (b)(4)	(b)(4)					Milestone 1.4.5 – Deliverable 1.4	
1.6 External pulse generator for (b)(4)							
1.6.3 System verification completed (b)(4)	(b)(4)					Milestone 1.6.3 – Deliverable 1.6.1 – Deliverable 1.6.2	
1.6.4 Documentation Supporting the IDE submission (b)(4)	(b)(4)					Milestone 1.6.4 – Deliverable 1.6.1	
Build sets of (b)(4) to	(b)(4)						
1.6.5 conduct GLP safety study in 4 pigs in Phase 1. (b)(4)	(b)(4)					Milestone 1.6.5 – Deliverable 1.6.2	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.7 (b)(4) electrodes for human use (b)(4)							(b)(4)
1.7.3 First prototypes of leads delivered for test (b)(4)						Milestone 1.7.3 – Deliverable 1.7	
1.7.4 Finalize design and production (b)(4)						Milestone 1.7.4 – Deliverable 1.7	
1.7.5 Biocompatibility test is completed. Documentations for FDA submission (b)(4)						Milestone 1.7.5 – Deliverable 1.7	
1.8 Physician/Investigator controller for (b)(4)							
1.8.2 Evaluation board fabricated/assembled and initial evaluation complete (b)(4)						Milestone 1.8.1 – Deliverable 1.8	
1.8.3 Integration with a simple enclosure with display and firmware (b)(4)						Milestone 1.8.1 – Deliverable 1.8	
1.8.4 Final documentation to support the FDA IDE as an accessory device (b)(4)						Milestone 1.8.4 – Deliverable 1.8	
1.9 Patient controller for human EPG (b)(4)							
1.9.2 Verification testing completed (b)(4)						Milestone 1.9.2 – Deliverable 1.9	
1.10 Software Development Kit (SDK) (b)(4)							
1.10.2 At the completion of the development, the SDK is complete with the 2 documentation on how to access the devices via the software API (b)(4)						Milestone 1.10.2 – Deliverable 1.10	

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 0881



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)						
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)					Milestone 1.13 – Deliverable 1.13	(b)(4)
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	
1.16 FDA IDE Submission (b)(4)							
1.16 FDA IDE application for the (b)(4) is submitted	(b)(4)					Milestone 1.16 – Deliverable 1.16	

Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats

Zhijun Shen ¹, Jicheng Wang ¹, Bing Shen ¹, Jianan Jian ¹, Khari Goosby ¹, William Wang ¹, Jonathan Beckel ², William C. de Groat ², Christopher Chermansky ¹, Changfeng Tai ^{1,2,3}

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

³ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Running Head: Penile Erection by Sacral Root Stimulation

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Conflict of Interest: Dr. Tai is the inventor of a patent related to this study. The other authors have no conflicts of interest.

Correspondence to:

Changfeng Tai, Ph.D.
Department of Urology
University of Pittsburgh
700 Kaufmann Building
Pittsburgh, PA 15213
Phone: 412-692-4142
Fax: 412-692-4380
Email: cftai@pitt.edu

ABSTRACT

Objective: This study aimed at determining if stimulation of sacral spinal roots can induce penile erection in cats.

Materials and Methods: In anesthetized cats, a 20-gauge catheter was inserted into the corpus cavernosum to measure the penile pressure. Stimulus pulses (5-80 Hz, 0.2 ms) were applied via bipolar hook electrodes to sacral ventral roots alone or to combined ventral and dorsal roots of a single S1-S3 segment to induce penile pressure increases and penile erection.

Results: Stimulation of the S1 or S2 ventral root at 30-40 Hz induced observable penile erection with rigidity and the largest increase (>150 cmH₂O) in penile pressure. Continuous stimulation (10 minutes) of afferent and efferent axons by simultaneous stimulation of the S1 or S2 dorsal and ventral roots at 30 Hz also produced a large (about 150 cmH₂O on average) increase in penile pressure that was sustainable during the entire stimulation period. After a complete spinal cord transection at the T9-T10 level, simultaneous stimulation of the S1 or S2 dorsal and ventral roots induced large (>100 cmH₂O) and sustainable increases in penile pressure.

Conclusion: This study indicates the possibility to develop a novel neuromodulation device to restore penile erection after spinal cord injury using a minimally invasive surgical approach to insert a lead electrode via the sacral foramen to stimulate a sacral spinal root.

Keywords: stimulation, sacral spinal root, erection, spinal cord injury, cat

INTRODUCTION

After spinal cord injury (SCI) erectile dysfunction presents as a short duration or an absence of penile erection, or poor penile rigidity that is insufficient for sexual intercourse (1,2). Although drug treatment is effective in restoring erectile function after SCI, it is not suitable for every SCI person due to the drug side effects and the health conditions of the SCI person (2). In addition to drug treatment, a fully implantable stimulator has also been used successfully in humans to restore penile erection after SCI by stimulating sacral anterior roots (3-5). However, sacral anterior root stimulation requires a major invasive spinal surgery to expose the sacral spinal roots for implantation of stimulation electrodes. Therefore, a minimally invasive neuromodulation approach that does not require invasive spinal surgery will be desirable for treatment of erectile dysfunction after SCI.

A minimally invasive surgical approach for stimulation of sacral spinal roots has been available for many years to treat patients with an overactive bladder (6). It employs a foramen needle to insert a tined lead electrode percutaneously under the guide of fluoroscopic imaging in proximity to the S3 spinal root that consists of both anterior (motor) and posterior (sensory) spinal roots. This minimally invasive sacral neuromodulation approach has also been evaluated in recent years to treat erectile dysfunction with a significant improvement in quality of sexual life for 20-40% subjects (7,8). However, the sacral neuromodulation stimulation parameters used in these studies to treat erectile dysfunction may not be optimal for improving penile erection because they were the same as those used to treat overactive bladder. Therefore, optimization of sacral neuromodulation parameters for treatment of erectile dysfunction is needed so that this minimally invasive surgical approach could be used as an effective therapy for SCI or non-SCI people with erectile dysfunction.

In this study using anesthetized cats, we investigated the penile response to electrical stimulation of an individual S1-S3 ventral root to determine the optimal stimulation frequency and the most effective spinal root in inducing penile erection. Then, combined stimulation of ventral and dorsal roots (termed spinal root stimulation) of the most effective spinal segment (S1 or S2) was used to induce sustainable (>10 minutes) penile erection. The information obtained in this preclinical study is important to design a human clinical trial to further evaluate sacral spinal root stimulation using the minimally invasive surgical approach as used in current clinical application (6) to treat erectile dysfunction in both SCI and non-SCI people.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical procedures

A total of 8 male cats (4.6 ± 0.2 kg) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder

via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (see Fig.1). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Experimental protocol

At the beginning of the experiment, stimulation (30 Hz frequency, 0.2 ms pulse width, and 1-2 min duration) was delivered via the hook electrode to sacral S1 ventral root at a minimal intensity (0.5 V). If no erection was observed, the stimulation intensity was increased and then tested again until a penile erection was observed. If S1 ventral root stimulation was not effective, then the stimulation electrode was moved to the S2 ventral root, and the test was repeated. A penile erection, which was evident as a full protrusion of the penis with rigidity resistant to bending, was always observed by stimulation of the individual S1 and S2 ventral roots. After the penile erection was observed, a 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (see

Fig.1). Since electrically evoked penile protrusion is very well correlated with increased corpus cavernosum pressure (9,10), the pressure recording was used as a quantitative measurement of penile erection in this study.

After placement of the penile catheter (N=8 cats), stimulation (30 or 40 Hz frequency, 0.2 ms pulse width, and 1 min duration) was delivered via the hook electrode to individual left or right sacral ventral roots (S1, S2, or S3) at a range of intensities (0.5-15 V) to determine the intensity threshold (T) for inducing an observable increase in penile pressure. Then, an intensity (1.5-3T) that induced the maximal penile pressure (>100 cmH₂O) was used to test penile responses to different stimulation frequencies (5-80 Hz). If no penile pressure >100 cmH₂O was induced by stimulation of an individual ventral root, the maximal stimulation intensity that did not produce movement of the animal's lower body was used to test the frequency response. It was our intention not to fix the hip and lower spine, so that sacral root stimulation by the hook electrode could produce local movement of the tail or hindlimb. However, the maximal stimulation intensity was limited by any movement of the lower spine, which would displace the hook electrode from the nerve. After testing each ventral root, the ventral root (S1 or S2) that elicited the largest penile pressure was combined with the dorsal root and the combination was stimulated continuously for 10 mins with the most effective frequency (30 Hz) and intensity to determine if the induced penile erection was sustainable during the entire stimulation period. Finally, the spinal cord was completely transected at the T9-T10 level (N=6 cats). Then, the continuous 10-minute stimulation of the same spinal root (ventral and dorsal roots together) was tested again to induce penile erection.

Data analysis

To compare the penile erection responses induced by stimulation of different ventral roots at different frequencies, the maximal amplitudes of the penile pressure induced by stimulation were measured and averaged across different animals for the same stimulation conditions. To determine the sustainability of the induced penile erection, the maximal penile pressure induced by the continuous 10-minute stimulations was compared to the pressure at the end of the 10-minute stimulation. To determine the effect of the spinal cord transection, the maximal penile pressures induced by the continuous 10-minute stimulation were compared before and after the spinal cord transection. The data from different animals are averaged and presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined by paired t-test or repeated-measures one-way ANOVA followed by Dunnett multiple comparison.

RESULTS

Penile erection dependent on stimulation frequency and the spinal segment stimulated.

Penile erection was observed as a full protrusion of the penis with rigidity in 6 cats by stimulation (30 Hz, 0.2 ms, 4-8 V) of S1 ventral root (S2 and S3 ineffective) and in 2 cats by stimulation of S2 ventral root (S1 and S3 ineffective). These responses which were quantified by penile pressure recordings showed that S1 or S2 ventral root stimulation in 6 cats and 2 cats, respectively, induced large increases in pressure (about 150 cmH₂O on average, Fig.2 A-C). The optimal stimulation frequency to induce penile erection by S1 or S2 ventral root stimulation was 30-40 Hz on average (Fig.2D), although lower frequencies (10-20 Hz, see Fig.2A) or higher frequencies (60-80 Hz, see Fig.2 A-B) could also induce large increases in penile pressure in

some cats. The stimulation intensity (0.6-12 V, mean 3.5 ± 1.4 V) for 30-40 Hz S1 or S2 ventral root stimulation to induce the largest increase in penile pressure was always above the motor threshold to induce leg muscle contraction (S1) or anal sphincter contraction (S2).

Sustainable penile erection induced by S1/S2 spinal root stimulation

Continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (i.e., ventral and dorsal roots together) induced a large increase (>150 cmH₂O) in penile pressure (Fig.3). The maximal penile pressure was either sustained (Fig.3A) or gradually developed (Fig.3B) during the 10-minute stimulation. On average (N=8 cats), the penile pressure at the end of 10-minute stimulation was not significantly ($p>0.05$) different from the maximal pressure developed during the stimulation (Fig.3C). The maximal penile pressure induced by stimulation of ventral and dorsal root together was also not significantly ($p>0.05$) different from the maximal pressure induced by stimulation of ventral root alone (Fig.2D and Fig.3C).

After a complete spinal cord transection at the T9-T10 level, continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (ventral and dorsal roots together) also induced a large increase (>100 cmH₂O) in penile pressure (Fig.4). As seen prior to spinal transection, the maximal penile pressure was also sustained or gradually developed (Fig.4B) during the 10-minute stimulation. However, on average (N=6 cats) the induced penile pressure was significantly ($p<0.05$) reduced to 113 ± 19 cmH₂O after spinal cord transection when compared to the penile response (186 ± 9 cmH₂O) before the spinal cord transection (Fig.4C). The blood pressure was also significantly ($p<0.05$) reduced from 229 ± 11 cmH₂O to 186 ± 7 cmH₂O by spinal cord transection, but it was not changed by sacral root stimulation.

DISCUSSION

This study in anesthetized cats shows that the penile erection induced by sacral root stimulation is dependent on stimulation frequency and the spinal segment stimulated. S1/S2 ventral root stimulation at 30-40 Hz is optimal to induce the largest increase (>150 cmH₂O) in penile pressure (Fig.2). Stimulation of the S1/S2 spinal root (ventral and dorsal roots together) is also effective in inducing a large penile pressure increase that is sustainable for the entire 10-minute stimulation (Fig.3). More importantly, large sustainable penile pressure increase (>100 cmH₂O) can also be induced by S1/S2 spinal root stimulation after an acute complete transection of the spinal cord (Fig.4). These results are important for the design of a new neuromodulation device to treat erectile dysfunction.

Previous studies have shown that penile erection can be induced by electrical stimulation of pelvic nerve, cavernous nerve, or dorsal penile nerve in mice, rats, cats, dogs, monkeys, or humans (11-15). However, the pelvic and cavernous nerves require invasive surgery for implantation of stimulation electrodes while the dorsal penile nerve is not a convenient location to attach a stimulation electrode during sexual intercourse. Therefore, these stimulation methods are not adopted for clinical application to treat erectile dysfunction. Sacral ventral root stimulation was also shown to be effective in inducing penile erection in this study and in previous studies using cats (16) and dogs (17). In addition, sacral anterior root stimulation was used successfully to restore bladder function and erectile function for SCI people (3-5,18). However, invasive spinal surgery is required to implant the stimulation electrodes on the sacral anterior roots, preventing this effective treatment to be used in SCI or non-SCI people for the sole purpose of treating erectile dysfunction. Our study in cats indicates that penile erection can be induced by stimulation of the sacral spinal roots. A previous study in dogs also showed a

similar effect (17). The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to employ a minimally invasive surgical approach for stimulation. Sacral spinal roots in humans are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder (6). Therefore, our study has significant implications for developing a new sacral neuromodulation therapy using a minimally invasive surgical approach to restore erectile function for both SCI and non-SCI people.

Previous studies using sacral neuromodulation to treat overactive bladder also evaluated the effects on penile erection in both SCI and non-SCI people (7,8). Questionnaires indicate that subjects with moderate-mild erectile dysfunction significantly improved their quality of sexual life by sacral neuromodulation therapy. However, these studies used stimulation parameters optimized to treat overactive bladder, i.e., stimulation frequency 20 Hz at the sensory threshold intensity. These stimulation parameters may not be optimal for the treatment of erectile dysfunction because our study indicates that a higher stimulation frequency (30-40 Hz) at intensities above sensory and motor thresholds can induce penile erection quickly once the stimulation is turned on and the erection can be sustained during the entire 10-minute stimulation. This type of on-demand erection should be much more satisfactory for the patient than the quality-of-life improvement produced by continuous (24 hours x 7 days) sacral neuromodulation at a lower frequency and weaker stimulation intensity. Therefore, it seems that a higher frequency (30-40 Hz) and a stronger stimulation intensity (above sensory and motor thresholds) should be tested in clinical studies to treat erectile dysfunction. Although leg movement will be induced by a stronger stimulation, the leg muscle contraction will be tonic and the movement will only occur at the beginning of the stimulation, which should not be a problem for successful

sexual intercourse. When anterior root stimulation was used to induce penile erection in SCI people, it also induced leg movement but did not prevent successful sexual intercourse (18).

Stimulation of the sacral spinal root (ventral and dorsal roots together) induced a large increase in penile pressure similar to that produced by stimulation of the ventral root alone (Fig.3). This result indicates that activation of additional sensory nerves in the dorsal root did not disrupt the penile erection induced by direct stimulation of the efferent nerves in the ventral root. A previous study in dogs (17) also reported no difference in penile erection elicited by ventral root stimulation alone or by stimulation of ventral and dorsal roots together. In human application under awake conditions, activation of sensory nerves may cause uncomfortable or painful sensation that can limit the stimulation intensity and therefore limiting the efficacy of the stimulation in treating erectile dysfunction. This potential issue can only be resolved by a clinical study in non-SCI human subjects. However, it should not be an issue for subjects with a complete SCI who have no sensation below the injury.

Our study shows that the penile erection induced by sacral root stimulation is sustainable or gradually develops during the 10-minute stimulation (Fig.3 A-B). This indicates that sacral root stimulation can produce long duration erections when it is applied for a longer time. It also indicates that stimulation of afferent nerves in the dorsal root did not reflexively activate the inhibitory sympathetic pathway that can suppress penile erection (19). Sacral root stimulation probably activated both parasympathetic and somatic pathways to produce the erection and penile rigidity (19, 20). However, after spinal cord transection the amplitude of the induced penile pressure was significantly reduced, possibly due to the spinal shock effect and the decrease in blood pressure after an acute spinal cord transection. Nevertheless, sacral root stimulation still induced a large (>100 cmH₂O on average, Fig.4) sustained increase in penile

pressure after acute spinal cord transection, indicating that this stimulation could be useful in restoring erectile function after SCI (21). Whether sacral root stimulation will be less effective in SCI people than in non-SCI people to induce penile erection is a question to be answered by human clinical studies. However, it is known that sacral anterior root stimulation is effective in inducing penile erection strong enough for sexual intercourse in SCI people (3-5).

In summary, this study in cats optimized the stimulation parameters for sacral spinal root stimulation to induce penile erection. The results have significant implications for design of a novel neuromodulation device to restore erectile function after SCI, and for optimizing sacral neuromodulation parameters to treat non-SCI people with erectile dysfunction.

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FIGURE CAPTION

Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

Fig.2: Penile pressure in the corpus cavernosum induced by electrical stimulation of sacral ventral roots at different frequencies and different spinal segments. A. In a cat, S1 ventral root

stimulation (10-80 Hz) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. B. In another cat, S2 ventral root stimulation (30-80 Hz) of short duration (1 minute) was most effective in inducing large increases (150 cmH₂O) in penile pressure. C. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. * indicates significantly different from S1 ventral root ($p < 0.05$, ANOVA). Stimulation: 30 Hz, 0.6-12 V, 0.2 ms. D. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root. N = 8 cats. * indicates significantly different from 5 Hz ($p < 0.05$, ANOVA). Stimulation: 0.6-12 V, 0.2 ms.

Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

Fig.4: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the 10 minutes of stimulation. B. In the same cat, 10 minutes after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure

during the first 3 minutes and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference ($p=0.0025$, paired t-test).

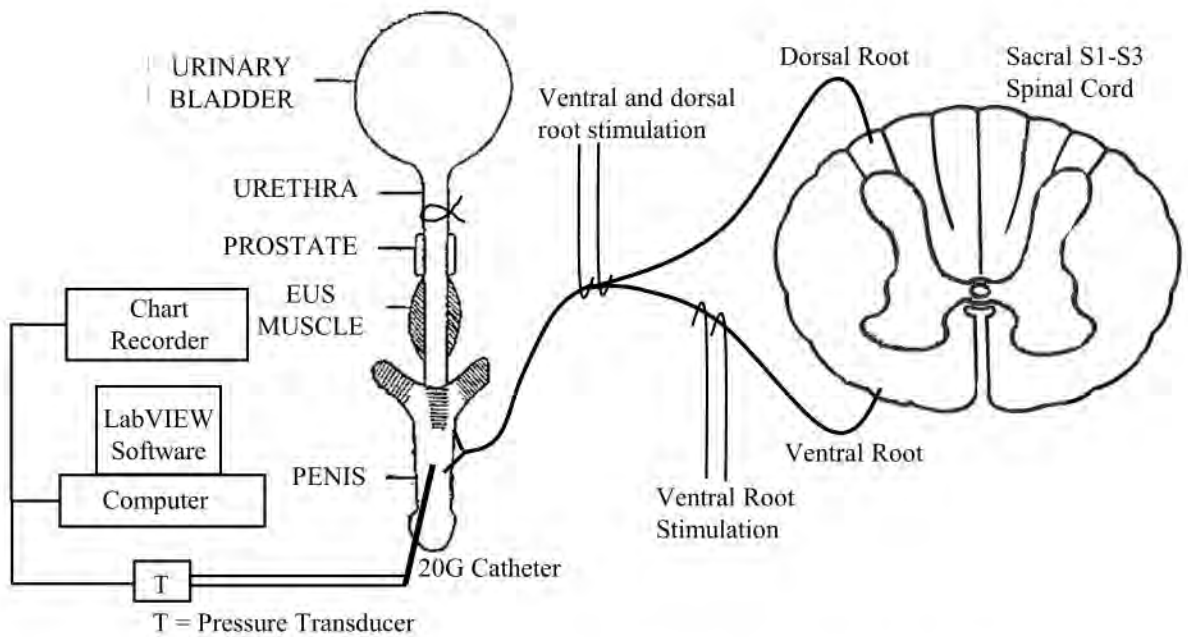
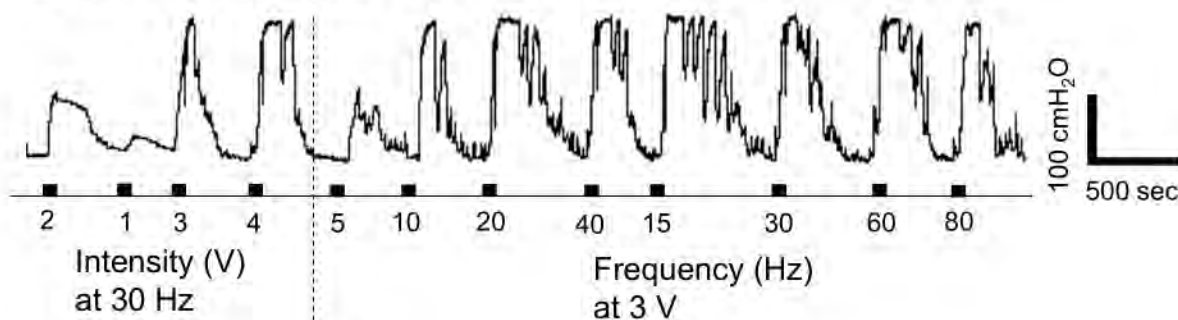
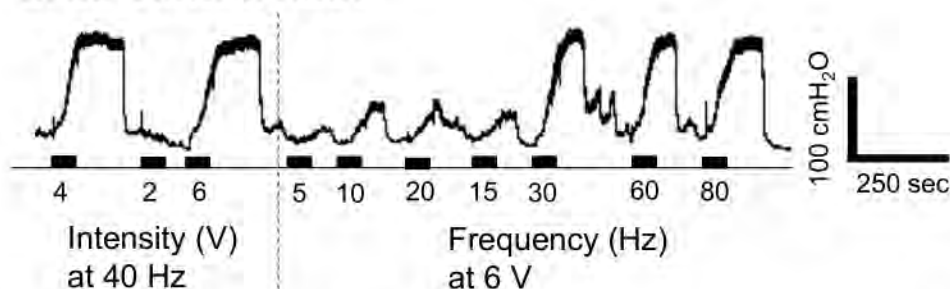


Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

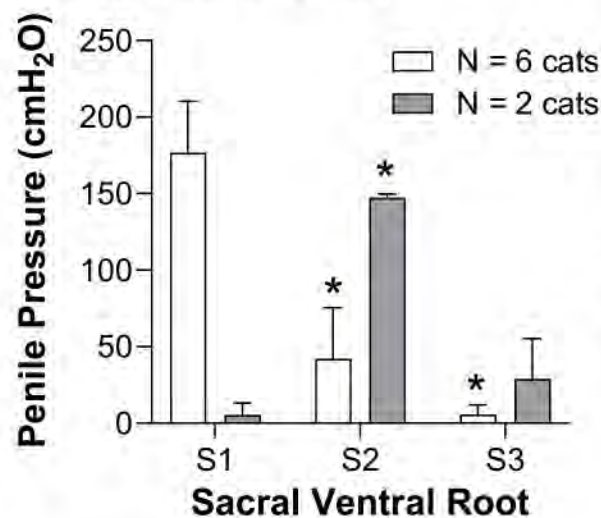
A. S1 Ventral Root



B. S2 Ventral Root



C. Segmental Effect



D. Frequency Effect

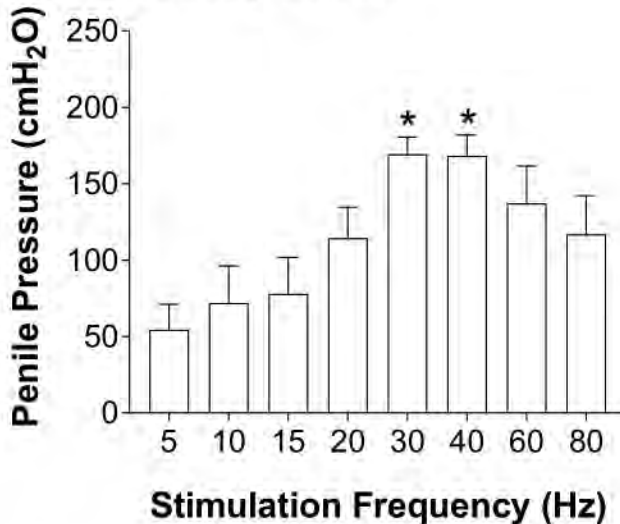
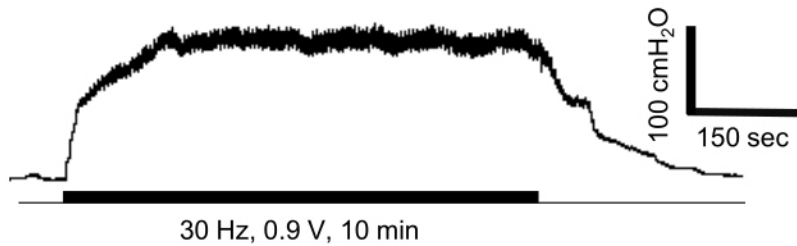
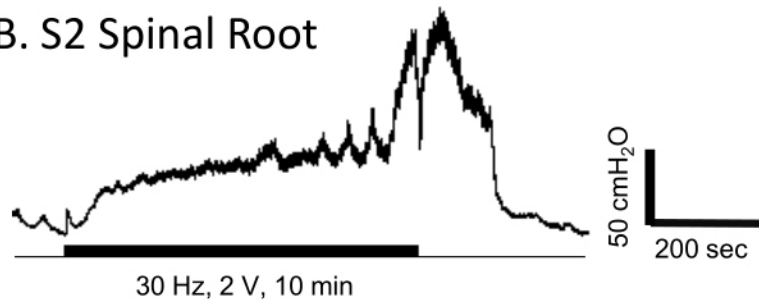


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A. S1 Spinal Root



B. S2 Spinal Root



C. Sustained Pressure

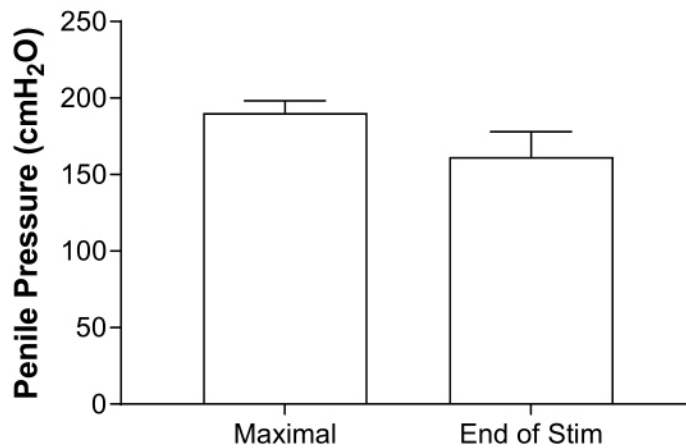
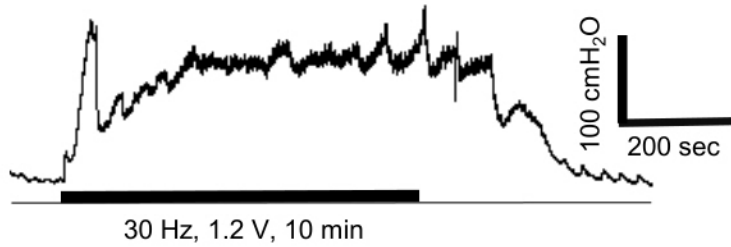
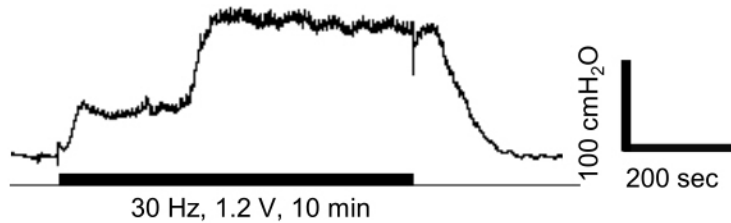


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A. Before Spinal Transection



B. After Spinal Transection



C. Maximal Pressure

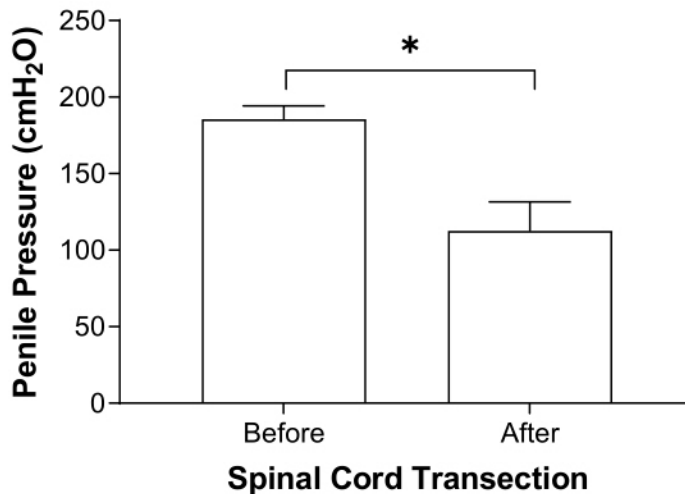


Fig.4: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the 10 minutes of stimulation. B. In the same cat, 10 minutes after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure during the first 3 minutes and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference (p=0.0025, paired t-test).

University of Pittsburgh

Bridging the GAP Plus (BG+) Technical / R&D

(b)(4) Final Report

Period Covered by the Report: October 1, 2020 through March 31, 2022 (b)(4)

Date of Report: March 31, 2022

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: (b)(4)

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(4), (b)(6)

Submitted by:

(b)(4), (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) period (b)(4), we have made major progresses in the following areas:

1. Scientific progress:

- **Bladder Function:** We have successfully demonstrated in anesthetized cats that pudendal nerve stimulation and block by lead electrodes can achieve a low-pressure (≤ 50 cmH₂O) voiding. This success indicates that a minimally invasive surgical approach by inserting lead electrodes close to the pudendal nerves can be developed to restore bladder function after spinal cord injury. Our first paper titled "Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats" has been published on Experimental Neurology (see attachment). **This is a Big Win for this DARPA BG+ project.**
- **Bowel Function:** We have successfully demonstrated in anesthetized cats that stimulation of sacral S2 spinal root at 7 Hz is most effective to induce colon contractions, while stimulation of S1/S3 root or pudendal nerve is not effective. In addition, defecation is successfully induced by stimulation of S2 spinal root using wire electrodes. This success indicates a minimally invasive surgical approach by inserting

lead electrodes close to the S2 spinal root can be developed to restore bowel function after spinal cord injury. Our second paper titled "Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats" has been published on American Journal of Physiology - Gastrointestinal and Liver Physiology (see attachment). **This is a Big Win for this DARPA BG+ project.**

- Male Sexual Function: We have successfully demonstrated in anesthetized male cats that stimulation of sacral S1/S2 spinal root at (b)(4) using wire electrodes is most effective to induce penile erection, while stimulation of S3 root or pudendal nerve is not effective. This success indicates that a minimally invasive surgical approach by inserting lead electrodes close to the S1/S2 spinal root can be developed to restore penile erection after spinal cord injury. Our third paper titled "Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats" has been submitted to Neuromodulation for publication (see attachment).
- Female Sexual Function: We have successfully demonstrated in anesthetized female cats that pudendal nerve stimulation at (b)(4) is most effective to induce vaginal lubrication and contractions. This success indicates that our pudendal stimulation/block device can also be used to restore female sexual function after SCI in addition to restoring bladder function. Our fourth paper titled "Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats" has been submitted to Journal of Sexual Medicine for publication (see attachment).

- (b)(4)

2. Engineering progress:

- Completed the full electrical design schematics of a (b)(4)

- (b)(4)

- (b)(4)

(b)(4)

- The (b)(4) programs were developed for (b)(4)
- (b)(4)
- The (b)(4) component placement has been finalized. All components fit on the board and have been shrunk down to fit in an (b)(4)
- All components, testing results, and documents of the (b)(4) for the IDE study were compiled and submitted to the FDA - **This is a Big Win for this DARPA BG+ project.**
- (b)(4)
- (b)(4)
-
-
-
-
-
- Multiple (b)(4) designs were created in house to trial if (b)(4)
- (b)(4)
- The (b)(4) design was reviewed by the internal team and an order was placed with a (b)(4) that should be delivered in (b)(4)
- The engineering for the (b)(4) has been completed and the (b)(4) company has moved on to the fabrication stage.
- The GUI successfully controlled the (b)(4) during a UPitt cat experiment wirelessly (b)(4)
- The (b)(4) successfully communicates and controls the (b)(4)
- (b)(4)

- Firmware features (b)(4) has been fully defined and created (b)(4)
 - The next steps will be to load the GUI and firmware onto a patient controller.

3. Regulatory progress:

- FDA IDE (b)(4) was submitted (b)(4) and informational meeting was requested.
 - (b)(4)
 -
 -
- Met with the FDA twice regarding (b)(4)
 - Received feedback on (b)(4) (b)(4)
 - Given FDA's positive feedback, we amended our (b)(4) studies accordingly
- Early feasibility human clinical protocol was outlined, revised, and included in FDA IDE submission.
- Finalized logistics, scheduled and performed (b)(4) (b)(4), (b)(6)
- FDA IDE (b)(4) was drafted, which focuses on (b)(4) (b)(4)
- Submitted and met with the FDA regarding (b)(4) (b)(4)
 -
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 -
- Fully drafted and submitted IDE to FDA on (b)(4)
 - FDA received the IDE submission on (b)(4)
 - Received and responded to (b)(4) from FDA
 - IDE unconditionally approved by FDA on (b)(4) (b)(4)
 - FDA thanked Thermaquil for quick and comprehensive responses
- Continued to identify user needs and voice of customer to inform device requirements, (b)(4)

4. Administrative progress:

- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
- Weekly meetings were held between University of Pittsburgh, (b)(4) and Thermaquil to coordinate the project activity.

- Monthly meeting was hold with DARPA to report our progress.
- (b)(4)

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.1 Institutional IACUC approval and DOD ACURO review (b)(4)

We have obtained IACUC approvals from University of Pittsburgh, DOD ACURO, and NAVY IACUC.

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.1 Restore bladder function (b)(4)

We have successfully demonstrated in anesthetized cats that pudendal nerve can be blocked by (b)(4) stimulation using (b)(4)

(b)(4) These results indicate that a minimally invasive surgical approach by inserting the (b)(4) close to the pudendal nerves can be used to (b)(4) the pudendal nerves to restore bladder function after spinal cord injury (SCI).

Nerve Block by a Lead Electrode

Our study in anesthetized cats has discovered that the pudendal nerve can be completely blocked by (b)(4) i.e., (b)(4) (Fig.1). Blocking nerve conduction by a lead electrode is novel because all previous studies require a major invasive surgery to implant a cuff electrode to block a peripheral nerve conduction. Our study demonstrates in the first time that a cuff electrode is not needed, and a nerve block can be induced by a (b)(4) which indicates that in clinical applications a (b)(4) could be used to block nerve conduction. This result is significant because a minimally invasive surgical approach could be developed to insert the (b)(4) close to a peripheral nerve, instead of a major invasive surgery to (b)(4) to achieve a (b)(4) for clinical applications.

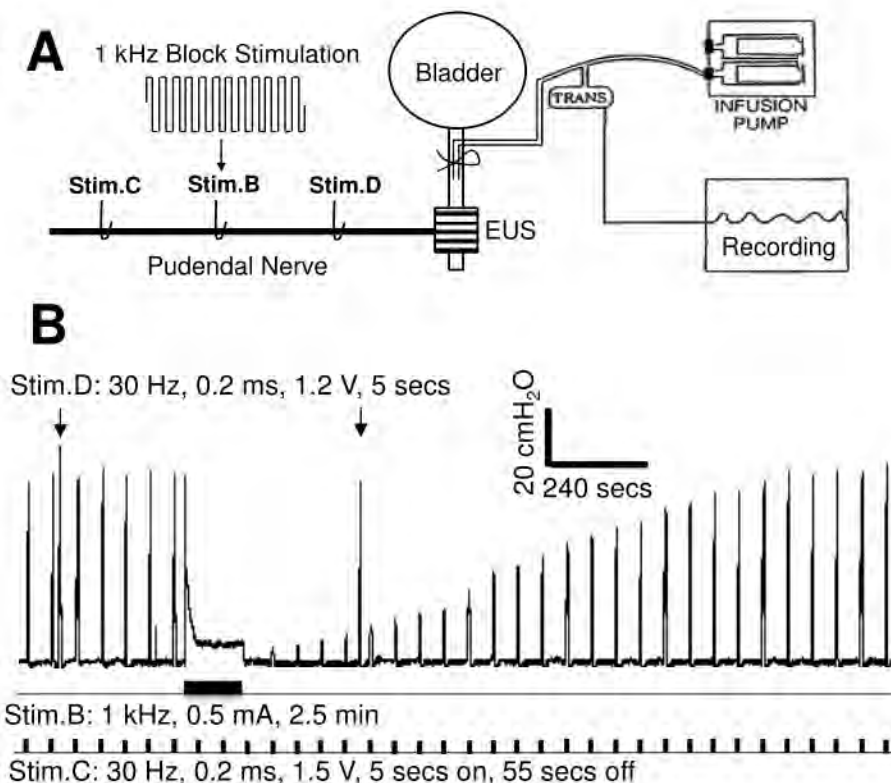


Fig.1: A. Experimental setup for pudendal nerve block by a hook electrode. B. Complete nerve block occurred after the end of the 1 kHz biphasic continuous stimulation. The arrows indicate the EUS contractions induced by the 30 Hz stimulation at the distal site (Stim.D). The thick bars under the EUS pressure trace indicate the stimulation durations for Stim.B and Stim.C. After ending the 1 kHz Stim.B, EUS contraction can still be induced by the distal Stim.D but the contractions induced by the central Stim.C are completely blocked initially and then gradually recover, indicating that nerve conduction block occurs locally at Stim.B and the EUS is not fatigued. EUS –External Urethral Sphincter.

As shown in Fig.1, Stim.B (b)(4) successfully blocked the periodic EUS contractions induced by short bursts of Stim.C (b)(4) during a period after terminating the (b)(4) i.e., a post-stimulation block. Since the EUS contractions induced by Stim.D (b)(4) was not changed while the contractions induced by Stim.C was blocked, it indicated that Stim.B blocked the pudendal nerve conduction instead of causing a EUS fatigue that should affect the responses induced by both Stim.C and Stim.D. For long-term bladder management after SCI, repeatedly fatiguing the EUS by pudendal nerve stimulation will cause the EUS to become more and more fatigue resistant and not relax sufficiently for a low-pressure voiding.

The duration of (b)(4) was dependent on the intensity and duration of the stimulation. As shown in Fig.2, the block duration was increased by increasing the intensity of 1 kHz stimulation (30 secs) from (b)(4). At the intensity of (b)(4) the duration for a complete post-stimulation block reached (b)(4) with a recovery duration of (b)(4). When the intensity of (b)(4) stimulation increased to 4 mA, the complete block duration increased to 6 mins, but the recovery duration increased dramatically to 100 mins (see the fourth trace in Fig.2). In the other 2 cats, a prolonged recovery duration (58 mins or 60 mins) was induced by 1 kHz stimulation at the intensity/duration combination of 4 mA/30 secs or 2 mA/60 secs, respectively.

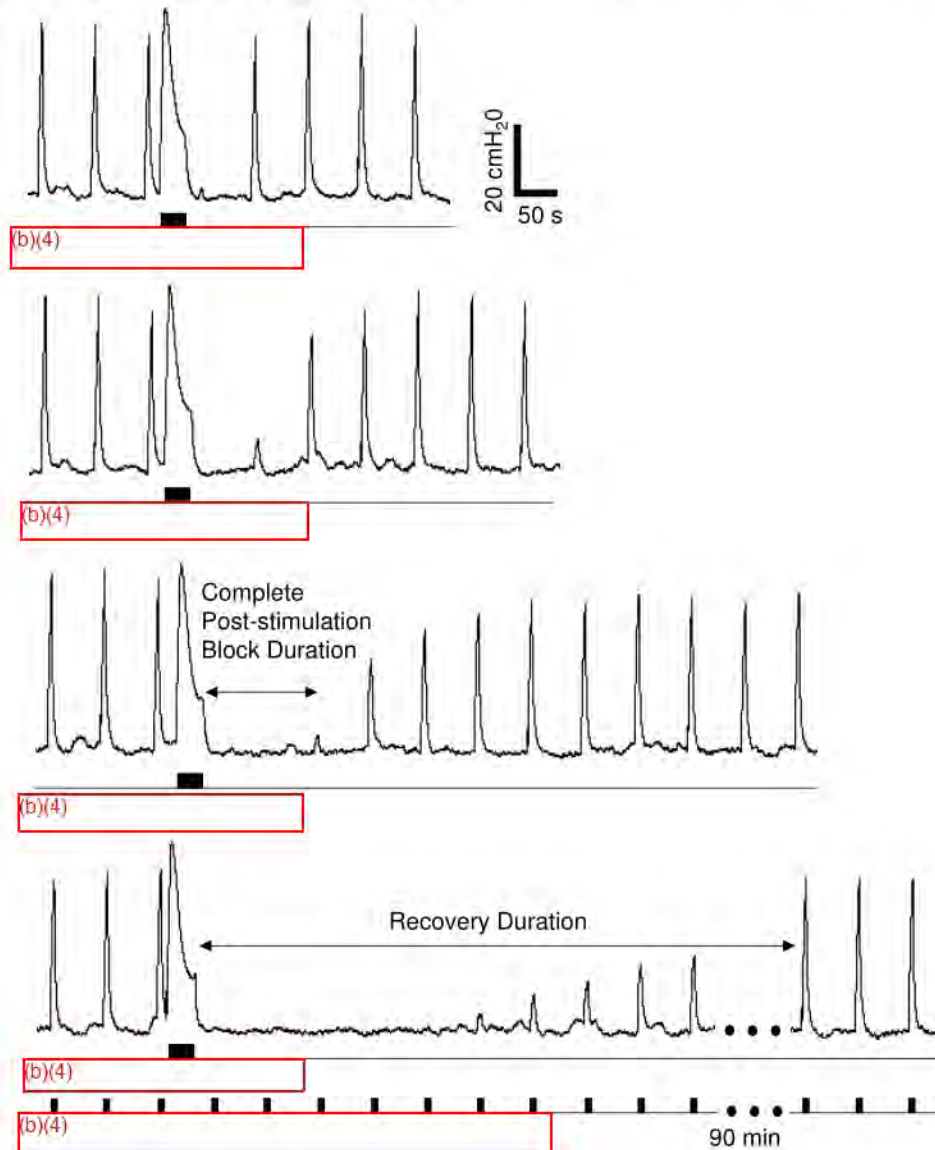


Fig.2: Block stimulation (Stim.B) produced a complete post-stimulation block of duration ≥ 2 minutes as the stimulation intensity gradually increased to 3 mA. At 4 mA intensity, Stim.B produced a prolonged post-stimulation block with a recovery period of 110 minutes. The short black bar under each pressure trace indicates the stimulation duration. The duration of Stim.C shown at the bottom applies to all pressure traces. The intensity for Stim.C is at 3 times of the threshold (T) intensity for inducing visible anal twitch to induce maximal contractions of the external urethral sphincter.

Fig.3 summarized the intensity and duration required for (b)(4) to induce a complete post-stimulation block of duration (b)(4) and the corresponding duration for the block and recovery (N=6 nerves from 3 cats). In one cat, (b)(4) duration failed to induce a complete post-stimulation of duration (b)(4) before its intensity increased to the level causing hindlimb movement. In this cat after increasing the stimulation duration to 60 secs, the (b)(4) required a much lower intensity (b)(4) see the black dots in Fig.3) than (b)(4) duration to induce the complete block. These results indicate that there is a combination of intensity and duration for (b)(4) to induce a complete post-stimulation block of duration ≥ 2 mins that can recover relatively quick within (b)(4) (Fig.3). However, if the intensity and/or the duration increased further, a prolonged recovery of (b)(4) may occur (see the fourth trace in Fig.2). The complete block was defined as the EUS contraction pressure induced by Stim.C was reduced to <10 cmH₂O that would allow a low-pressure voiding to occur. The complete post-stimulation block of duration (b)(4) would allow a duration long enough for voiding to complete.

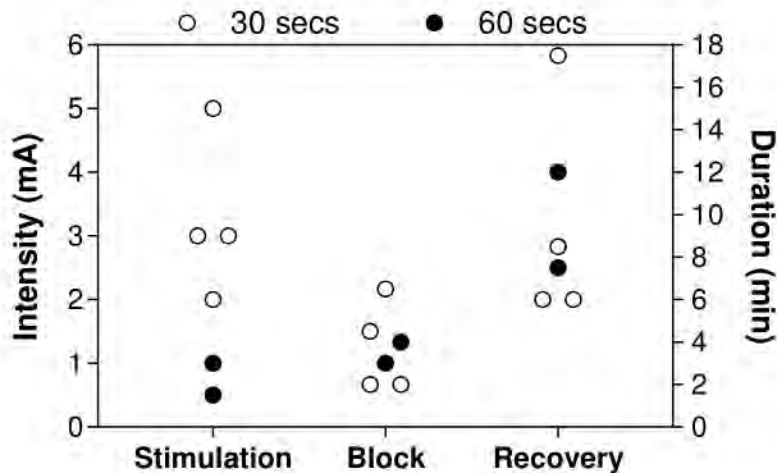


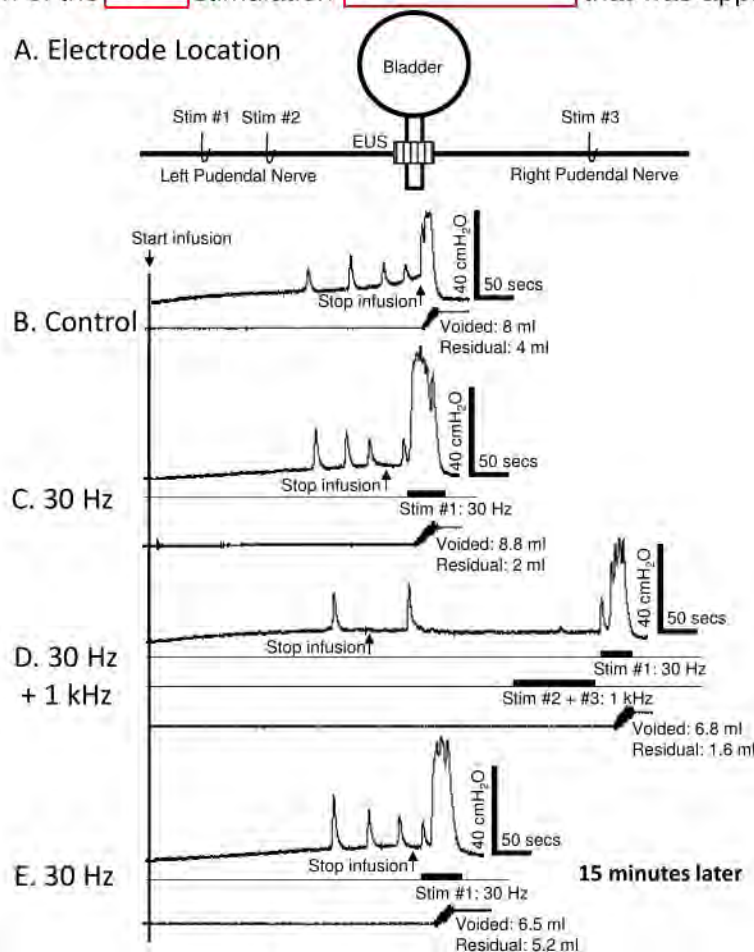
Fig.3: The intensity and duration (30 secs or 60 secs) required for 1 kHz stimulation to produce a complete post-stimulation block of duration ≥ 2 mins and the corresponding duration for the block and recovery. Stimulation intensity is shown on the left Y-axis. Duration for the block and recovery is shown on the right Y-axis. N = 6 nerves from 3 cats.

Low-Pressure Voiding by Lead Electrodes

More importantly, we further demonstrated that a low-pressure (≤ 50 cmH₂O) voiding can be successfully induced by a (b)(4) pudendal nerve stimulation during the post-stimulation block of bilateral pudendal nerves induced by lead electrodes (Fig.4). Low-pressure voiding induced by pudendal nerve stimulation and block is critical for long-term management of bladder dysfunction after SCI, because high-pressure voiding can cause renal failure in a long-term clinical application. Therefore, the (b)(4) pudendal nerve block as shown in our study is very important because it can prevent detrusor sphincter dyssynergia (DSD) after SCI and reduce

urethral outlet resistance for a low-pressure voiding to occur. The clinical significance of using lead electrodes to induce a low-pressure voiding is that a minimally invasive surgical approach by inserting the lead electrodes close to the pudendal nerves, instead of a major invasive surgery to implant the cuff electrodes, can be used to block and stimulate the pudendal nerves to restore bladder function after SCI.

Fig.4 shows that using monopolar hook electrodes the (b)(4) pudendal nerve stimulation combined with (b)(4) post-stimulation block successfully induced a low-pressure (≤ 50 cmH₂O) voiding. During the control CMG (Fig.4B), slow infusion of the bladder triggered a micturition reflex producing a low-pressure (≈ 40 cmH₂O) voiding. With the bladder partially full (Fig.4C), Stim #1 (30 Hz) alone induced a voiding of a high pressure (≈ 60 cmH₂O). However, the same Stim #1 successfully induced a low-pressure (≈ 40 cmH₂O) voiding when applied immediately after termination of the (b)(4) stimulation (b)(4) that was applied to both Stim #2



(b)(4)

and #3 (see Fig.4D). The high-pressure (≈ 60 cmH₂O) voiding was returned in about 15 minutes after termination of the (b)(4) (Fig.4E).

Fig.5 shows that a low-pressure voiding can also be induced by combination of (b)(4) and (b)(4) using bipolar hook electrodes. However, the recovery from a low-pressure voiding to a high-pressure voiding was prolonged since the 1 kHz block stimulation was too strong. As shown in Fig.5A-B, micturition reflex induced a low-pressure (<50 cmH₂O) voiding while Stim #1 (b)(4) alone induced a high-pressure (≈ 100 cmH₂O) voiding. After applying (b)(4) stimulation (2 mA, 1 min) to both Stim #2 and #3, the same Stim #1 lost the ability to induce a bladder contraction in about 10 mins, and then it successfully induced a low-pressure (<50 cmH₂O) voiding (see Fig.5C). The low-pressure voiding induced by Stim

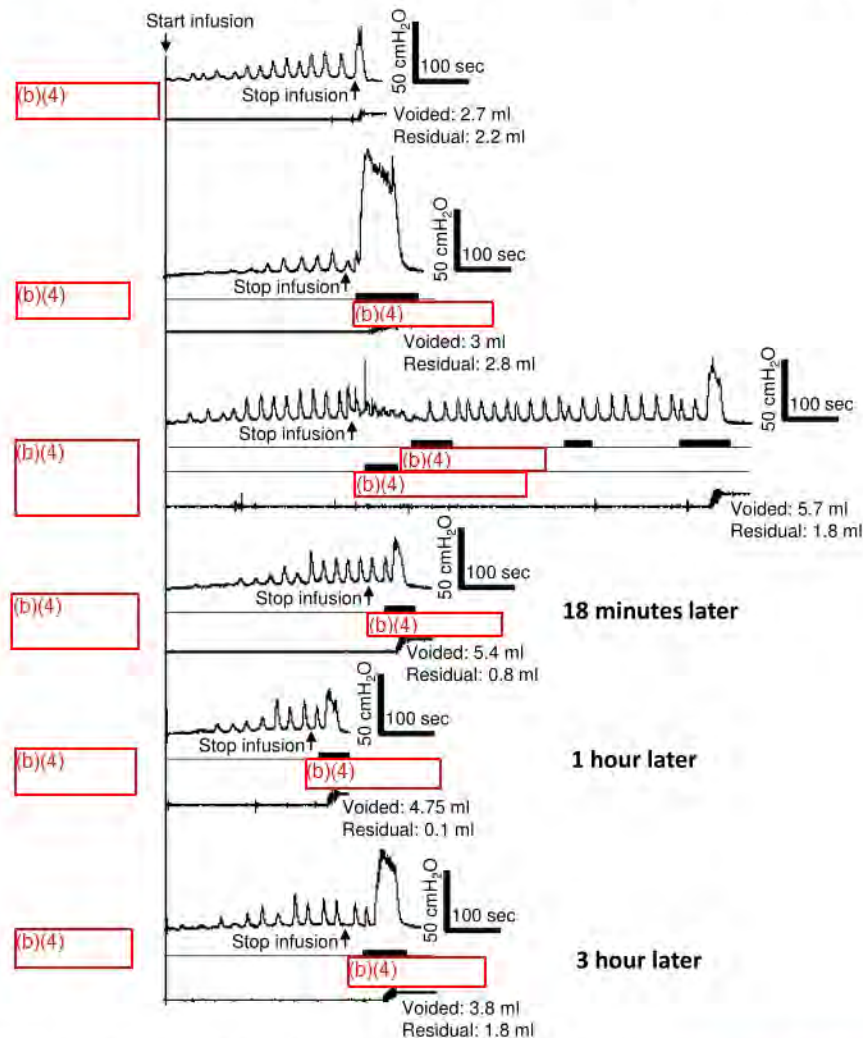


Fig.5. Long-lasting effect of 1 kHz post-stimulation block. A. Control cystometrogram showing a low-pressure (<50 cmH₂O) voiding produced by micturition reflex. B. Stim #1 (b)(4) alone produced a high-pressure (≈ 100 cmH₂O) voiding. C. After Stim #2 and #3 delivered the 1 kHz stimulation (b)(4), Stim #1 lost the ability to induce a bladder contraction for about 10 minutes. Then, Stim #1 induced a low-pressure (≈ 50 cmH₂O) voiding. D-E. The low-pressure voiding by Stim #1 lasted for at least 1 hour. F. At 3 hours after the (b)(4) the voiding pressure induced by Stim #1 increased to about 70 cmH₂O. The black bar under the pressure trace indicates the stimulation duration. Infusion rate = 1 ml/min.

#1 alone lasted for at least an hour (Fig.5 D-E). In 3 hours, the voiding pressure returned to about 70 cmH₂O (Fig.5F). Please note that there was no leakage of fluid from the bladder during the period immediately following termination of the (b)(4) block stimulation although many non-voiding contractions occurred during this period (Fig.5C).

It is worth noting that the 1 kHz post-stimulation block is reversible within minutes if an appropriate combination of stimulation intensity and duration is used (Figs.3-4). This reversibility is needed because the pudendal nerve needs to be functional after each voiding to maintain continence during urine storage. There is an optimal combination of stimulation intensity and duration for achieving a short duration (~2 mins) post-stimulation block to allow a low-pressure voiding to occur while also allowing the block to recovery within 20 mins (Fig.3). In future clinical application, this optimal combination of stimulation intensity and duration will have to be determined for each individual subject after implanting the pudendal nerve stimulation and block device. If the (b)(4) stimulation is too strong or lasts too long, the pudendal nerve block will need a long time to recovery (see forth trace in Fig.3), which will result in a prolonged period of low-pressure voiding (Fig.5).

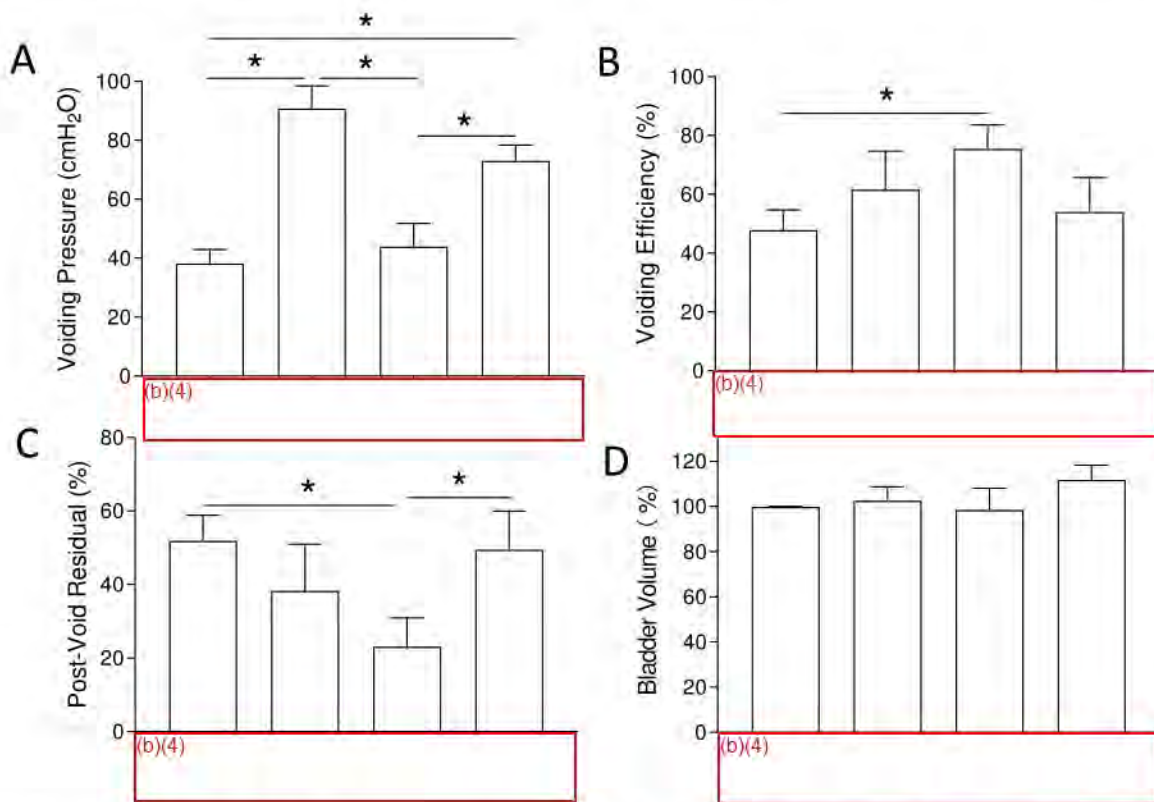


Fig.6. Summary of the urodynamic parameters for control condition, (b)(4) stimulation alone, (b)(4) stimulation after (b)(4) stimulation, and recovery from the (b)(4) blocking effect (Post-kHz recovery). A. Voids pressure. B. Voids efficiency. C. Post-void residual. D. Bladder volume. * indicates a significant difference ($p < 0.05$, ANOVA). N = 5 cats.

Fig.6 summarizes the results from 5 cats. On average, in the control condition the micturition reflex induced by filling the bladder produced low-pressure (≈ 40 cmH₂O) voiding (Fig.6A) of a 50% voiding efficiency (Fig.6B) with a post-void residual volume of 50% (Fig.1C) of the control bladder capacity. A slightly higher voiding efficiency, but not significantly different from control, and a lower post-void residual volume were produced by (b)(4) stimulation alone (Fig.6 B-C) at a significantly ($p < 0.05$) higher voiding pressure (90 cmH₂O, Fig.6A). However, (b)(4) stimulation combined with (b)(4) block significantly ($p < 0.05$) increased voiding efficiency (80%, Fig.1B) and reduced post-void residual volume (20%, Fig.6C) but at a significantly ($p < 0.05$) lower pressure (≈ 40 cmH₂O) that was same as in the control condition (Fig.6A). After a recovery period ranging from 15 mins to 3 hours (see Fig.4 and Fig.5, the voiding pressure induced by (b)(4) alone increased to about 70 cmH₂O (Fig.6A) while voiding efficiency declined (50%, Fig.6B) and post-void residual volume significantly ($p < 0.05$) increased (50%, Fig.6C) above that measured immediately after the 1 kHz stimulation. On average a similar amount of saline was infused into the bladder under the different conditions (Fig.6D).

Sub-Task 1.2.2 Restore bowel function (b)(4)

A total of 9 cats (4 females and 5 male, 4.0 ± 0.3 kg; Liberty Research, Waverly, NY) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, one balloon catheter (G15766, Cook Urological, Spencer, IN) 1.4 cm in diameter and 5 cm in length was inserted into the proximal colon via a small incision at the proximal end of the colon (Fig.7). The second balloon catheter of the same size was inserted into the distal colon via the anus. The two balloons were filled with water (5-7 ml) to a resting pressure of 10-15 cmH₂O to measure proximal and distal colon contractions in the first group of 7 cats. In the second group of 2 cats, a large balloon catheter made of a condom (3.5 cm in diameter and 15 cm in length) was inserted via the small incision at the proximal end of the colon to occupy the entire colon so that both proximal and distal colon contractions were recorded. The condom was filled with water (60-70 ml) to a resting pressure of 10-15 cmH₂O. In these 2 cats, 1-4 glass marbles (diameter 1.5 cm) were also inserted into the rectum via the anus so that evacuation of these marbles could be videotaped simultaneously with the colon pressure recording.

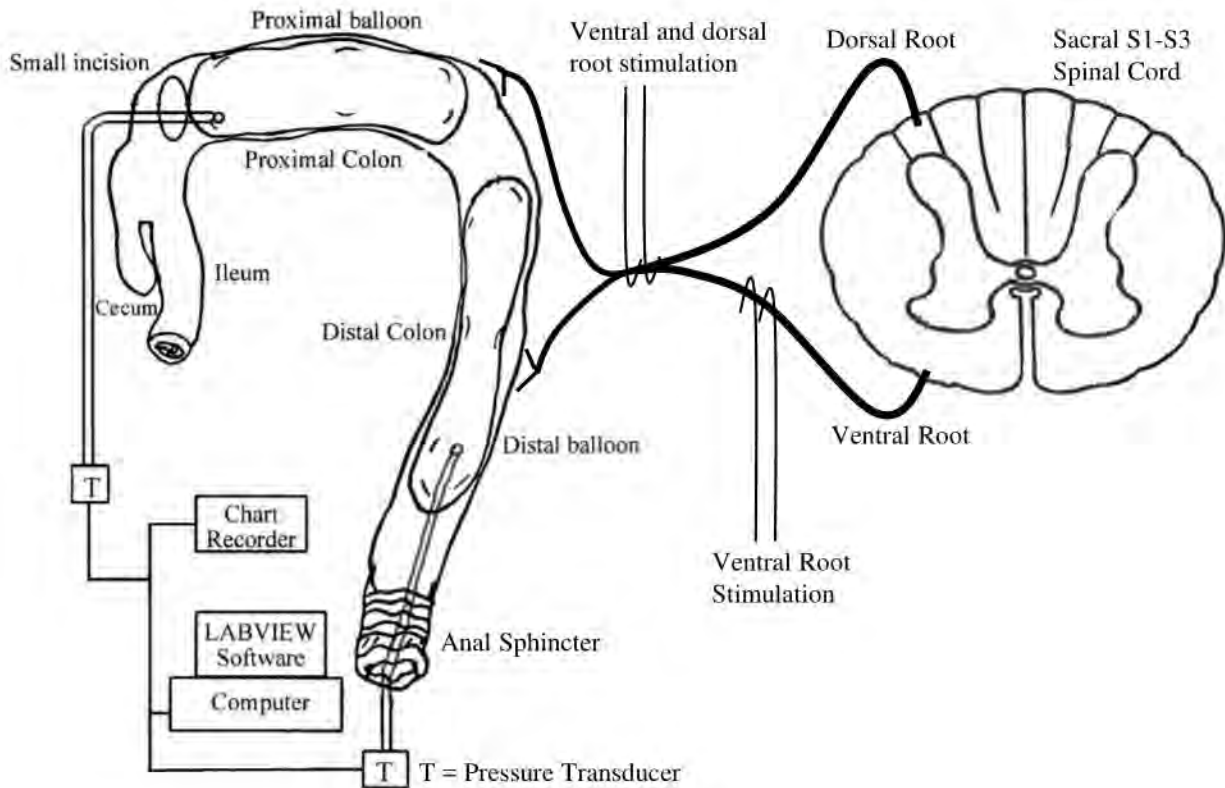


Fig.7. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce colon contractions.

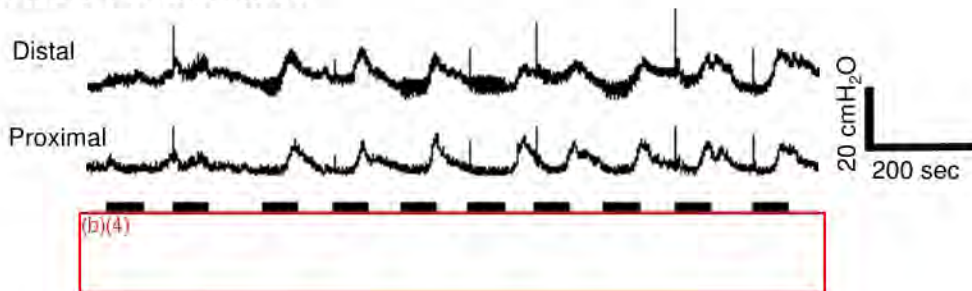
The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the (b)(4) was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (Fig.7). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Colon contraction induced by sacral ventral root stimulation is dependent on stimulation frequency and the spinal segment stimulated.

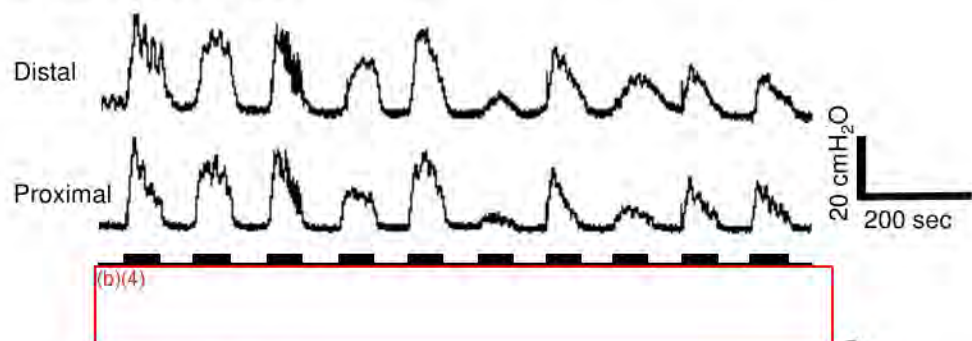
Stimulation of S2 ventral root induced larger colon contractions than stimulation of S1 or S3 ventral roots, and the most effective stimulation frequency was (b)(4) (Figs.8-9). Fig.8 shows the distal and proximal colon pressure traces recorded from the same cat. Stimulation of S2 ventral root induced colon contractions >20 cmH₂O at frequencies (b)(4) with a similar contraction amplitude in the distal and proximal colon (Fig.8B). Stimulation of the S1 or S3 ventral root did not induce colon contractions larger than the small (<10 cmH₂O) rhythmic baseline colon contractions that were occurring asynchronously during the periods of

stimulation (Fig.8 A and C). In this cat, the stimulation intensity used for S2 ventral root stimulation is 2 times the threshold intensity used during (b)(4) stimulation to induce an observable proximal colon contraction. Due to the weak colon response to S1 and S3 ventral root stimulation, the maximal stimulation intensity just below the threshold for inducing lower spine movement was applied to these roots. Hindlimb (mainly S1/S2 stimulation) and/or tail (mainly S2/S3 stimulation) movements were induced by stimulation of each of the three roots. Fig.9 summarizes the results from 7 cats showing that S2 ventral root stimulation at 7-10 Hz was optimal for producing significantly ($p < 0.05$) larger colon contractions than those elicited by S1 or S3 ventral root stimulation. There was no significant difference between the contraction amplitudes of distal and proximal colon (Fig.9).

A. S1 Ventral Root



B. S2 Ventral Root



C. S3 Ventral Root

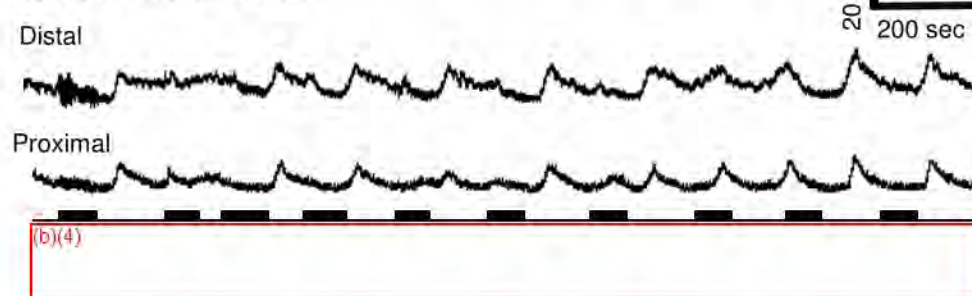
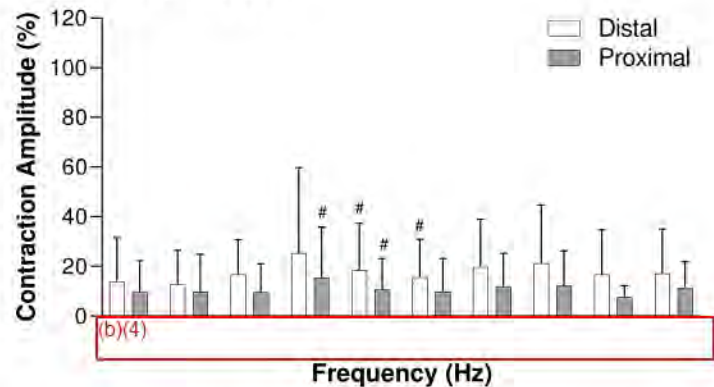
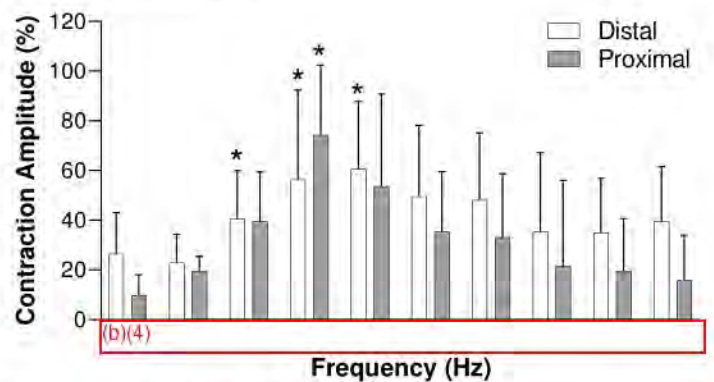


Fig.8. Distal and proximal colon responses to stimulation of sacral S1-S3 ventral roots at different frequencies (b)(4). A. S1 ventral root. B. S2 ventral root. C. S3 ventral root. Data were obtained from the same animal.

A. S1 Ventral Root



B. S2 Ventral Root



C. S3 Ventral Root

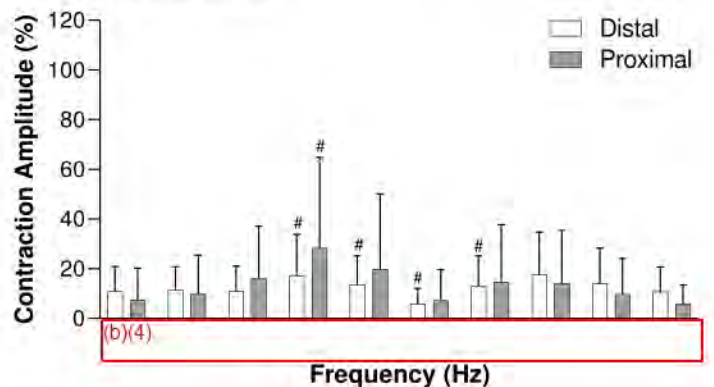
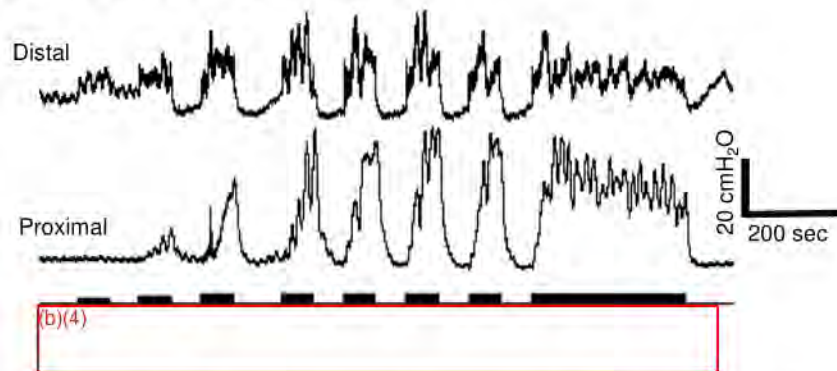


Fig.9. Distal and proximal colon responses to sacral ventral root stimulation are frequency and spinal segment dependent. A. S1 ventral root. B. S2 ventral root. C. S3 ventral root. The contraction amplitude induced by different stimulation frequencies in different sacral segments is normalized to the maximal response in the same animal. Stimulation intensity (4-16 V) for S2 ventral root was 1.5-3 times threshold intensity for (b)(4) stimulation to induce an observable contraction of proximal colon. When S1 and S3 ventral root stimulation did not induce a colon contraction, the maximal stimulation intensity (8-12 V) without causing lower body movement was used to test different frequencies. * significantly different from (b)(4) (one-way ANOVA); # significantly different from S2 ventral root (two-way ANOVA). N = 7 cats.

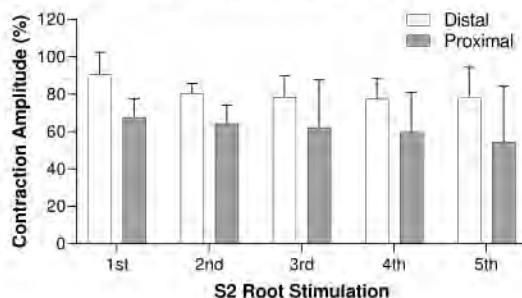
Fatigue of colon contractions induced by S2 root stimulation

Stimulation (b)(4) of the S2 ventral and dorsal roots together induced large amplitude (>20 cmH₂O) contractions in both distal and proximal colon (Fig.10). Fig.10A shows that intermittent (b)(4) threshold intensity for inducing a proximal colon contraction produced a stronger contraction during the second period of stimulation and that the large amplitude contraction was maintained throughout the next three 1-minute stimulations. However, the contraction amplitude gradually declined during a continuous 5-minute stimulation, indicating a fatigue in the colon contractions (Fig.10A). On average the contraction amplitude for both the distal and proximal colon was maintained during the 5 intermittent 1-minute stimulations (N=4 cats, Fig.10B), but was significantly (p<0.05) reduced from the mean maximal amplitude of 30 cmH₂O to about 18 cmH₂O at the end of the 5-minute continuous stimulation (N=7 cats, Fig.10C).

A. S2 Ventral and Dorsal Roots



B. Intermittent 1-min Stimulation



C. 5-min Continuous Stimulation

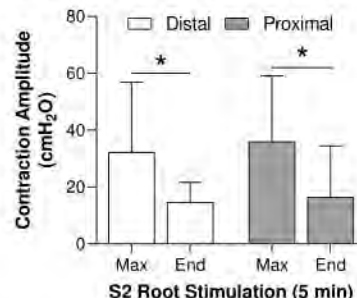
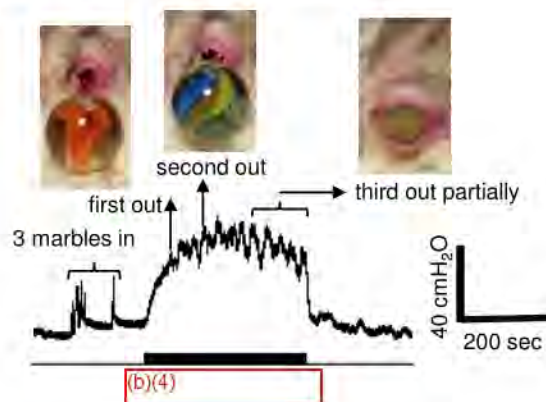


Fig.10. Distal and proximal colon responses to intermittent (5 x 1 minute) or long duration (5 minutes) continuous stimulation of both S2 ventral and dorsal roots. A. Pressure traces showing distal and proximal colon contractions. The intensity threshold (T) for proximal colon contraction was 4 V, and 6 V (1.5T) was used to induce the intermittent or continuous contractions. B. Summarized results (N=4 cats) showing that the contraction amplitudes were maintained during intermittent stimulation (7 Hz, 0.2 ms, 1.5-4T, T = 2-4 V). C. Summarized results (N = 7 cats) showing that the maximal (Max) contraction amplitude was significantly reduced from mean 30 cmH₂O to about 18 cmH₂O at the end of the continuous 5-minute stimulation (7 Hz, 0.2 ms, 1.5-3T, T = 2-4 V). * significantly different (p<0.05, paired t-test).

Defecation induced by S2 root stimulation

Stimulation of the S2 ventral and dorsal roots together induced a maximal amplitude colon contraction of 60 cmH₂O in cat #1 (Fig.11A) and 40 cmH₂O in cat #2 (Fig.11B). In cat #1 after 3 marbles were inserted into the rectum, S2 root stimulation (b)(4) induced a successful defecation that eliminated the first marble after 1 minute of stimulation and the second marble after 2 minutes of stimulation (Fig.11A). The elimination of the third marble which started during the last 2 minutes of the 6-minute stimulation was incomplete (Fig.11A). In cat #2 after 1 marble was inserted into the rectum, the S2 root stimulation (7 Hz, 6 V, 0.2 ms) eliminated the marble after 2.5 minutes of stimulation (Fig.11B). However, after 4 marbles were inserted into the rectum, the stimulation eliminated only 1 of the 4 marbles after (b)(4) (b)(4) in the rectum at the end of (b)(4) (Fig.11B). Although stimulation of S2 ventral and dorsal roots together only eliminated 1-2 marbles in these two cats, stimulating the S2 ventral root alone without the dorsal root successfully eliminated all 4 marbles in both cats.

A. Cat #1



B. Cat #2

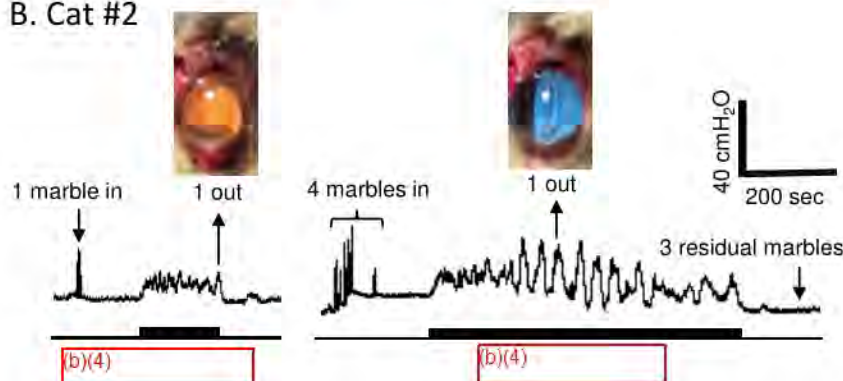


Fig.11. Defecation induced by stimulation of S2 ventral and dorsal roots. A. In cat #1 after inserting three marbles into the rectum, the defecation induced by sacral S2 root stimulation eliminated 2 marbles after (b)(4) of stimulation, respectively, with the last marble partially evacuated during the (b)(4). B. In cat #2 after inserting 1 marble into the rectum, it was defecated after (b)(4). After inserting 4 marbles, stimulation of 11 minutes produced defecation of 1 marble at 4.5 minutes with 3 residual marbles in the rectum. Colon contraction pressure was measured by a large condom catheter with the condom covering the proximal and distal colon.

Sub-Task 1.2.3 Restore sexual function (b)(4)

During this quarter and previous quarter, a total of 8 male cats were used to study the penile erection by stimulation of sacral roots or pudendal nerve. The animals were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (see Fig.12). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

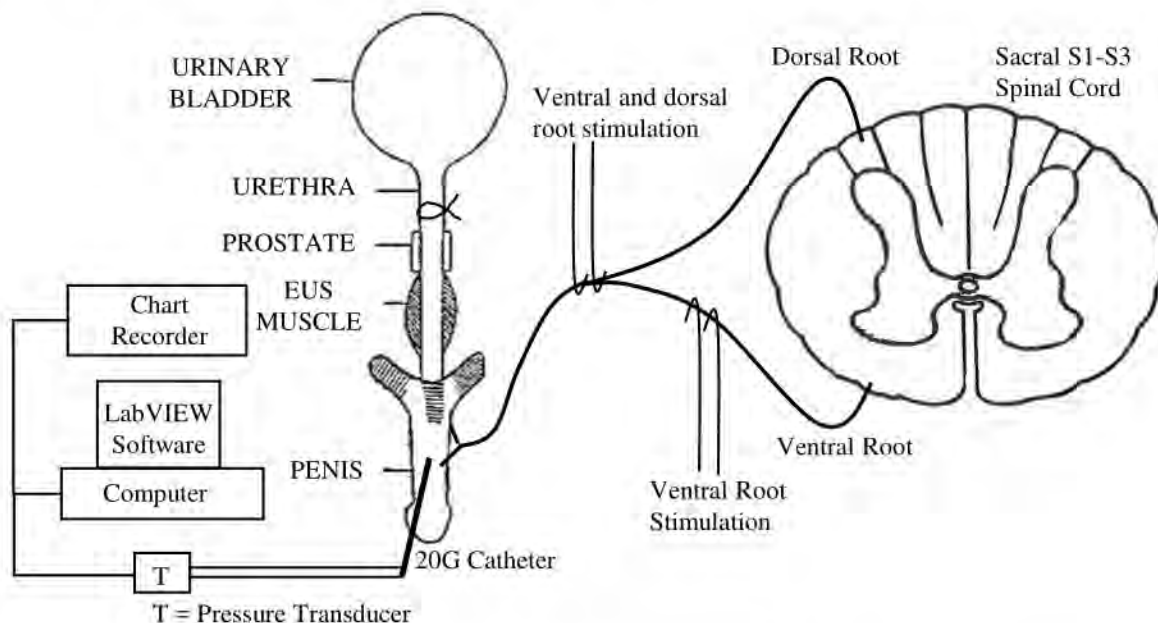


Fig.12. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

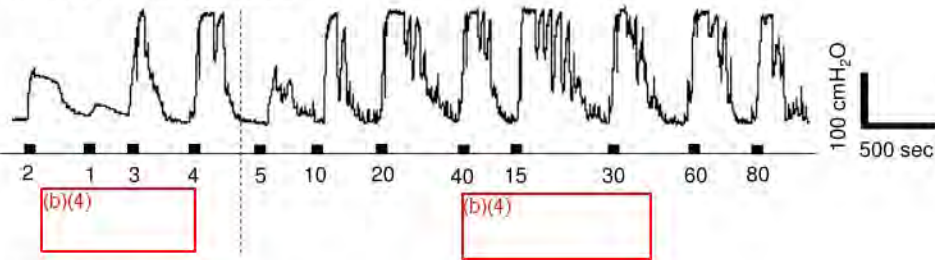
A 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (see Fig.12). Since electrically evoked penile erection is very well correlated with increased corpus cavernosum pressure, pressure recording from the corpus cavernosum was used as a quantitative measurement of penile erection in this study.

Based on the results obtained from the 8 male cats, penile erection could be induced by stimulation of S1/S2 root but stimulation of S3 root or pudendal root was not effective. Penile erection was observed as a full protrusion of the penis with rigidity in 6 cats by stimulation (b)(4) of S1 ventral root (S2 and S3 ineffective) and in 2 cats by stimulation of S2 ventral root (S1 and S3 ineffective). These responses which were quantified by penile pressure recordings showed that S1 or S2 ventral root stimulation in 6 cats and 2 cats, respectively, induced large increases in pressure (about 150 cmH₂O on average, Fig.13 A-C). The optimal stimulation frequency to induce penile erection by S1 or S2 ventral root stimulation was (b)(4) (Fig.13D), although lower frequencies (b)(4) Fig.13A) or higher frequencies (60-80 Hz, see Fig.13 A-B) could also induce large increases in penile pressure in some cats. The stimulation intensity (b)(4) (b)(4) ventral root stimulation to induce the largest increase in penile pressure was always above the motor threshold to induce leg muscle contraction (S1) or anal sphincter contraction (S2).

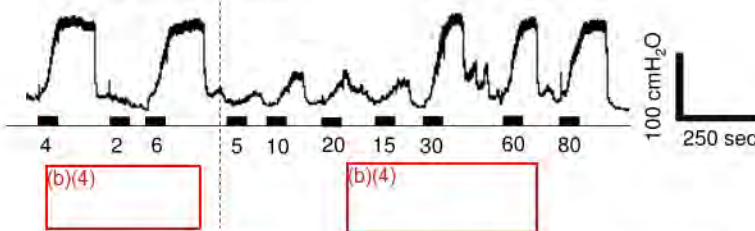
Continuous (b)(4) of the S1 or S2 spinal root (i.e., ventral and dorsal roots together) induced a large increase (>150 cmH₂O) in penile pressure (Fig.14). The maximal penile pressure was either sustained (Fig.14A) or gradually developed (Fig.14B) during the 10-minute stimulation. On average (N=8 cats), the penile pressure at the end of 10-minute stimulation was not significantly ($p>0.05$) different from the maximal pressure developed during the stimulation (Fig.14C). The maximal penile pressure induced by stimulation of ventral and dorsal root together was also not significantly ($p>0.05$) different from the maximal pressure induced by stimulation of ventral root alone (Fig.13D and Fig.14C).

After a complete spinal cord transection at the T9-T10 level, continuous 10-minute stimulation (b)(4) of the S1 or S2 spinal root (ventral and dorsal roots together) also induced a large increase (>100 cmH₂O) in penile pressure (Fig.15). As seen prior to spinal transection, the maximal penile pressure was also sustained or gradually developed (Fig.15B) during the 10-minute stimulation. However, on average (N=6 cats) the induced penile pressure was significantly ($p<0.05$) reduced to 113 ± 19 cmH₂O after spinal cord transection when compared to the penile response (186 ± 9 cmH₂O) before the spinal cord transection (Fig.15C). The blood pressure was also significantly ($p<0.05$) reduced from 229 ± 11 cmH₂O to 186 ± 7 cmH₂O by spinal cord transection, but it was not changed by sacral root stimulation.

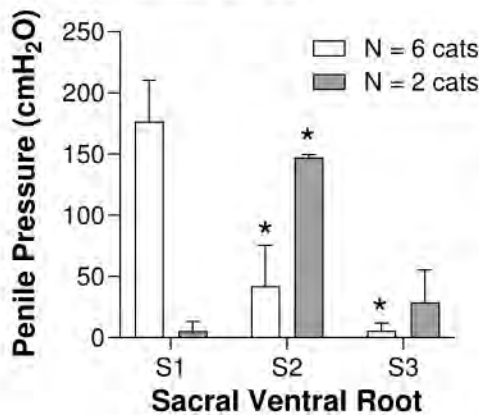
A. S1 Ventral Root



B. S2 Ventral Root



C. Segmental Effect



D. Frequency Effect

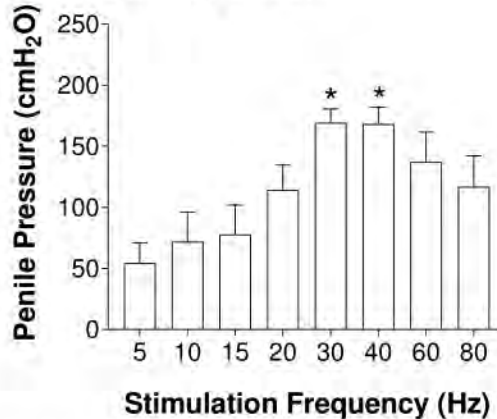
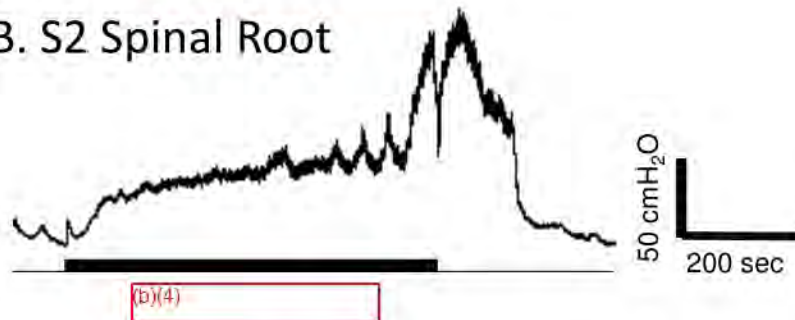


Fig.13: Penile pressure in the corpus cavernosum induced by electrical stimulation of sacral ventral roots at different frequencies and different spinal segments. A. In a cat, S1 ventral root stimulation (b)(4) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. B. In another cat, S2 ventral root stimulation (b)(4) of short duration (1 minute) was most effective in inducing large increases (150 cmH₂O) in penile pressure. C. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. * indicates significantly different from S1 ventral root ($p < 0.05$, ANOVA). Stimulation: (b)(4). D. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root. N = 8 cats. * indicates significantly different from (b)(4) ($p < 0.05$, ANOVA). Stimulation: (b)(4).

A. S1 Spinal Root



B. S2 Spinal Root



C. Sustained Pressure

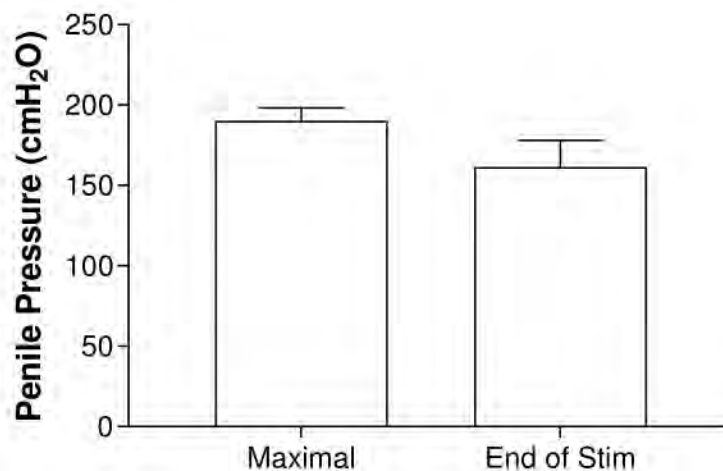
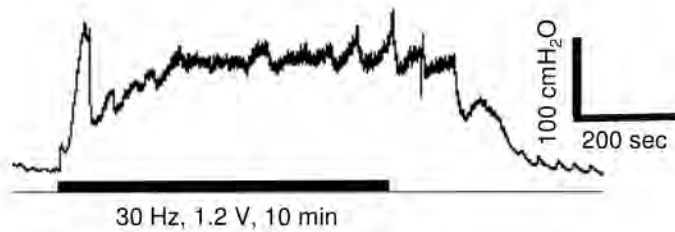
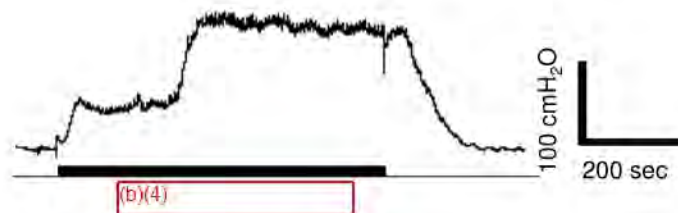


Fig.14: Penile pressure in the corpus cavernosum induced by prolonged (b)(4) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of (b)(4) stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

A. Before Spinal Transection



B. After Spinal Transection



C. Maximal Pressure

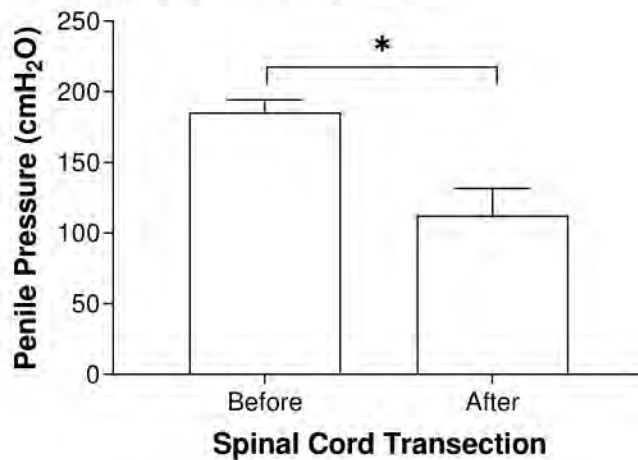


Fig.15: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the (b)(4) of stimulation. B. In the same cat, (b)(4) after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure during the first (b)(4) and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference (p=0.0025, paired t-test).

In addition to restore male sexual function, we also successfully restored female sexual functions in anesthetized female cats by pudendal nerve stimulation to increase the vaginal lubrication and produce vaginal contractions. The surgical procedure is similar to the male cats as described above, but the experimental protocol is different. At the beginning of the experiment, a small piece of dry cotton was weighted and inserted into the vagina for 10 minutes without any stimulation. After the (b)(4) the cotton was pulled out and weighted again to determine the increase in the cotton's weight that is used as the control value to indicate the vaginal wetness. Then, the same (b)(4) cotton test was repeated using another piece of dry cotton while PNS (b)(4) at the 2 times intensity threshold for inducing anal twitching was also applied intermittently (b)(4) during the (b)(4) to increase the vaginal wetness.

After testing the vaginal wetness, a small catheter (7 French) with a balloon at the tip was inserted into the vagina with the balloon slightly distended by 0.2 ml air to measure vaginal contraction. PNS (b)(4) was applied for (b)(4) each time at different intensities to determine the intensity response. Then, PNS at an intensity of 2 times the threshold (T) for inducing a small observable vaginal contraction was applied at different frequencies (b)(4) to determine the frequency response. Finally, PNS (b)(4) was applied for 4 minutes either continuously or intermittently (b)(4) in a random order to determine vaginal fatigue during the (b)(4) contraction. A time interval of (b)(4) minutes was inserted between the two (b)(4) PNS to avoid any carry-over fatigue effect.

PNS applied intermittently (b)(4) significantly increased vaginal wetness (Fig.16). Without PNS, the vaginal wetness as indicated by the increase in

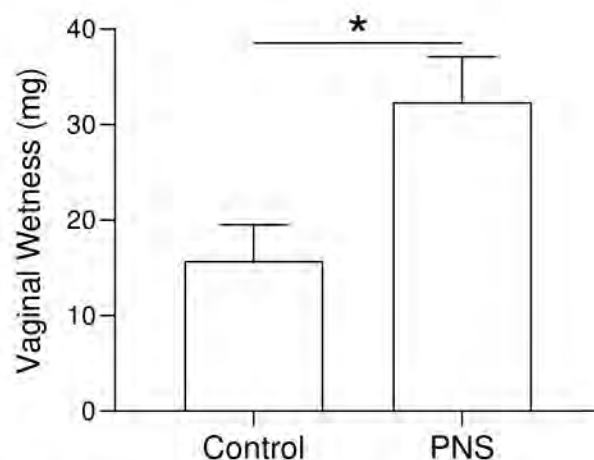


Fig.16. Pudendal nerve stimulation (PNS) increased vaginal wetness. PNS: (b)(4) (b)(4) T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p=0.0327$). N = 4 cats.

the vaginal cotton weight during a (b)(4) is 15.8 ± 3.8 mg (control in Fig.16). After (b)(4) the vaginal wetness was significantly ($p=0.0327$) increased to 32.4 ± 4.7 mg (PNS in Fig.16).

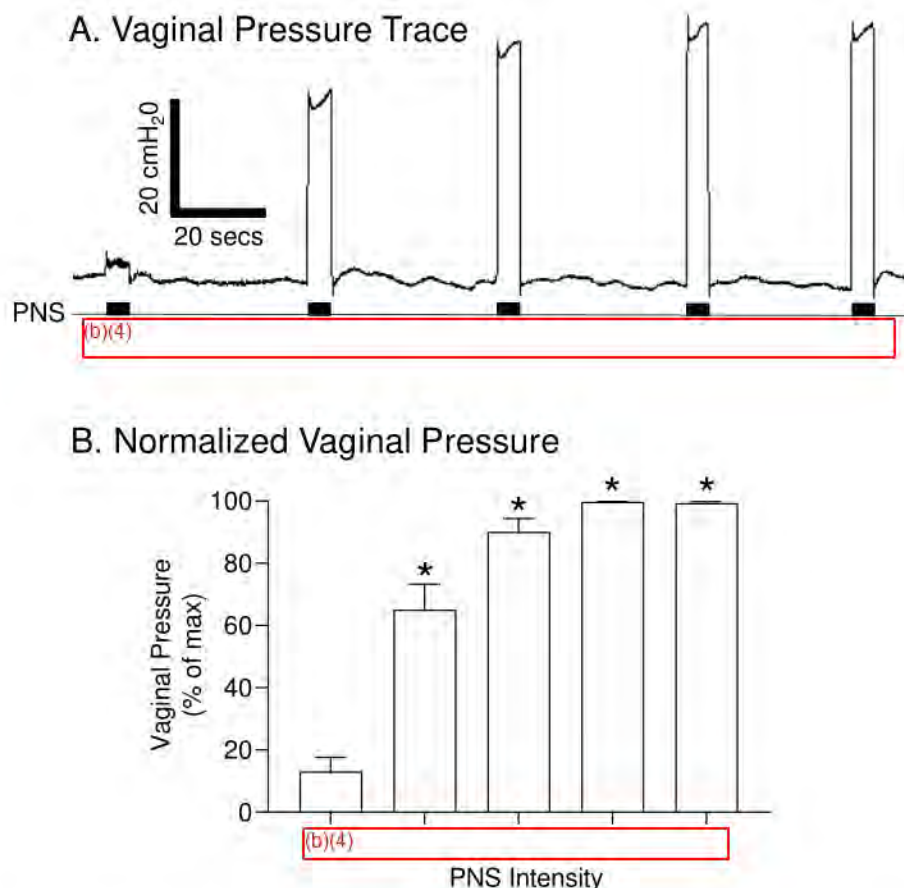
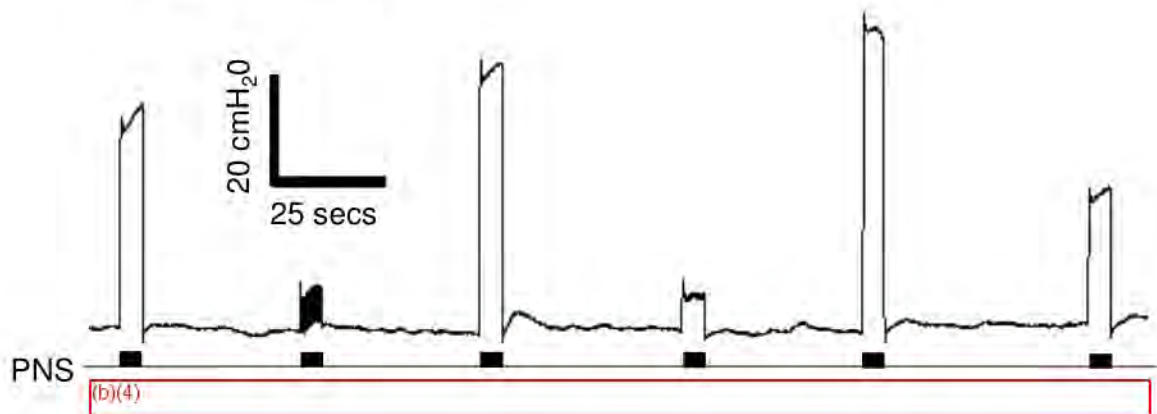


Fig.17. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation intensities. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: (b)(4) T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 30 Hz, (b)(4) * indicates significantly different from (b)(4) (one-way ANOVA). N =4 cats.

Vaginal contraction was significantly ($p<0.05$) increased as the PNS intensity increased (Fig.17). PNS (b)(4) at an intensity $\geq T$ induced vaginal contraction stronger than 90% of the maximal contraction (Fig.17). Meanwhile, at intensity of (b)(4) the PNS induced significantly ($p<0.05$) large vaginal contraction at frequencies (b)(4) (Fig.18). At frequencies (b)(4) PNS induced vaginal contraction larger than 80% of the maximal contraction (Fig.18). When PNS (b)(4) was applied for (b)(4) it produced less fatigue when applied intermittently than continuously (Fig.19). On average ($N=4$), intermittent PNS induced fatigue that is significantly ($p=0.0354$) smaller than continuous PNS Fig.19).

In summary, our studies in anesthetized male and female cats have shown successfully that sexual functions could be restore after SCI by stimulation of the sacral roots or pudendal nerve.

A. Vaginal Pressure Trace



B. Normalized Vaginal Pressure

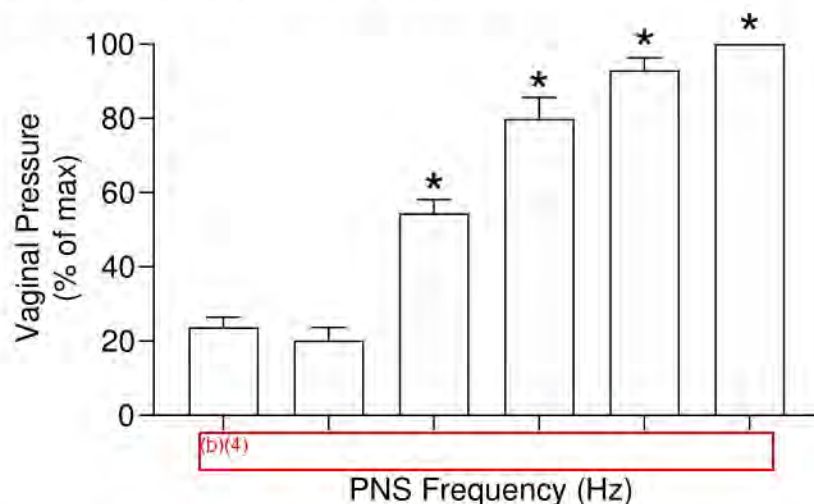


Fig.18. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation frequencies. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: (b)(4) T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: (b)(4) (b)(4) * indicates significantly different from (b)(4) (one-way ANOVA). N =4 cats.

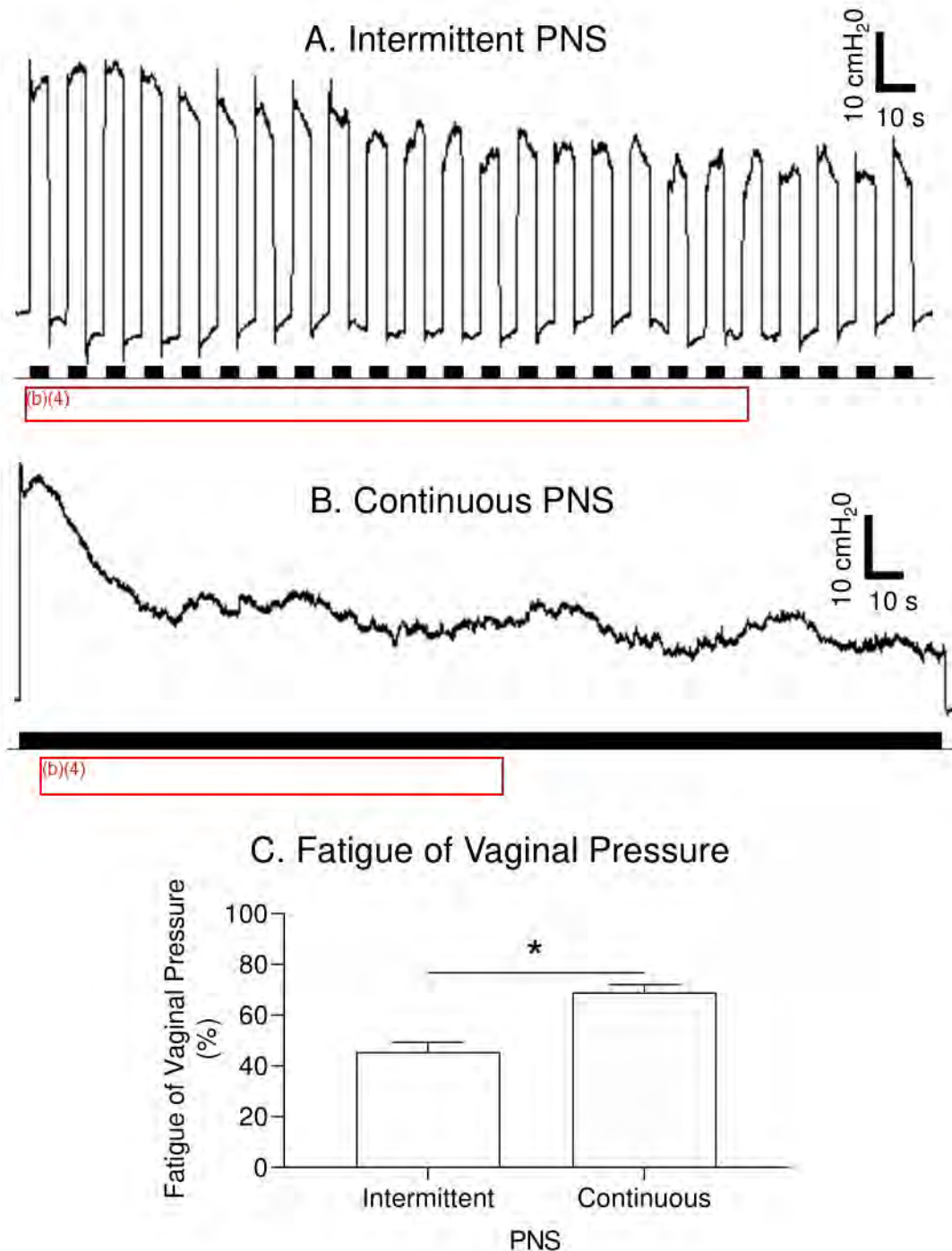


Fig.19. Fatigue of vaginal pressure increases induced by prolonged (4 minutes) pudendal nerve stimulation (PNS). A. Vaginal pressure responses induced by intermittent PNS. B. Vaginal pressure responses induced by continuous PNS. Black bars under the pressure trace indicate the PNS duration. C. Fatigue of vaginal pressure responses. The reduction in vaginal pressure responses at the end of 4-minutes PNS was normalized to the maximal response to indicate the muscle fatigue. PNS: (b)(4) (b)(4) T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, p = 0.0354). N = 4 cats.

Sub-Task 1.2.4 Measure fullness of bladder and bowel (b)(4)

Our studies in cats show that bladder and bowel fullness (b)(4)

(b)(4)

(b)(4)

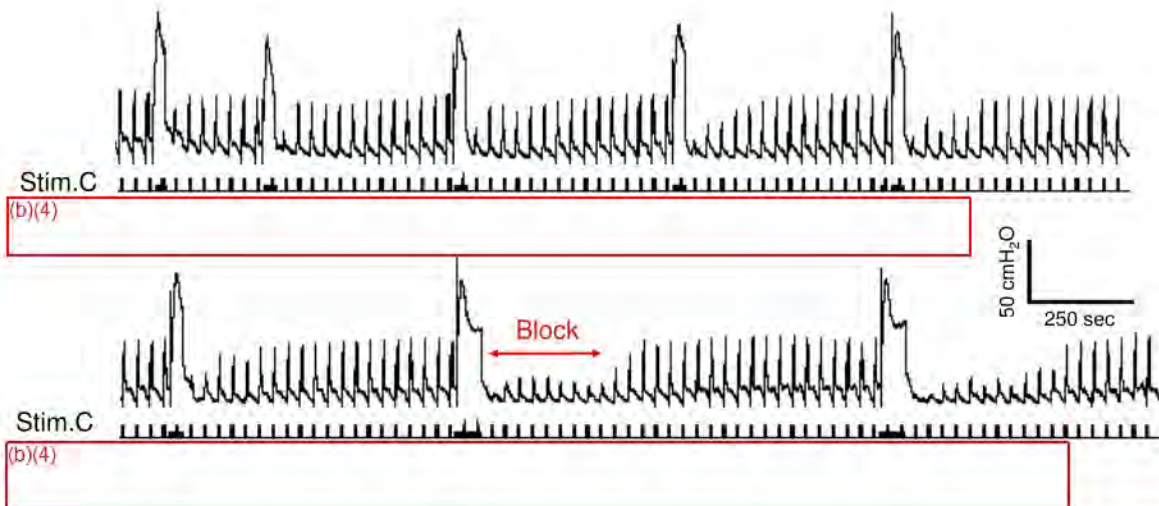
(b)(4)

Sub-Task 1.2.5 Test electrode and device prototypes (b)(4)

We have successfully tested the (b)(4) provided by (b)(4) to block pudendal nerve conduction in an anesthetized cat. Fig.22 shows the experimental setup and Fig.23 shows the results that the pudendal nerve can be successfully blocked using (b)(4)

(b)(4)

(b)(4)



(b)(4)

We also successfully tested the (b)(4) in 2 cats. The (b)(4) generated different types of stimulation signals to achieve the functions of inhibiting bladder, exciting bladder, or blocking the pudendal nerve. Fig. 24 shows that the (b)(4) inhibited bladder contractions by generating a (b)(4) stimulation of the pudendal nerve, excited the bladder by generating (b)(4) stimulation of the pudendal, and blocked the pudendal nerve conduction by generating (b)(4) stimulation waveform. This (b)(4) is now fully tested and documented for FDA IDE submission.

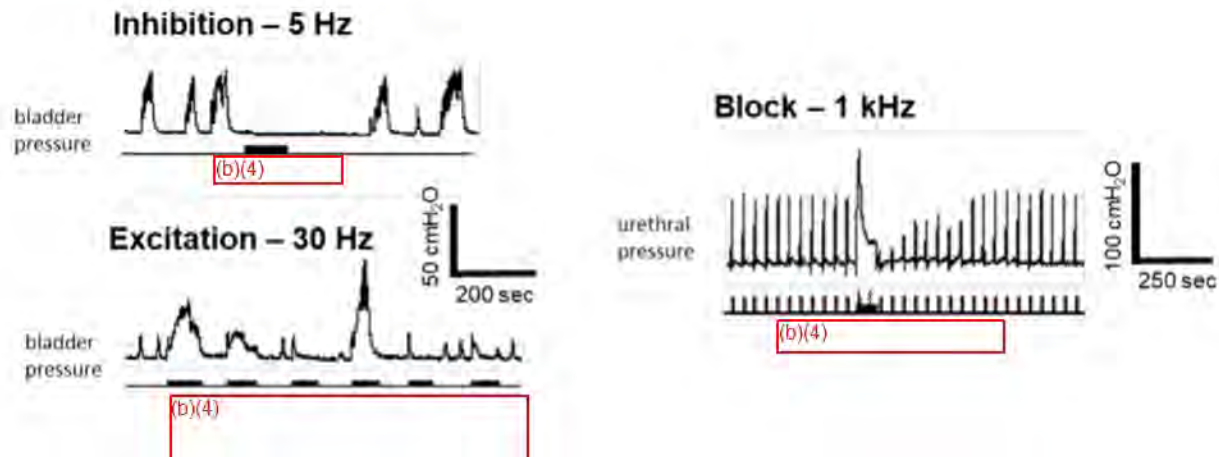


Figure 24. The (b)(4) successfully generated the required stimulation signals for bladder inhibition or excitation, and for pudendal nerve block.

Task 1.3 Test Bed Environment (b)(4)

Sub-Task 1.3.1 Complete initial PCB schematic design with design review (b)(4)

The development of the test bed environment is based on (b)(4). The original (b)(4) schematic had to be modified to achieve the desired design output requirements. Two test beds were chosen to be adapted to demonstrate specific attributes of the final (b)(4). The block diagram for the modified (b)(4) can be seen in Figure 25.

Sub-Task 1.3.2 Complete initial PCB board layout, fabrication/assembly and evaluation (b)(4)

The two adapted test beds were fabricated and assembled. These are referred to as Alpha and Beta prototypes, whereby the Alpha is a simple proof of technical feasibility. During evaluation, hardware and software limitations were identified. The Alpha was successfully modified to meet the design requirements subject to inherent hardware limitations. The second test bed is a more in-depth modification. The block diagram for visualizing the modified (b)(4) can be seen in Figure 25.

Sub-Task 1.3.3 PC test application with board connectivity (b)(4)

(b)(4) for graphical user interface of the Beta prototype is re-programmed to interface with the test bed system. (b)(4) Firmware has been updated and is functioning as desired.

Sub-Task 1.3.4 Integration of (b)(4) with firmware and (b)(4) connectivity (b)(4)

The Alpha prototype test bed was delivered and evaluated. The Alpha test bed was modified to include (b)(4) and easy (b)(4) (Figure 26). The Alpha test bed was successful in demonstrating a (b)(4)

(b)(4)

more the hardware is pushed past its limitations, which was anticipated. The Alpha test bed only has (b)(4) The knowledge gained from the Alpha pathway provided valuable inputs for the Beta prototype and ultimately the final (b)(4)

The Beta prototype is a modified (b)(4) test board configured for the (b)(4)

(b)(4)

The delivery of the Beta test bed was (b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.4 Implantable pulse generator with custom (b)(4)

(b)(4)

Sub-Task 1.4.1 Can/Header design with design review complete (b)(4)

The (b)(4) designs were optimized to meet the design requirements of (b)(4)

(b)(4)

Sub-Task 1.4.2 Can samples developed and fully evaluated (b)(4)

(b)(4)

1.4.3 Integrate pulse generator circuit into can (b)(4)

The (b)(4) (Figures 29-32). Currently, all components fit on the board and the last parts are being placed. Routing is the next step and then the boards will be sent off to be fabricated. (b)(4)

(b)(4)

(b)(4)

1.4.4 Final (b)(4) test application with wireless board connectivity (b)(4)

The (b)(4) application designed to control (b)(4) wirelessly for the IDE study is complete. The graphical user interface (GUI) controls the various stimulation parameters to (b)(4)

(b)(4) application required to wirelessly control (b)(4)
(b)(4) is also functioning as intended. The GUI currently utilizes (b)(4)
graphics provided (b)(4)
(b)(4) but both applications can control the (b)(4) with (b)(4)
(b)(4)

1.4.5 Full integration and test completed for initial prototype (b)(4)

The complete integration of the (b)(4) into an (b)(4) is currently underway. As mentioned before, (b)(4)

(b)(4) All parts (b)(4) are ordered. The (b)(4) PCB will get ordered (b)(4)
now that the (b)(4) schematic is complete. Now, the battery (b)(4)
(Figure 29) to permit (b)(4)

(b)(4) For the same reason, the (b)(4) were moved (b)(4)
(b)(4) (Figures 33-34). Fitting the battery within the
enclosure (b)(4)

The (b)(4) enclosure sample fit the (b)(4) perfectly (Figure 35).
(b)(4) (Figure 36).

(b)(4)

(b)(4)



(b)(4)

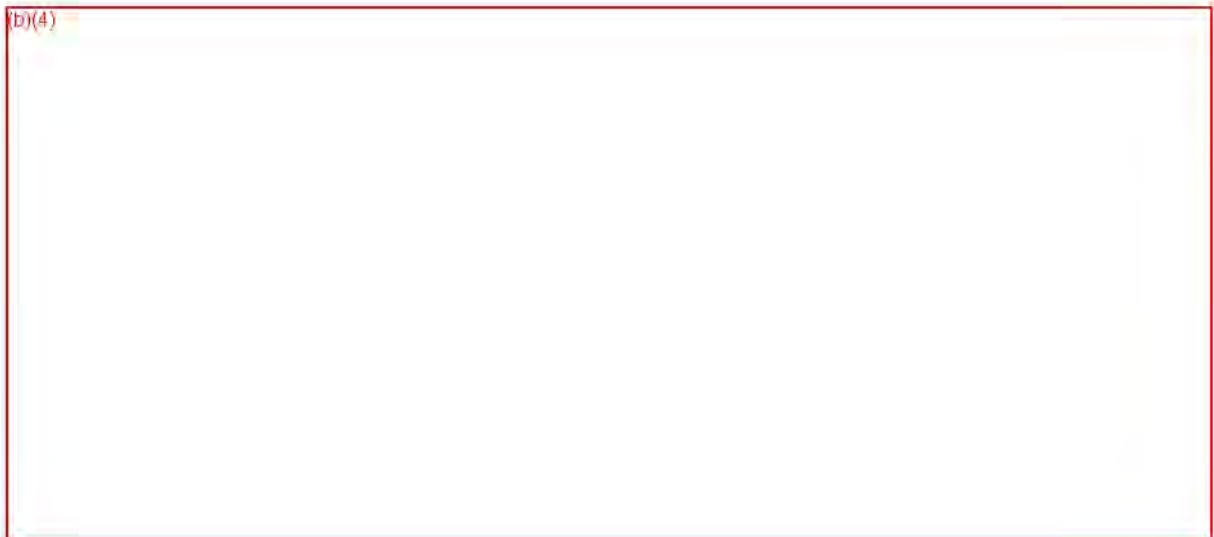
Task 1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)

Sub-Task 1.5.1 Finalize on the lead/cuff size and length (b)(4)



Sub-Task 1.5.2 Finalize design of cat lead/cuff (b)(4)

The cuff electrode design has been finalized to follow those specifications as determined in our previous cat studies. The (b)(4)



(b)(4)

Sub-Task 1.5.3 First prototypes of leads/cuffs delivered for test (b)(4)

The first SNS percutaneous lead prototypes was delivered early for evaluation. The desired effect was achieved using this prototype lead. Incremental leads were ordered. A cuff lead prototype to be used in animal studies (cats) has been conceived and a prototype was delivered.

Sub-Task 1.5.4 Finalize design and lead/cuff electrode production (b)(4)

A (b)(4) was purchased to bring the (b)(4) in house. The (b)(4) machine was delivered during (b)(4). It passed in-house validation process and quality standards. A protocol was developed for operation. Designs have been approved and released for the (b)(4). The final (b)(4) product will need to meet quality and performance standards and testing.

Task 1.6 External pulse generator for (b)(4)

Sub-Task 1.6.1 Industrial design and CAD drawings for the (b)(4)

A general (b)(4) was designed to be worn by a person with SCI for a (b)(4).
(b)(4)

Sub-Task 1.6.2 Integration of the (b)(4) with firmware and the

(b)(4)

The Boards have been received, fully populated, after (b)(4).

(b)(4)

(b)(4) Once received, firmware was loaded on the Boards and bring up was started immediately (Figure 44).

(b)(4)

Sub-Task 1.6.3 System verification completed (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.6.4 Documentation supporting the IDE submission (b)(4)

This task is complete. All documentation supporting the IDE was submitted. Please see the submitted IDE documents for the details.

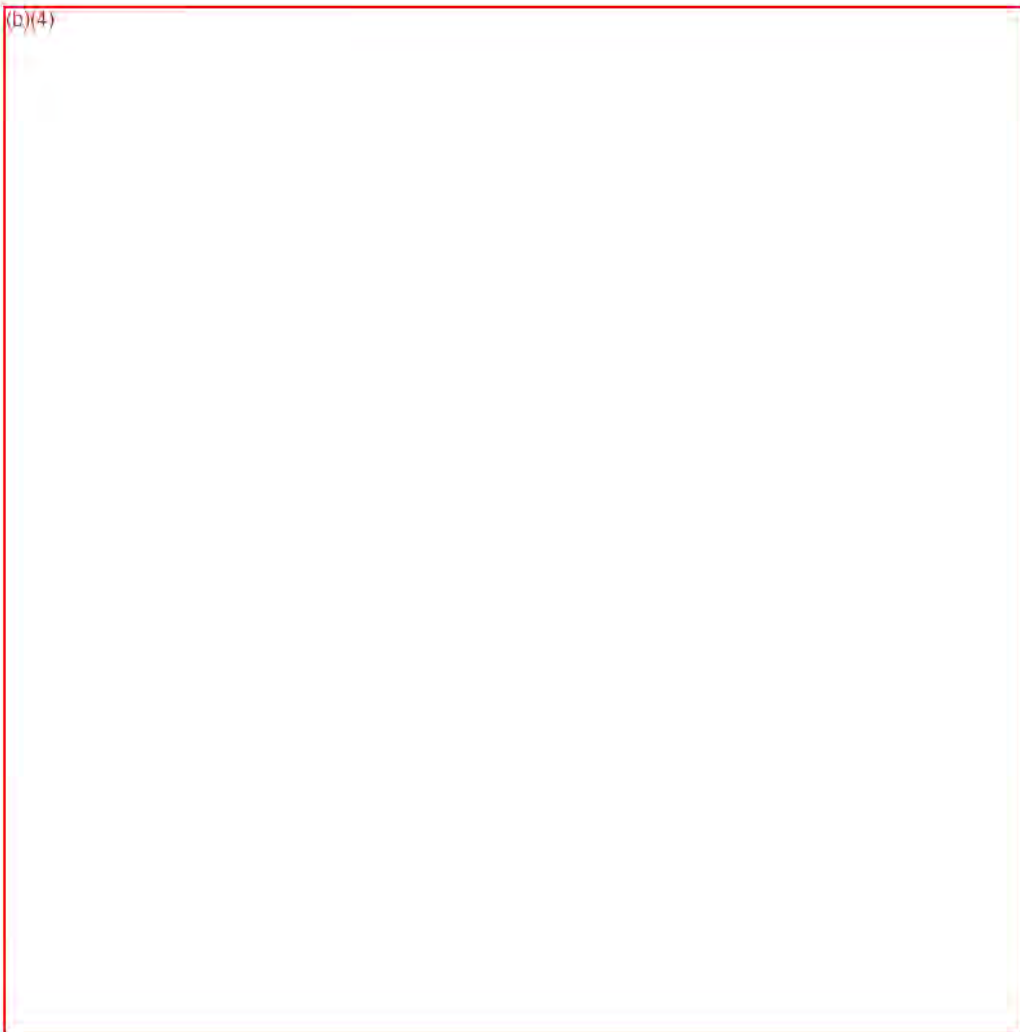
(b)(4)

(b)(4) The process starts with a signed contract between Thermaquil and Med-Ally. Med-Ally is Thermaquil's main subcontractor responsible for the assembly process and supplier management. Med-Ally is certified to ISO13485:2016. Med-Ally orders components through Med-Ally's supplier management procedures. Components are properly identified and documented during assembly in the device history files. Med-Ally assembles the (b)(4)

(b)(4) (Table 1). The assembly and tests are performed to Med-Ally work instructions and documented in the device history record forms.

(b)(4)

Final device assembly, acceptance and packaging, which includes application of product labels, occurs at (b)(4) (Table 3, Figures 47,48). Final product acceptance is recorded on device history record forms.



(b)(4)

(b)(4)

(b)(4)

Sub-Task 1.6.5 Build sets of (b)(4) to conduct GLP safety study in pigs in (b)(4)

(b)(4)

(b)(4)

built for the human IDE study coming up in (b)(4) (Figures 49-57).

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.7 (b)(4) electrodes for human use (b)(4)

Sub-Task 1.7.1 Finalize on the (b)(4)

The various (b)(4) configurations have been identified for the (b)(4)

(b)(4)



Sub-Task 1.7.2 Finalize design of (b)(4)



(b)(4)

(b)(4)

Sub-Task 1.7.3 First prototypes of (b)(4) delivered for test (b)(4)

(b)(4)

(b)(4) We have made and tested hundreds of (b)(4) The very specialized process of

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Sub-Task 1.7.4 Finalize design and (b)(4) production (b)(4)

(b)(4)

Sub-Task 1.7.5 Biocompatibility test is completed. Documentation for FDA submission

(b)(4)

Biocompatibility quotes from three test houses for the leads were received. The biocompatibility discussions with the test houses are ongoing while we prepare the materials and assemblies to be tested. (b)(4)

(b)(4)

(b)(4)

Task 1.8 Physician/Investigator controller (b)(4)

(b)(4)

Sub-Task 1.8.2 Evaluation board fabricated/assembled and initial evaluation complete

(b)(4)

The (b)(4) GUI for the (b)(4) IDE study is complete (Figures 71,72). The

(b)(4)

(b)(4)

Sub-Task 1.8.3 Integration with (b)(4) and firmware (b)(4)

The firmware for the (b)(4) IDE study was created for the researcher, clinician and patient (Figure 73). (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.8.4 Final documentation to support the FDA IDE as an accessory device (b)(4)

All documentation required for the IDE was submitted. Please refer to submitted documents for details. (b)(4)

(b)(4)

have a lot of the pertinent information.

Task 1.9 Patient controller (b)(4)

(b)(4)

Sub-Task 1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)

(b)(4)

Sub-Task 1.9.2 Verification testing completed (b)(4)

Verification testing continues as well as electrical safety and efficacy. While the systems are currently being used in an animal lab setting, we are exhausting every test in anticipation of upcoming clinical human work.

(b)(4)

firmware has been verified. (b)(4)

(b)(4)

Task 1.10 Software Development Kit (SDK) (b)(4)

Sub-Task 1.10.1 Requirements understood for SDK (b)(4)

The SDK will be developed as part of the firmware development for the (b)(4). The requirements for the SDK are in the process of being established early.

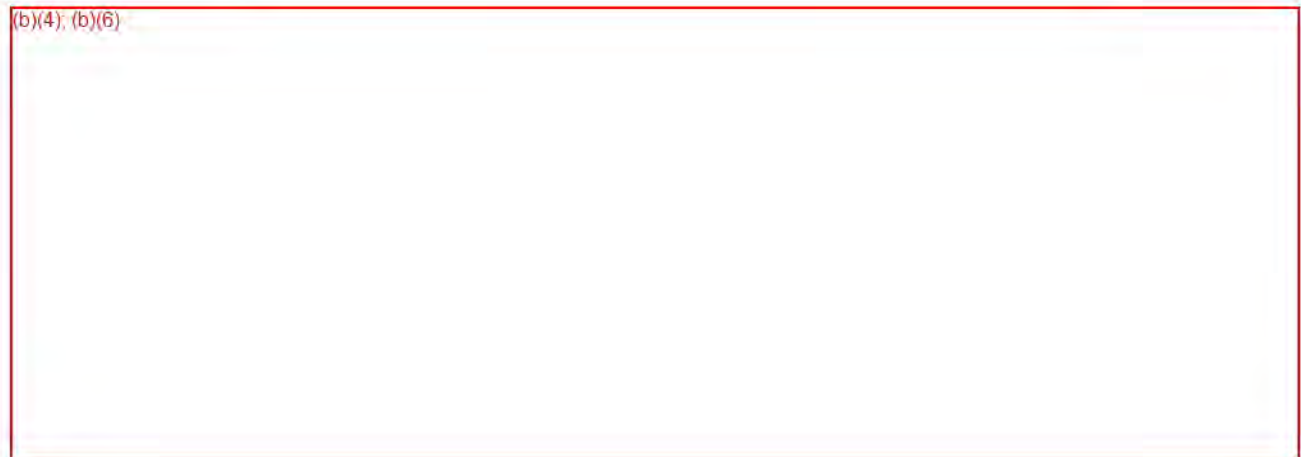
Sub-Task 1.10.2 At the completion of the development, the SDK is complete with the documentation on how to access the devices via the software API (b)(4)

The SDK and API are in the process of being developed. A detailed instruction for use (IFU) was written to document how to use the software application prepared for the IDE study (b)(4)

(b)(4)

Task 1.11 GLP animal study (b)(4)

(b)(4)



(b)(4), (b)(6)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.14 FDA IDE (b)(4)

- Task 1.14.1 FDA IDE (b)(4)
- Task 1.14.2 FDA IDE (b)(4)

Thermaquil submitted FDA IDE (b)(4) and requested an information meeting with the FDA for the (b)(4) The Informational Meeting with the FDA is scheduled for

(b)(4)

(b)(4) Fig.80 shows a part of the table of content for this submitted (b)(4) Fig.81 shows the FDA email confirming the (b)(4) and Fig.82 shows (b)(4)

(b)(4)

(b)(4)

(b)(4), (b)(6)

Task 1.15 FDA IDE (b)(4)

- Task 1.15.1 FDA IDE (b)(4)

Thermaquil has drafted and submitted FDA IDE (b)(4) which focuses on the (b)(4) (Fig.83). We have scheduled a meeting with FDA for (b)(4) (Fig.84, Fig.85).

(b)(4); (b)(6)



Task 1.16 FDA IDE Submission (b)(4)

Task 1.16.1 Prepare and submit FDA IDE application for (b)(4)
(b)(4)

Thermaquil has fully drafted and submitted our IDE submission (Fig 86). FDA confirmed that the IDE was received on (b)(4) (Fig.87). In response to FDA's feedback, we are included a detailed justification of (b)(4)
(b)(4). We responded to (b)(4) (Fig. 89-99). FDA

Thermaquil

**Nerve Block and Stimulation External
Pulse Generator (NBS-EPG)
Early Feasibility Study**

Original Investigation Device Exemption

(b)(4)

Sponsor	Thermaquil, Inc. 3401 Market St. Suite 200 Philadelphia, PA 19104
Primary Correspondent	(b)(4), (b)(6) CEO, Co-Founder Thermaquil, Inc. Phone: (b)(4), (b)(6) Email: (b)(4), (b)(6)
Secondary Correspondent	(b)(4), (b)(6) Senior Consultant, Medical Devices Biologics Consulting Group, Inc. 1555 King Street, Suite 300 Alexandria, VA 22314 Phone: (b)(4), (b)(6) Email: (b)(4), (b)(6)

This submission contains CONFIDENTIAL material and information and should be restricted in its distribution. Do not copy without the permission of the Submitter.

Fig.86. First page of submitted IDE.



Acknowledgment Letter

(b)(4)

(b)(4); (b)(6)

Thermaquil, Inc.
3401 Market St.
Suite 200
Philadelphia, PA 19104
UNITED STATES

Dear (b)(4); (b)(6)

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has received your submission. This submission has been assigned the unique document control number below. All future correspondence regarding this submission should be identified prominently with the number assigned and should be submitted to the Document Control Center at the address listed below. Failure to do so may result in processing delays. If you believe the information identified below is incorrect, please contact the Office of Product Evaluation and Quality (OPEQ) submission support at (301) 796-5640 or OPEQSubmissionSupport@fda.hhs.gov.

Submission Number: (b)(4)

Received: (b)(4)

Applicant: Thermaquil, Inc.

Device: Nerve Block and Stimulation External Pulse Generator (NBS-EPG)

We will notify you when the review of this document has been completed or if any additional information is required. If you are submitting new information about a submission for which we have already made a final decision, please note that your submission will not be re-opened. For information about CDRH review regulations and policies, please refer to <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm>.

U.S. Food & Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
www.fda.gov

Fig.87. FDA letter acknowledging that the IDE was received (b)(4)

(b)(4); (b)(6)

From: **Widstrom, Matthew** <Matthew.Widstrom@fda.hhs.gov>
Date: Thu, Feb 24, 2022 at 3:08 PM
Subject: G220048 - Interactive Review Expectations, Interactive request #1
To: Stephen Popielarski <steve@thermanujil.com>

Dear Stephen,

We received your IDE submission G220048 for the Nerve Block and Stimulation External Pulse Generator (NBS-EPG). As we proceed with our review, we may send you questions to address interactively. Because the limited statutory review time for an IDE (30 days), it is critical that you respond to any interactive requests as soon as possible and no later than the FDA requested due date. Please be aware that FDA may request information within a short turn-around time to ensure we have the information needed to make a final decision. Accordingly, please ensure the necessary resources are available on your end to ensure timely responses to our questions, including an alternate contact if we are unable to reach you.

Thank you in advance for your cooperation.

Please find our first interactive deficiency below:

In Section 6.5 of the IDE, you state that the safety of the software programs used in the NBS-EPG device and controller PC used for programming the stimulation settings are documented in test results and risk analyses. For the test results, you cite system-level functional testing performed to verify that the stimulation settings can be programmed (via the wireless link) and that the pulse generator delivers the intended stimulation waveform. However, the provided documents contain only the test procedure (Appendix 10-01) and a blank test form (Appendix 10-02). No test results are provided. Please provide the results of this functional testing. This information is needed to verify that the software used in the investigational device performs as intended, and will not deliver unintended stimulation that could result in patient harm.

Please provide a response by **COB 2/25/22**, if possible. We look forward to hearing from you.

Best Regards,

Matthew Widstrom, Ph.D.
General Engineer, Incontinence and Female Urological Devices Team

DHT3B: Division of Reproductive, Gynecology, and Urology Devices
OHT3: Office of Gastrorenal, ObGyn, General Hospital and Urology Devices
Office of Product Evaluation and Quality

From: **Widstrom, Matthew** <Matthew.Widstrom@fda.hhs.gov>
Date: Mon, Feb 28, 2022 at 2:02 PM
Subject: G220048 - Interactive request #2
To: Stephen Popielarski <steve@thermanujil.com>

Hello Steve,

Please address our interactive question below:

Please consider aspects of IEC 60601-2-10, safety and essential performance of nerve and muscle stimulators, in your analysis of what is relevant from a safety perspective for this IDE. Please determine if your electrical safety test procedure, WI 11-52, needs to be updated (based on consideration of 2-10), and please provide the test results or identify where they are in your submission.

Please provide a response by **COB 3/2/22**, if possible. We look forward to hearing from you.

Regards,

Matthew Widstrom, Ph.D.
General Engineer, Incontinence and Female Urological Devices Team

DHT3B: Division of Reproductive, Gynecology, and Urology Devices
OHT3: Office of Gastrorenal, ObGyn, General Hospital and Urology Devices
Office of Product Evaluation and Quality

CDRH | Food and Drug Administration
White Oak, Bldg. 66, Rm. 2620A | 10903 New Hampshire Avenue | Silver Spring, MD 20993
Phone: (301) 837-7139

matthew.widstrom@fda.hhs.gov


Excellent customer service is important to us. Please take a moment to provide feedback regarding the customer service you have received:
<https://www.research.natls.io/hc/customer-service/?ID=15248&S=E>

Fig.89. First two interactive requests made by FDA

From: Wiestrom, Matthew <Matthew.Wiestrom@hhs.gov>
Date: Tue, Mar 8, 2022 at 6:00 PM
Subject: G220348 - Interactive request #3
To: Stephen Popewinski <step@broadcom.com>

Hello Steve:
Please address our interactive questions below.

1) You state the following Exclusion Criterion in Doc 002 on page 5476:
"History of autonomic dysreflexia (symptoms include headache, flushing, and sweating)."
However, while the exclusion is appropriate it is not sufficient to protect this subpopulation of subjects (will complete SCI). Since some of these patients may experience autonomic dysreflexia even without prior history this issue could still arise. This is important because autonomic dysreflexia is a serious and even life-threatening condition. Therefore, please provide what mitigations you will put in place as part of your proposed protocol to address this significant subject safety concern should it arise. Alternatively, if it is already addressed, please provide where it can be found in your protocol for FDA review.
This EFS study is an in-office study that is conducted under physician supervision. If autonomic dysreflexia should occur during the study, the study will be stopped. This will be added to the protocol. This topic will also be considered for mitigation for future studies that are not as closely controlled as this proposed EFS.

2) You provided the following Exclusion Criterion in Doc 002 on page 5576 apparently to address our concern of vulnerable populations of subjects:
"Subjects who are cognitively challenged."
However, this does not adequately address our concern of including vulnerable subjects since there are other subpopulations to whom this concern may apply such as incarcerated individuals or those in whom there may be a language barrier (which could be spoken, written or both). This is important since such subjects may be unduly induced to participate in a clinical study either against their will or because of lack of understanding of the risks and/or benefits involved, adversely impacting subject rights. In such cases the ethical integrity of the study may be compromised. Therefore, please use terminology which more generally excludes vulnerable populations of subjects.
We will add an exclusion criteria that specifies that vulnerable (subject populations) (incarcerated, cognitively challenged), non-native English language skills, pediatric are excluded from participation.
3) You provided in Vol 001, Doc 027 your IFU (Instruction for Use). However, you do not appear to have provided a section for "Precautions." The Precautions Section includes information regarding any special care to be exercised by the practitioner and/or patient for the safe and effective use of the device. This is important even at this early stage of device development to ensure the safe and effective use of the investigational device. Therefore, please revise your IFU to include a section on Precautions (even if the section only states there are no special precautions needed).
Update with updates and state precautions.
Here is the requested information relating to (b)(4) & (b)(6):
Precautions are on pages 3-5. Many of these will not be applicable to your device at this point.
https://www.accessdata.fda.gov/cdrh_docs/research_docs/SP770004EDMAC.pdf

4) You state the following in Vol 001, Doc 003 Cadaver Report on page 407:
"Foramen needles were used to deploy leads near the pudendal nerves bilaterally. Two leads were placed on one side and the third lead was placed on the contralateral side."
However, it is not clear why two foramen needles are placed on one side and whether you intend this procedure to be used in living subjects. This is important to the Agency's understanding of the proposed procedure in which implant leads will be placed in living subjects and any safety implications that may arise therefrom. Therefore, please clarify this portion of the procedure that was performed as part of your cadaveric study.

Please provide a response by **CDB 3/9/22**, if possible. We look forward to hearing from you.
Regards,
Matthew Wiestrom, Ph.D.
General Engineer, Incontinence and Female Urological Devices Team

Fig.90. Interactive request #3





(b)(4), (b)(6)

(b)(4), (b)(6)

On Tue, Mar 15, 2022 at 2:09 PM Widstrom, Matthew <Matthew.Widstrom@fda.hhs.gov> wrote:

Hello Stephen,

Can you please fill out the maximum simulation parameters table below, so that I have all of the information clearly in one place?

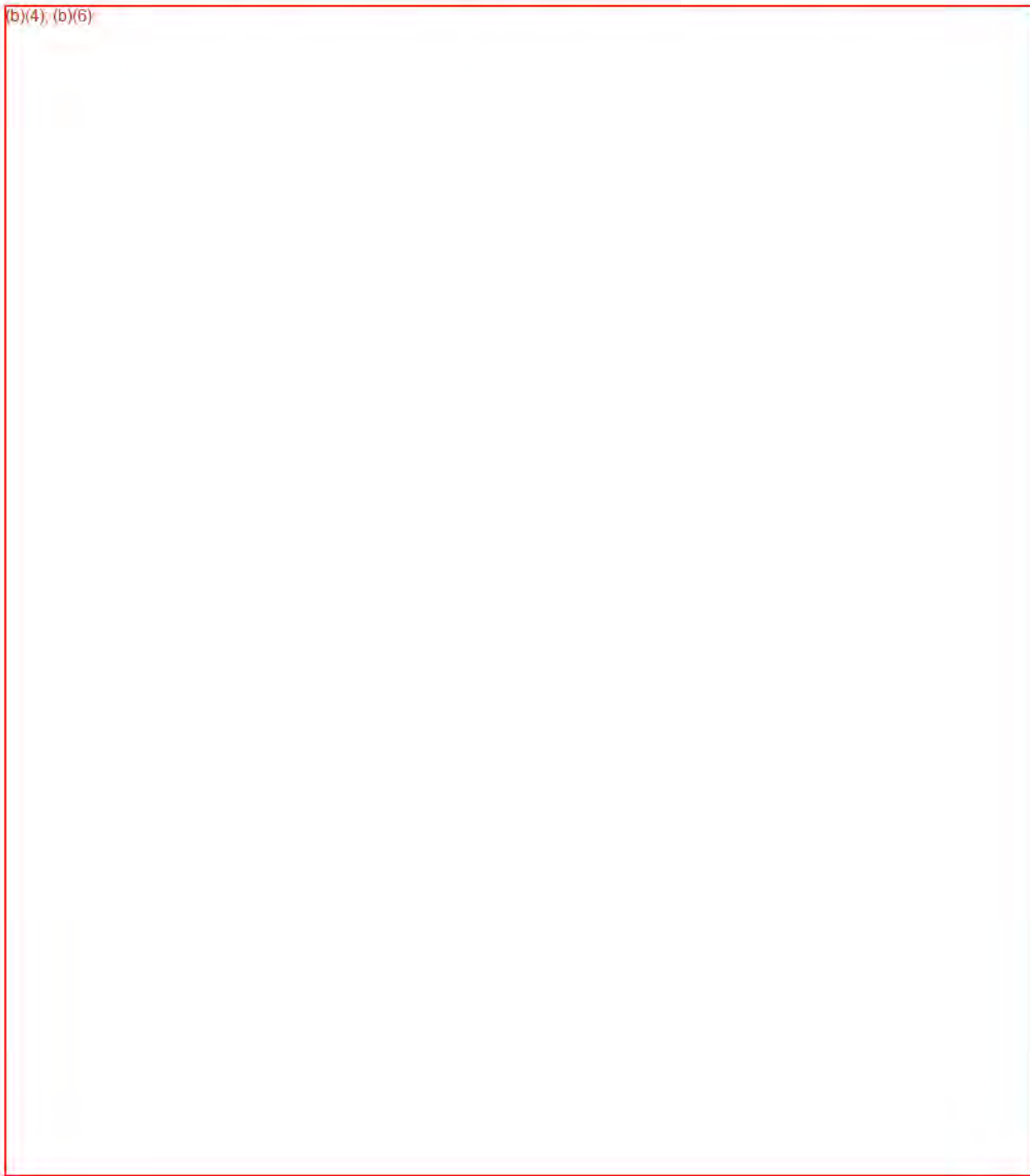
Signal Usage	Frequency, Hz	Amplitude, mA	Pulse width, μ s	Charge/phase, μ C	Charge density, μ C/cm ²	Current density, mA/cm ²
Nerve Finding	5					
Bladder Stimulation	20 to 30					
Nerve Block	1,000					
Nerve Block	10,000					

Please provide a response by **COB 3/15/22**, if possible. Thank you.

Fig.95. Interactive request #8

(b)(4); (b)(6)

(b)(4), (b)(6)



(b)(4); (b)(6)

(b)(4); (b)(6)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Weekly meetings with the team members from University of Pittsburgh, (b)(4) and Thermaquil were hold regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4) is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermaquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Peer-Reviewed Journal Papers

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"

Experimental Neurology, 2021; 346:113860.

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.
"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".

American Journal of Physiology - Gastrointestinal and Liver Physiology, 2021; 321: G735-G742

Zhijun Shen, Jicheng Wang, Bing Shen, Jianan Jian, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

"Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats"

Neuromodulation, 2022. Submitted for review and publication.

Jialiang Chen, Yihua Zhong, Jicheng Wang, Bing Shen, Zhijun Shen, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats"

Journal of Sexual Medicine, 2022. Submitted for review and publication.

Meeting Abstracts

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
(b)(4)	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

BG+ (b)(4) Report, University of Pittsburgh, Period Report Covers: (b)(4)

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Urologist	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4), (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	



Milestones and Deliverables Summary

University of Pittsburgh – (b)(4) Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.1 Institutional IACUC approval and DOD ACURO review (b)(4)	(b)(4)						(b)(4)
1.1.1 IACUC approval	(b)(4)					Milestone 1.1.1 – Deliverable 1.1.1	
1.1.2 DOD ACURO approval	(b)(4)					Milestone 1.1.2 – Deliverable 1.1.2	
1.2 Animal studies to develop the external system and the implantable system (b)(4)	(b)(4)						
1.2.1 Restore bladder function (b)(4)	(b)(4)					Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.2.2 Restore bowel function (b)(4)	(b)(4)					Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.2.3 Restore sexual function (b)(4)	(b)(4)					Milestone 1.2.3(1-4) – Deliverable 1.2.3	
1.2.4 Measure fullness of bladder and bowel (b)(4)	(b)(4)						
1.2.5 Test electrode and device prototypes (b)(4)	(b)(4)					Milestone 1.2.5	
1.3 Test Bed Environment (b)(4)	(b)(4)						
1.3.1 Complete initial PCB schematic design with design review (b)(4)	(b)(4)					Milestone 1.3.1 – Deliverable 1.3	
1.3.2 Complete initial PCB board layout, fabrication/assembly and evaluation (b)(4)	(b)(4)					Milestone 1.3.2 - Deliverable 1.3	
1.3.3 (b)(4) test application with board connectivity (b)(4)	(b)(4)					Milestone 1.3.3 - Deliverable 1.3	
1.3.4 Integration of EPG in enclosure with firmware and PC application connectivity (b)(4)	(b)(4)					Milestone 1.3.4 - Deliverable 1.3	
1.4 Implantable pulse generator with (b)(4)	(b)(4)						
1.4.1 Can/Header design with design review complete (b)(4)	(b)(4)					Milestone 1.4.1 – Deliverable 1.4	

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Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
Can samples developed and fully evaluated (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.4.2 – Deliverable 1.4	(b)(4)
(b)(4)							
1.4.3 Integrate pulse generator circuit into can (b)(4)						Milestone 1.4.3 – Deliverable 1.4	
Final PC test application with 1.4.4 wireless board connectivity (b)(4)						Milestone 1.4.4 – Deliverable 1.4	
Full integration and test 1.4.5 completed for initial prototype (b)(4)						Milestone 1.4.5 – Deliverable 1.4	
1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)							
1.5.1 Finalized on lead/cuff size and length (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.5.1 – Deliverable 1.5	
1.5.2 Finalize design of cat lead/cuff (b)(4)						Milestone 1.5.2 – Deliverable 1.5	
1.5.3 First prototypes of leads/cuffs delivered for test (b)(4)						Milestone 1.5.3 – Deliverable 1.5	
1.5.4 Finalize design and lead/cuff electrode production (b)(4)						Milestone 1.5.4 – Deliverable 1.5	
1.6 External pulse generator for human study (b)(4)							
Industrial design and CAD 1.6.1 drawings for the custom enclosure (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
Integration of the (b)(4) (b)(4) components with 1.6.2 firmware and the enclosure (b)(4)							
System verification completed 1.6.3 (b)(4)							
Documentation Supporting the 1.6.4 IDE submission (b)(4)							
Build 8 sets of (b)(4) to 1.6.5 conduct GLP safety study in 4 pigs in Phase 1. (b)(4)							



Milestones and Deliverables Summary

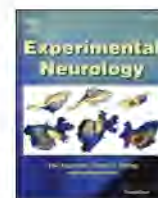
SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.7 electrodes for human use	(b)(4)	(b)(4)					(b)(4)
1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.1 – Deliverable 1.7	
1.7.2 Finalize design of human (b)(4) (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.2 – Deliverable 1.7	
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.3 – Deliverable 1.7	
1.7.4 Finalize design and (b)(4) production (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.4 – Deliverable 1.7	
1.7.5 Biocompatibility test is completed. Documentations for FDA submission (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.5 – Deliverable 1.7	
1.8 Physician/Investigator controller for	(b)(4)	(b)(4)					
1.8.1 Schematic design complete (b)(4) (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1 – Deliverable 1.8	
1.8.2 Evaluation board fabricated/assembled and initial evaluation complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.2 – Deliverable 1.8	
1.8.3 Integration with a simple enclosure with display and firmware (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.3 – Deliverable 1.8	
1.8.4 Final documentation to support the FDA IDE as an accessory device (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.4 – Deliverable 1.8	
1.9 Patient controller for	(b)(4)	(b)(4)					
1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)	(b)(4)	(b)(4)				Milestone 1.9.1 – Deliverable 1.9	
1.9.2 Verification testing completed (b)(4)	(b)(4)	(b)(4)				Milestone 1.9.2 – Deliverable 1.9	
1.10 Software Development Kit (SDK)	(b)(4)	(b)(4)					
1.10.1 Requirements understood for 1 SDK (b)(4)	(b)(4)	(b)(4)				Milestone 1.10.1 – Deliverable 1.10	
1.10.2 The SDK is complete with the 2 documentation (b)(4)	(b)(4)	(b)(4)				Milestone 1.10.2 – Deliverable 1.10	

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Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.11 GLP animal study (b)(4)							(b)(4)
1.11 GLP animal study	(b)(4)					Milestone 1.11 – Deliverable 1.11	
1.12 (b)(4)							
1.12 (b)(4)						Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)					Milestone 1.13 – Deliverable 1.13	
1.14 FDA IDE pre-submission #1 (b)(4)							
1.14. FDA IDE (b)(4) #1 for 1 the (b)(4)	(b)(4)					Milestone 1.14.1 – Deliverable 1.14.1	
1.14. FDA IDE (b)(4) 2 meeting						Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)							
1.15. FDA IDE (b)(4) for 1 the (b)(4)	(b)(4)					Milestone 1.15.1 – Deliverable 1.15.1	
1.15. FDA IDE (b)(4) 2 meeting						Milestone 15.2.1 – Deliverable 15.1.2	
1.16 FDA IDE Submission (b)(4)							
1.16 FDA IDE application for the (b)(4) 1 (b)(4) submitted	(b)(4)					Milestone 1.16 – Deliverable 1.16.1	
1.16. FDA IDE approval for the 2 (b)(4)						Milestone 1.6 – Deliverable 1.16.2	



Research paper

Low pressure voiding induced by stimulation and 1 kHz post-stimulation block of the pudendal nerves in cats

Jialiang Chen^{a,b}, Jianan Jian^a, Jicheng Wang^a, Zhijun Shen^a, Bing Shen^a, William Wang^a, Jonathan Beckel^c, William C. de Groat^c, Christopher Chermansky^a, Changfeng Tai^{a,c,d,*}

^a Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

^b Department of Urology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, PR China

^c Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

^d Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

Keywords:

Stimulation
Pudendal nerve
Bladder
Spinal cord injury
Cat

ABSTRACT

The goal of this study is to induce low-pressure voiding by stimulation and bilateral 1 kHz post-stimulation block of the pudendal nerves. In anesthetized cats, wire hook electrodes were placed on the left and/or right pudendal nerves. Stimulus pulses (30 Hz, 0.2 ms) were applied to one pudendal nerve to induce a reflex bladder contraction and to produce contractions of the external urethral sphincter (EUS). High frequency (1 kHz) biphasic stimulation was applied to block axonal conduction in both pudendal nerves and block EUS activity. In 4 cats, a catheter was inserted into the distal urethra to perfuse and measure the back pressure caused by the EUS contraction. In another 5 cats, a catheter was inserted into the bladder dome and the urethra was left open to allow voiding. The 1 kHz stimulation (30–60 s, 0.5–5 mA) delivered via a wire hook electrode completely blocked pudendal nerve conduction for ≥ 2 min after terminating the stimulation, i.e., a post-stimulation block. The block gradually disappeared in 6–18 min. The block duration increased with increasing amplitude or duration of the 1 kHz stimulation. Without the 1 kHz block, 30 Hz stimulation alone induced high-pressure (90 cmH₂O) voiding. When combined with the 1 kHz block, the 30 Hz stimulation induced low-pressure (≤ 50 cmH₂O) voiding with a high voiding efficiency (80%). In summary, a minimally invasive surgical approach might be developed to restore voiding function after spinal cord injury by stimulation and block of the pudendal nerves using lead electrodes.

1. Introduction

Normal micturition is elicited by a bladder contraction and a simultaneous relaxation of the external urethral sphincter (EUS), which reduces urethral outlet resistance and results in low-pressure (≤ 50 cmH₂O) voiding. However, after spinal cord injury (SCI) the coordination between bladder and EUS is lost, causing simultaneous contractions of the bladder and EUS, termed detrusor sphincter dyssynergia (DSD). This generates high-pressure (> 50 cmH₂O) in the bladder and prevents complete bladder emptying. High bladder pressure is harmful because it can cause kidney failure if not treated. Currently, DSD is often managed by daily intermittent urethral catheterization so that the bladder can be emptied without generating high bladder pressure. However, repeated urethral catheterization can cause frequent infections of the lower urinary tract (Jamil, 2001). A better treatment for DSD will significantly

improve the quality of life for SCI people who are currently suffering from the problem of bladder emptying (Hallin et al., 2000).

Our previous studies (Tai et al., 2006, 2007a, 2007b; Yang et al., 2013) in cats developed a novel method to prevent DSD by blocking the pudendal nerves bilaterally using 6–10 kHz high frequency biphasic stimulation. Recently, we developed a fully implantable stimulator that successfully produced highly efficient ($> 90\%$) low-pressure (≤ 50 cmH₂O) voiding in awake chronic SCI cats during a 3–4 month period using 10 kHz pudendal nerve block in combination with a 20–30 Hz pudendal nerve stimulation to induce a reflex bladder contraction (Cai et al., 2019; Guo et al., 2021). However, pudendal nerve block by 6–10 kHz stimulation can only be achieved by implantation of cuff electrodes on the nerves (Apovian et al., 2017; Boger et al., 2008; Sojin et al., 2015; Tai et al., 2005), which requires major invasive surgery to expose the pudendal nerves bilaterally. Therefore, a method to block the pudendal

* Corresponding author at: Department of Urology, University of Pittsburgh, 700 Kaufmann Building, Pittsburgh, PA 15213, USA.

E-mail address: ctai@pitt.edu (C. Tai).

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nerve using a lead electrode without a cuff could be very beneficial since a lead electrode could be percutaneously inserted close to the pudendal nerve by a minimally invasive surgical approach.

In this study using spinal cord intact cats under anesthesia, we discovered that high frequency (1 kHz) biphasic electrical stimulation delivered by a wire hook electrode without a cuff could block pudendal nerve conduction during a short period immediately after termination of the stimulation, i.e., a post-stimulation block. In addition, low-pressure voiding could also be induced by applying 30 Hz pudendal nerve stimulation to induce a reflex bladder contraction during the post-stimulation block. These results indicate that a minimally invasive surgical approach might be developed by inserting percutaneous lead electrodes close to the pudendal nerves to achieve pudendal nerve block and treat DSD after SCI.

2. Materials and methods

The experimental protocol and animal use in this study were

approved by the Animal Care and Use Committee at the University of Pittsburgh.

2.1. Surgical procedures

A total of 9 cats (8 females and 1 male, 3.4 ± 0.2 kg, 7–12 months old, Liberty Research, Waverly, NY) were anesthetized with isoflurane (2–5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. The left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847 V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, the ureters were isolated, tied, and cut for external drainage. In the first experimental group of 4 female cats, a plastic catheter (7F) was inserted via a small cut in the proximal urethra to a location 2–3 cm distal to the

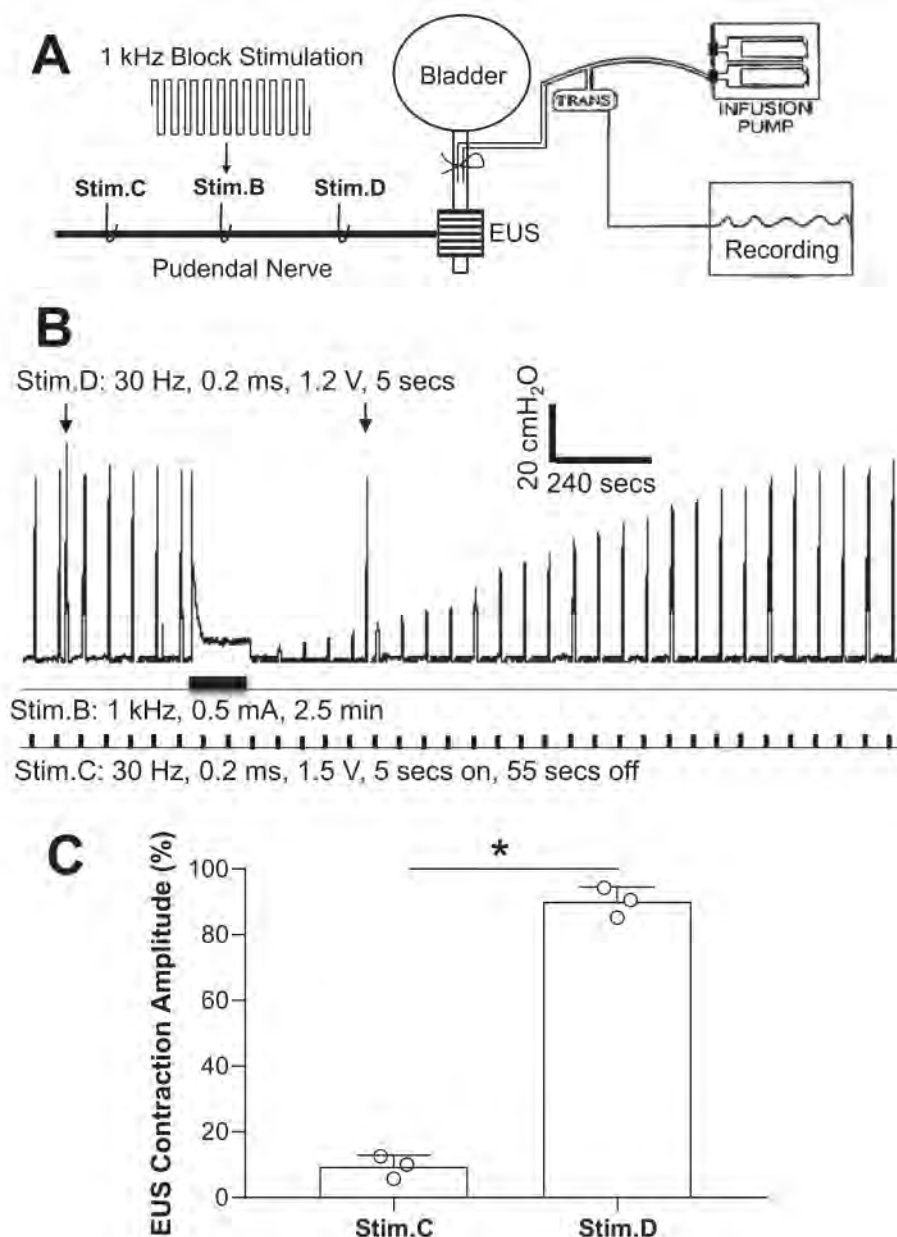


Fig. 1. A. Experimental setup for pudendal nerve block elicited by 1 kHz stimulation with a wire electrode. B. Complete nerve block occurred after the end of the 1 kHz biphasic continuous stimulation. The arrows indicate the EUS contraction pressures induced by the 30 Hz stimulation at the distal site (Stim.D). The thick bars under the EUS pressure trace indicate the stimulation durations for Stim.B and Stim.C. After ending the 1 kHz Stim.B, the EUS contraction can still be induced by the distal Stim.D but the contractions induced by the central Stim.C are completely blocked initially and then gradually recover, indicating that nerve conduction block occurs locally at Stim.B and the EUS is not fatigued. C. The normalized EUS contraction amplitude induced by Stim.C or Stim.D during the post-stimulation period. $N = 3$ nerves. * indicates a significant difference ($p = 0.0026$, paired t -test). EUS = External Urethral Sphincter.

bladder neck and secured by a ligature. The urethra was slowly perfused with saline via the catheter to measure the intraurethral back pressure caused by urethral contractions (Fig. 1A). The pudendal nerves were dissected bilaterally via 3- to 4-cm incisions in the sciatic notch lateral to the tail for placement of monopolar wire hook electrodes (Fig. 1A). A 16-gauge needle was inserted in the shoulder muscle as the reference electrode. The nerve and electrodes were covered by mineral oil during the study. In the second experimental group of 5 cats (4 female and 1 male), a double lumen plastic catheter (7F) was inserted into the bladder via a small puncture on the bladder dome and secured by a ligature so that voiding could occur without a physical urethral outlet obstruction. One lumen of the catheter was connected to a pump to infuse the bladder with saline, and the other lumen filled with saline was attached to a pressure transducer to record intravesical pressure (Fig. 4A). A funnel was used to collect the voided fluid into a beaker that was attached to a force transducer to record the voided volume. The pudendal nerves were exposed bilaterally for placement of either monopolar ($N = 2$ cats) or bipolar ($N = 3$ cats) wire hook electrodes (Fig. 4A). The nerve and electrodes were covered by saline or mineral oil during the study to confirm that low-pressure voiding could be achieved with the electrode surrounded by media of either low (saline) or high (oil) impedance.

2.2. Experimental protocol

In the first group of 4 cats, the urethra was slowly (1 ml/min) perfused to test the pudendal nerve block induced by a 1 kHz biphasic stimulation waveform (see Fig. 1A). In the first cat, three monopolar stainless-steel hook electrodes (diameter 0.5 mm and 5 mm long) were placed on either the right or left pudendal nerve with 3–5 mm between the electrodes (Fig. 1A). The blocking electrode (Stim.B) always delivered the 1 kHz block stimulation that was generated by a computer running LabView program and delivered via a constant current stimulus isolator (A395, World Precision Instruments, Sarasota, FL). The central electrode (Stim.C) delivered short bursts of stimulation (30 Hz, 0.2 ms, 5 s on and 55 s off) at an amplitude of 3 times the threshold (T) amplitude for inducing anal twitch to produce maximal EUS contractions. The 30 Hz stimulation was generated by an electrical stimulator (S88, Grass Medical Instruments, Quincy, MA) and delivered via a constant voltage stimulus isolator (SIU5, Grass Medical Instruments). The distal electrode (Stim.D) delivered a single short burst (5 s) of stimulation (30 Hz, 0.2 ms, 3 T) to confirm that Stim.B suppressed the EUS contractions induced by Stim.C via a local nerve block instead of causing EUS fatigue. The 1 kHz stimulation (Stim.B) at various stimulation amplitudes and durations was explored in the first cat to determine the appropriate range of stimulation parameters for further testing.

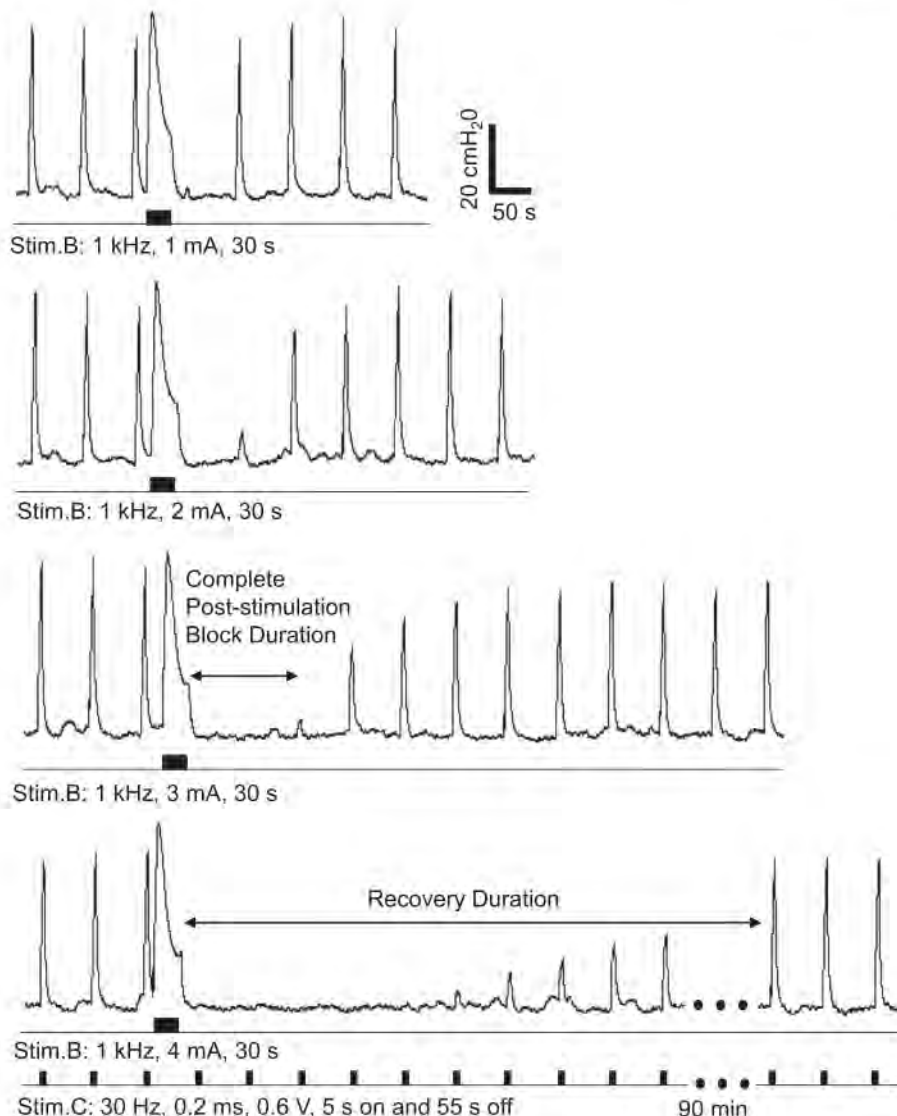


Fig. 2. Block stimulation (Stim.B) produced a complete post-stimulation block of duration ≥ 2 min as the stimulation amplitude gradually increased from 1 mA to 3 mA. At 4 mA amplitude, Stim.B produced a prolonged post-stimulation block with a recovery period of 110 min. The black bar under each urethral pressure trace indicates the stimulation duration. The duration of Stim.C shown at the bottom by short black bars applies to all urethral pressure traces. The amplitude for Stim.C which is at 3 times the threshold (T) amplitude for inducing visible anal twitch induces maximal contractions of the external urethral sphincter.

In the next 3 cats, two monopolar hook electrodes (Stim.C and Stim.B) were placed initially on left pudendal nerve with 3–5 mm between the electrodes (Fig. 1A). Stim.C delivered short bursts of stimulation (30 Hz, 0.2 ms, 3 T, 5 s on and 25/55 s off) to induce periodic EUS contractions during which Stim.B (1 kHz, 30 s) was applied at an increasing amplitude starting at 1 mA with 1 mA increase each time until a complete post-stimulation block of duration ≥ 2 min was observed. This duration was considered to be sufficient to allow complete voiding. Complete block was defined as a reduction in the Stim.C evoked EUS contraction pressure to <10 cmH₂O that would allow low-pressure voiding to occur (see third trace in Fig. 2). After each application of the 1 kHz stimulation, a waiting period was given to allow the EUS contractions induced by Stim.C to recover to the level preceding the application of the 1 kHz stimulation. If a complete post-stimulation block of duration ≥ 2 min was not observed before the amplitude of 1 kHz stimulation reached the level causing hindlimb movement, the duration of 1 kHz was doubled to 60 s and the test was repeated (Fig. 3). Then, the Stim.D electrode (Fig. 1A) was added to confirm Stim.B suppressed the EUS contractions induced by Stim.C via a local nerve block instead of causing EUS fatigue. After testing the left pudendal nerve, the electrodes were moved to the right pudendal nerve and the same test was repeated. However, on the right pudendal nerve the amplitude of 1 kHz stimulation was increased above the level that produced complete post-stimulation block of ≥ 2 min duration to elicit a more prolonged (>30 min) recovery duration that is defined as the time for the response to recover to at least 80% of the level prior to the application of 1 kHz stimulation (see fourth trace in Fig. 2).

In the second group of 5 cats with the bladder catheterized through the bladder dome, multiple voiding CMGs were performed initially to determine the control values for bladder capacity, voiding pressure, voiding efficiency, and post-void residual volume. Then, in the following repeated CMGs the bladder was infused to a volume close to the control capacity without triggering reflex voiding. With the bladder partially full, pudendal nerve stimulation (Stim #1 in Fig. 4A: 30 Hz, 0.2 ms, 3–4 T) was first applied to induce a pudendal-to-bladder reflex contraction for voiding. Our previous studies (Cai et al., 2019; Guo et al., 2021; Tai et al., 2006, 2007a, 2007b; Yang et al., 2013) in both normal and chronic spinal cord injured cats showed that 30 Hz stimulation was effective in inducing large reflex bladder contractions. In the next CMG with the bladder partially full, the 1 kHz stimulation (1 mA, 30 s) was applied bilaterally to the pudendal nerves via Stim #2 and Stim #3 (see Fig. 4A) to induce a post-stimulation block. Immediately following the termination of the 1 kHz block stimulation, the Stim #1 was applied to induce a bladder contraction for voiding. If the voiding pressure was not reduced, the same block/stimulation protocol was repeated in the

following CMGs with bladder partially full, but the 1 kHz stimulation was applied at the maximal amplitude without triggering hindlimb movement or at an increased stimulation duration starting at 1 min and with 1 min increments each time. Once a low-pressure (≤ 50 cmH₂O) voiding was achieved, only the Stim #1 was applied in the following repeated CMGs to monitor the recovery of the voiding pressure. At the end of each CMG, post-void residual volume was determined by withdrawing fluid from the bladder catheter.

2.3. Data analysis

In the first group of cats, the amplitude and duration for the 1 kHz stimulation to induce a complete post-stimulation block of duration ≥ 2 min were determined, and the related block and recovery durations were measured. The amplitudes of the EUS contractions induced during the post-stimulation period by Stim.D and Stim.C within a 30 s interval as shown in Fig. 1B were compared by normalizing them to their control amplitudes before the 1 kHz stimulation. In the second group of cats, the bladder volume, voiding pressure, voiding efficiency, and post-void residual volume were measured for each CMG. The bladder volume was measured as the voided volume + the post-void residual volume. The bladder volume and post-void residual volume were normalized to the control bladder capacity in the same animal. The data from different animals are presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined using repeated-measures one-way ANOVA followed by Dunnett's multiple comparison or using Welch's or paired *t*-test.

3. Results

3.1. Post-stimulation block of the pudendal nerve by 1 kHz biphasic stimulation waveform

As shown in Fig. 1, Stim.B (1 kHz, 0.5 mA, 2.5 min) successfully blocked the periodic EUS contractions induced by short bursts of Stim.C (30 Hz, 0.2 ms, 1.5 V, 5 s on and 55 s off) during a period after terminating the 1 kHz stimulation, i.e., a post-stimulation block. Since the EUS contractions induced by Stim.D (30 Hz, 0.2 ms, 1.2 V, 5 s) were not changed while the contractions induced by Stim.C were blocked, it is clear that Stim.B blocked pudendal nerve conduction instead of causing EUS fatigue that should affect the responses induced by both Stim.C and Stim.D. On average the EUS contraction amplitude induced by Stim.D during the post-stimulation period was maintained at about 90% of its control amplitude measured before the 1 kHz stimulation while the contraction amplitude induced by Stim.C was significantly ($p = 0.0026$, paired *t*-test) reduced to only 10% of its control (Fig. 1C). The large difference between the reductions in the amplitude of the two evoked responses indicates a local nerve block occurred at the Stim.B and that EUS fatigue which should reduce the responses induced by Stim.C and Stim.D equally was not involved.

The duration of 1 kHz post-stimulation block was dependent on the amplitude and duration of the stimulation. As shown in Fig. 2, the block duration was increased by increasing the amplitude of 1 kHz stimulation (30 s) from 1 mA to 4 mA. At the amplitude of 3 mA, the duration for a complete post-stimulation block reached 2 min with a full recovery occurring at 6 min (see the third trace in Fig. 2). When the amplitude of 1 kHz stimulation increased to 4 mA, the complete block duration increased to 6 min, but the time for complete recovery increased dramatically to 100 min (see the fourth trace in Fig. 2). In the other 2 cats, a prolonged recovery (58 min or 60 min) was induced by 1 kHz stimulation at the amplitude/duration combination of 4 mA/30 s or 2 mA/60 s, respectively.

Fig. 3 summarizes the amplitude and duration required for 1 kHz stimulation to induce a complete post-stimulation block of duration ≥ 2 min, the corresponding duration of the complete block, and the time for full recovery from block ($N = 6$ nerves from 3 cats). In one cat, 1 kHz stimulation of 30 s duration failed to induce a complete post-stimulation

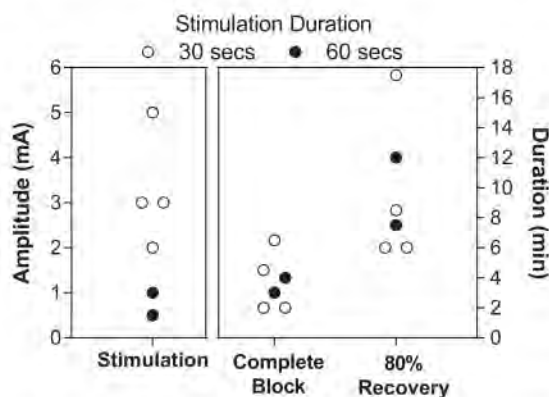
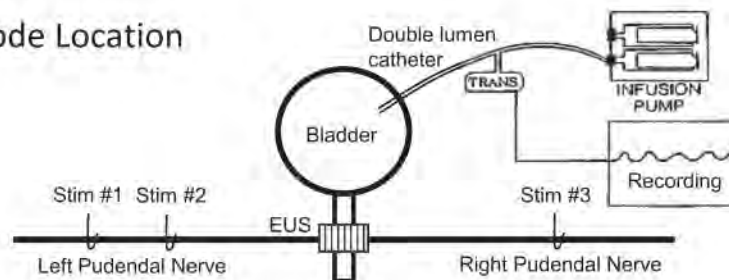
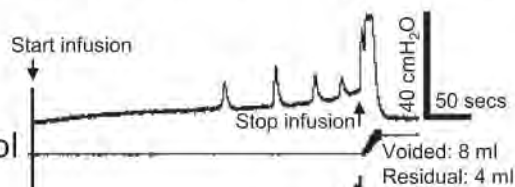


Fig. 3. The amplitude and duration (30 s or 60 s) required for 1 kHz stimulation to produce a complete post-stimulation block of duration ≥ 2 mins, the corresponding duration for the complete block, and the duration for an 80% recovery from the block. Stimulation amplitude is shown on the left Y-axis. Duration for the complete block and 80% recovery is shown on the right Y-axis. $N = 6$ nerves from 3 cats.

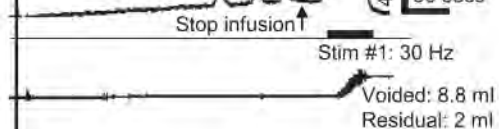
A. Electrode Location



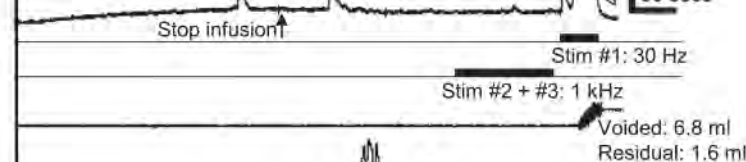
B. Control



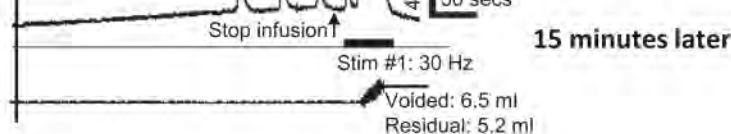
C. 30 Hz



D. 30 Hz + 1 kHz



E. 30 Hz



block of duration ≥ 2 min before its amplitude increased to the level causing hindlimb movement. In this cat after increasing the stimulation duration to 60 s, the 1 kHz stimulation-induced complete block occurred at a much lower stimulation amplitude (0.5–1 mA, see the black dots in Fig. 3) than the 30 s stimulation ($p = 0.0236$, Welch's t -test). These results indicate that there is a combination of amplitude and duration for 1 kHz to induce a complete post-stimulation block of duration ≥ 2 min that can gradually disappear within 20 min (Fig. 3). However, if the amplitude and/or the duration are increased further, a prolonged recovery of 1–2 h can occur (see the fourth trace in Fig. 2).

3.2. Low-pressure voiding induced by 1 kHz block and 30 Hz stimulation

Fig. 4 shows that when using monopolar hook electrodes in a saline pool the 30 Hz pudendal nerve stimulation combined with 1 kHz post-stimulation block successfully induced low-pressure (≤ 50 cmH₂O) voiding. During the control CMG (Fig. 4B), slow infusion of the bladder triggered a micturition reflex producing a low-pressure (≈ 40 cmH₂O) voiding. With the bladder partially full (Fig. 4C), Stim #1 (30 Hz) alone induced voiding at a high pressure (≈ 60 cmH₂O). However, the same

Fig. 4. Low-pressure voiding induced by stimulation and 1 kHz post-stimulation block of axons in the pudendal nerves. A. Monopolar wire electrodes were placed on pudendal nerves bilaterally to deliver stimulations at three locations (#1, #2, #3). Stim #1 (30 Hz, 0.2 ms) was delivered to induce a reflex bladder contraction after Stim #2 and #3 delivered a short burst of 1 kHz stimulation to produce a post-stimulation block of the pudendal nerves. EUS - external urethral sphincter. B. Low-pressure voiding induced by infusion of saline into the bladder without stimulation. C. Stim #1 (30 Hz, 0.2 ms, 9 V) alone produced high-pressure (≈ 60 cmH₂O) voiding. D. When Stim #1 was applied immediately after Stim #2 and #3 delivered the 1 kHz stimulation (1.75 mA, 2 mins), it induced a low-pressure (≈ 40 cmH₂O) voiding. E. In 15 mins after the 1 kHz stimulation, Stim #1 again induced a high-pressure voiding. The black bar under the bladder pressure trace indicates the stimulation duration. Infusion rate = 2 ml/min.

Stim #1 successfully induced low-pressure (≈ 40 cmH₂O) voiding when applied immediately after termination of the 1 kHz axonal blocking stimulation (1.75 mA, 2 min) at both Stim #2 and #3 (see Fig. 4D). The high-pressure (≈ 60 cmH₂O) voiding returned in about 15 min after termination of the 1 kHz blocking stimulation (Fig. 4E).

Fig. 5 shows that low-pressure voiding can also be induced by a combination of 1 kHz block and 30 Hz stimulation using bipolar hook electrodes in a mineral oil pool. However, the reversal from low-pressure voiding to high-pressure voiding was slower due to a stronger effect of the 1 kHz post-stimulation block in this experiment. As shown in Fig. 5A–B, the micturition reflex elicited by filling the bladder induced low-pressure (< 50 cmH₂O) voiding; while Stim #1 (30 Hz) alone induced high-pressure (≈ 100 cmH₂O) voiding. After applying 1 kHz stimulation (2 mA, 1 min) to both Stim #2 and #3, the response to Stim #1 was completely blocked for about 10 min, after which it recovered to successfully induce low-pressure (< 50 cmH₂O) voiding (see Fig. 5C). The low-pressure voiding induced by Stim #1 alone lasted for at least an hour after the 1 kHz blocking stimulation (Fig. 5D–E); but at 3 h, the high-pressure voiding (70 cmH₂O) returned (Fig. 5F). Note that there was no leakage of fluid from the bladder during the period immediately

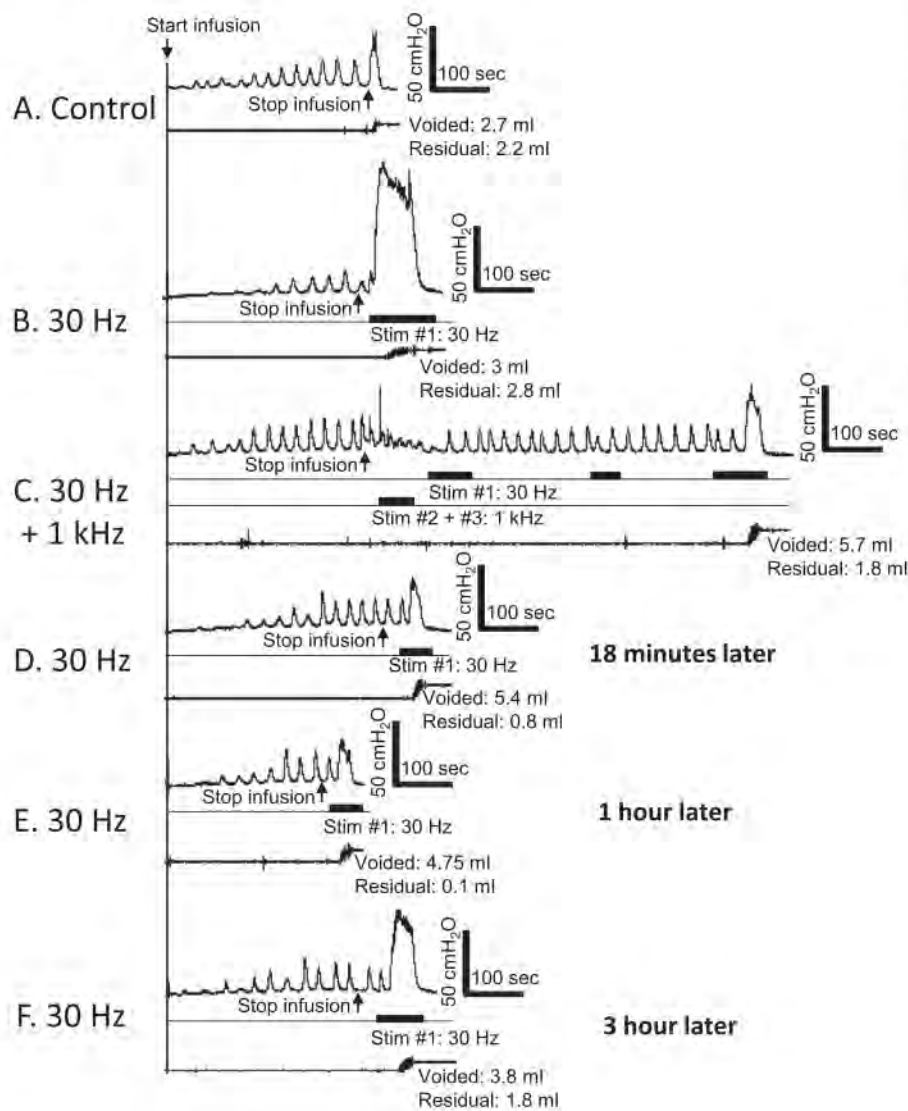


Fig. 5. Long-lasting effect of 1 kHz post-stimulation block of axons in the pudendal nerves. A. Control cystometrogram showing a low-pressure (<50 cmH₂O) voiding produced by a micturition reflex induced by infusion of saline into the bladder. B. Stim #1 (30 Hz, 0.2 ms, 0.48 V) alone produced a high-pressure (~100 cmH₂O) voiding. C. After the 1 kHz stimulation (2 mA, 1 min) at Stim #2 and #3, Stim #1 lost the ability to induce a bladder contraction for about 10 min, after which Stim #1 induced a low-pressure (~50 cmH₂O) voiding. D-E. The low-pressure voiding induced by Stim #1 lasted for at least 1 h. F. At 3 h after the 1 kHz stimulation, the voiding pressure induced by Stim #1 increased to about 70 cmH₂O. The black bar under the bladder pressure trace indicates the stimulation duration. Infusion rate = 1 ml/min.

following termination of the 1 kHz blocking stimulation although many non-voiding contractions occurred during this period (Fig. 5C).

Fig. 6 summarizes the results from 5 cats. On average, in the control condition the micturition reflex induced by filling the bladder produced low-pressure (~40 cmH₂O) voiding (Fig. 6A) and a 50% voiding efficiency (Fig. 6B) with a post-void residual volume of 50% (Fig. 6C) of the control bladder capacity. A slightly higher voiding efficiency, but not significantly different from control, and a lower post-void residual volume were produced by 30 Hz stimulation alone (Fig. 6 B–C) at a significantly ($p = 0.0149$) higher voiding pressure (90 cmH₂O, Fig. 6A). However, 30 Hz stimulation combined with 1 kHz post-stimulation block significantly ($p = 0.0203$) increased voiding efficiency (80%, Fig. 6B) and reduced post-void residual volume (20%, Fig. 6C) but at a significantly ($p = 0.0303$) lower pressure (~40 cmH₂O) that was same as in the control condition (Fig. 6A). After a recovery period ranging from 15 mins to 3 h (see Fig. 4 and Fig. 5), the voiding pressure induced by 30 Hz stimulation alone increased to about 70 cmH₂O (Fig. 6A) while voiding efficiency declined (50%, Fig. 6B) and post-void residual volume significantly ($p = 0.0354$) increased (50%, Fig. 6C) above that measured immediately after the 1 kHz stimulation. On average a similar amount of saline was infused into the bladder under the different conditions (Fig. 6D).

4. Discussion

This study shows that application of 1 kHz stimulation to the pudendal nerve by a wire electrode elicits a block of axonal conduction that persists following the stimulation, i.e., a post-stimulation block (Fig. 1) and that the block duration increases with increasing amplitude and duration of the stimulation (Fig. 2 and Fig. 3). All previous studies (Apovian et al., 2017; Boger et al., 2008; Sooin et al., 2015; Tai et al., 2005) applied 5–10 kHz stimulation that requires a cuff electrode to successfully block the nerve conduction during the stimulation. The 5–10 kHz stimulation also requires a very long stimulation duration (>10 min) to induce a post-stimulation block (Wang et al., 2020). However, our recent study using cuff electrodes (Shapiro et al., 2020) discovered that a post-stimulation block could be induced by low-frequency (100 Hz – 1 kHz) stimulation of a short duration (30–60 s). The current study further demonstrates for the first time that a cuff electrode is not needed, and that post-stimulation block can be induced by 1 kHz stimulation using a monopolar or bipolar wire electrode, raising the possibility that a lead electrode without a cuff might also be useful in clinical applications to block nerve conduction. This result is significant because instead of a major invasive surgical procedure to implant the cuff electrodes a minimally invasive surgical approach could be developed to insert lead electrodes close to the pudendal nerves to

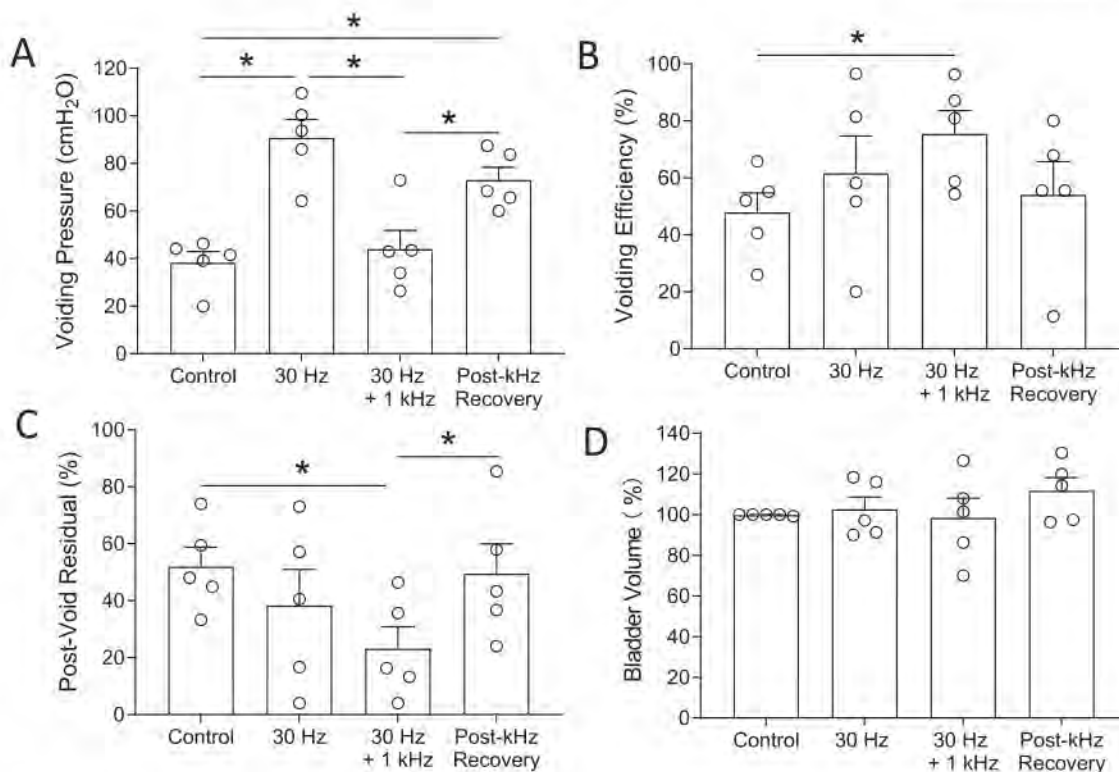


Fig. 6. Summary of the urodynamic parameters for control condition, 30 Hz stimulation alone (30 Hz), 30 Hz stimulation after 1 kHz stimulation (30 Hz + 1 kHz), and recovery from the 1 kHz blocking effect (Post-kHz recovery). A. Voiding pressure. B. Voiding efficiency. C. Post-void residual. D. Bladder volume infused. * indicates a significant difference ($p < 0.05$, ANOVA). $N = 5$ cats.

achieve pudendal nerve block. More importantly, during the bilateral post-stimulation block of the pudendal nerves, low-pressure reflex voiding can be induced by 30 Hz stimulation of afferent axons in the pudendal nerve (Figs. 4–6). Nerve block used in combination with stimulation of pudendal nerve afferent axons has a possible clinical application to reduce voiding pressure and improve voiding efficiency after SCI (Cai et al., 2019; Gup et al., 2021; Yang et al., 2013). Because high-pressure voiding after SCI can damage the kidneys, the low-pressure voiding induced by pudendal nerve stimulation and block (Figs. 4–6) would contribute to the long-term preservation of upper and lower urinary tract function and thus be a significant advance in the care of people with SCI.

However, it is important to recognize that the present study was performed in anesthetized spinal cord intact cats with normal lower urinary tract reflexes that produced relatively efficient reflex voiding at low pressures in response to bladder filling (Fig. 4B). This is due to coordinated bladder and EUS activity that produces a relaxation of the EUS during the bladder voiding contraction. After SCI the EUS relaxation is lost and the EUS contracts during voiding, a condition termed DSD, which increases urethral outlet resistance, impedes urine flow, increases voiding pressure and impairs complete bladder emptying. Block of the efferent input to the EUS by 1 kHz pudendal nerve stimulation would prevent DSD after SCI and normalize voiding. In the present study DSD was mimicked by applying pudendal nerve stimulation at 30 Hz, which caused a reflex bladder contraction and a simultaneous contraction of the EUS resulting in a high-pressure voiding. The efficacy of 1 kHz blocking stimulation in reducing the high pressure during the voiding induced by 30 Hz stimulation in the present study predicts that the blocking stimulation would be similarly effective in reversing the DSD-induced inefficient high-pressure voiding after SCI. It is worth noting that the 30 Hz stimulation combined with 1 kHz block significantly increased voiding efficiency over the control condition but not over the 30 Hz stimulation alone (Fig. 6B). However, 30 Hz stimulation alone

produced a very high voiding pressure reaching about 90 cmH₂O on average (Fig. 6A) that can increase voiding efficiency but is also harmful to the kidney and useless for a long-term clinical application. Therefore, the 1 kHz nerve block is very important because it allows a low-pressure voiding (40 cmH₂O as shown in Fig. 6A) to be induced by the 30 Hz stimulation.

This study examined two mechanisms (nerve block and EUS muscle fatigue) that might contribute to the prolonged suppression of neurally evoked EUS contractions by 1 kHz stimulation. The possibility that 1 kHz induces muscle fatigue instead of a local nerve block is not supported by the observation that when the response induced by Stim.C is blocked (see Fig. 1B–C) the response induced by Stim.D (see Fig. 1A) at a site distal to the blocking electrode is not blocked (see Fig. 1B–C). This method (Stim.C/Stim.D) has been used in many previous studies by different neurophysiologists to distinguish local nerve block from muscle fatigue (Bowman and McNeal, 1986; Kilgore and Bhadra, 2004). The initial muscle response to 1 kHz stimulation in our current study is similar to that elicited by 10 kHz stimulation (Tai et al., 2005) that is known to block axonal conduction without producing muscle fatigue (Bowman and McNeal, 1986; Kilgore and Bhadra, 2004). Therefore, the initial fast decline in response (see Fig. 1B) may not necessarily be muscle fatigue. Furthermore, the EUS is striated muscle that is more fatigue resistant than skeletal muscle. A previous study showed that prolonged (40 min) electrical nerve stimulation (12–24 Hz) of maximal intensity failed to relax the EUS and allow passage of urine (Brindley et al., 1974). This result is completely different from the result in our current study showing a complete relaxation of EUS (Fig. 1 and Fig. 2) that allowed low-pressure efficient voiding to occur (Figs. 4–6). In addition, it is certainly not typical that a short duration (30 s) neurally evoked EUS contraction produces complete EUS fatigue lasting 5 min followed by a very long (1.5 h) period for a full recovery of the EUS contractions (see the bottom trace in Fig. 2). Therefore, we believe that the prolonged 1 kHz induced suppression of the EUS contractions

observed in our current study is due to axonal block and not due to muscle fatigue.

More importantly, the 1 kHz post-stimulation block is reversible within minutes if an appropriate combination of stimulation amplitude and duration is used (Figs. 3–4). This reversibility is needed because the pudendal nerve needs to be functional after each voiding to maintain continence during urine storage. There is an optimal combination of stimulation amplitude and duration for achieving a short duration (~2 min) post-stimulation block to allow a low-pressure voiding to occur while also allowing the block to recover within 20 min (Fig. 3). In future clinical application, this optimal combination of stimulation amplitude and duration will have to be determined for each individual subject after implanting the pudendal nerve stimulation and block device. If the 1 kHz stimulation is too strong or lasts too long, axonal conduction in the pudendal nerve will need a long time to recover from the block (see fourth trace in Fig. 2), which will result in a prolonged period of low-pressure voiding (Fig. 5). It is worth noting that during the prolonged period of pudendal nerve block there was no leakage of fluid from the bladder although many small amplitude non-voiding contractions were occurring during this period (Fig. 5). This result indicates that a basal urethral outlet resistance was maintained during the prolonged pudendal nerve block that prevented leakage from the bladder. Furthermore, the 1 kHz stimulation elicited an initial strong EUS contraction (Fig. 2) but did not elicit a large reflex bladder contraction before it blocked the pudendal nerve (Fig. 4D and Fig. 5C), which eliminates the possibility for the 1 kHz stimulation to generate high bladder pressures in a long-term clinical application causing kidney damage.

Wire electrodes were used in this study to block the pudendal nerve. The electrical current can spread over a large area around the electrode because a nerve cuff was not used to limit the current spread. Since the sciatic nerve is very close to the pudendal nerve in cats, leg movement caused by the spreading current prevented further increasing the amplitude of the 1 kHz stimulation. Although this may not be an issue in clinical applications due to the larger distance between the sciatic nerve and the pudendal nerve in humans, our study shows that the limitation of using an increased stimulation amplitude can be overcome by increasing the stimulation duration to achieve the nerve block at a lower intensity (Fig. 3). To reduce the spread of the electrical current, a bipolar lead electrode configuration may be preferred over a monopolar lead electrode configuration in clinical applications to block a peripheral nerve.

The nerve block by continuous kHz stimulation has been approved by FDA for human application to treat obesity (Apovian et al., 2017) or amputation limb pain (Soin et al., 2015). Therefore, we expect that the 1 kHz post-stimulation block of pudendal nerve is safe for restoring voiding function after SCI because it will only be applied 5–8 times per day and a few minutes each time for voiding. This study has shown that the 1 kHz block is safe in acute application since it is always reversible (Figs. 1–2). However, for a long-term clinical application the safety of the 1 kHz block still needs to be determined in a chronic animal study using a fully implantable device.

To prevent DSD and improve voiding after SCI, the commercially available Brindley stimulator (Brindley, 1977; van Kerrebroeck et al., 1996) requires sacral posterior root rhizotomy, which is destructive and irreversible and results in the loss of reflex sexual and defecation functions that are very important residual reflexes for SCI people. The long-term goal of our project is to develop a novel fully implantable pudendal neuromodulation device to restore lower urinary tract functions for SCI people without sacral posterior root rhizotomy. Our approach will preserve the spinal reflex functions of the bowel and sexual organs, and more importantly provide the opportunity for SCI people to benefit from advances in neural regeneration and repair techniques in the future.

The mechanism of post-stimulation block induced by 1 kHz stimulation is currently unknown. Our recent modeling studies (Zhong et al., 2021a, 2021b) indicate that the changes induced by high-frequency stimulation in intracellular and extracellular sodium and potassium

concentrations could cause the post-stimulation block. As shown in this study the recovery following stimulation is slow requiring many minutes because after ending the stimulation the ion pump can only slowly restore the intracellular and extracellular sodium and potassium concentrations to normal levels. More animal studies are needed to better understand the mechanism underlying post-stimulation block.

Previous studies (Bowman and McNeal, 1986; Crosby et al., 2017; Kilgore and Bhadra, 2004; Tai et al., 2005) found that a stimulation frequency of 1 kHz is too low to produce nerve conduction block during the stimulation. However, these studies never investigated the possibility of post-stimulation block after termination of the stimulation, which the current and more recent studies showed can occur at frequencies >5 kHz (Bhadra et al., 2018; Wang et al., 2020; Yang et al., 2017) as well as at 100–1000 Hz (Shapiro et al., 2020). In addition, previous studies (Solomonow et al., 1983a, 1983b) also showed that muscle fatigue or partial nerve block could be induced during a 600 Hz stimulation, but the post-stimulation effect was not investigated. Therefore, our result showing post-stimulation block by 1 kHz stimulation is not in conflict with previous studies.

In summary, this study in anesthetized normal cats demonstrated for the first time that the pudendal nerve can be blocked by kHz stimulation with a wire electrode without using a nerve cuff. When post-stimulation block by 1 kHz stimulation is combined with a 30 Hz pudendal nerve stimulation, a low-pressure and highly efficient voiding can be induced. These results indicate that neuromodulation to restore voiding function after SCI might be achieved by insertion of lead electrodes close to the pudendal nerves using a minimally invasive surgical approach instead of implanting nerve cuff electrodes on the pudendal nerves which requires major surgery.

Declaration of Competing Interest

Dr. Tai is the inventor of a patent related to this study. The other authors have no conflicts of interest.

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RESEARCH ARTICLE

Defecation induced by stimulation of sacral S2 spinal root in cats

Jicheng Wang,¹ Zhijun Shen,¹ Bing Shen,¹ Jianan Jian,¹ Travis Hannan,¹ Khari Goosby,¹ William Wang,¹ Jonathan Beckel,² William C. de Groat,² Christopher Chermansky,¹ and Changfeng Tai^{1,2,3}

¹Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania; ²Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania; and ³Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract

The aim of this study was to determine whether stimulation of sacral spinal nerve roots can induce defecation in cats. In anesthetized cats, bipolar hook electrodes were placed on the S1–S3 dorsal and/or ventral roots. Stimulus pulses (1–50 Hz, 0.2 ms) were applied to an individual S1–S3 root to induce proximal/distal colon contractions and defecation. Balloon catheters were inserted into the proximal and distal colon to measure contraction pressure. Glass marbles were inserted into the rectum to demonstrate defecation by videotaping the elimination of marbles. Stimulation of the S2 ventral root at 7 Hz induced significantly ($P < 0.05$) larger contractions (32 ± 9 cmH₂O) in both proximal and distal colon than stimulation of the S1 or S3 ventral root. Intermittent (5 times) stimulation (1 min on and 1 min off) of both dorsal and ventral S2 roots at 7 Hz produced reproducible colon contractions without fatigue, whereas continuous stimulation of 5-min duration caused significant fatigue in colon contractions. Stimulation (7 Hz) of both dorsal and ventral S2 roots together successfully induced defecation that eliminated 1 or 2 marbles from the rectum. This study indicates the possibility to develop a novel neuromodulation device to restore defecation function after spinal cord injury using a minimally invasive surgical approach to insert a lead electrode via the sacral foramen to stimulate a sacral spinal root.

NEW & NOTEWORTHY This study in cats determined the optimal stimulation parameters and the spinal segment for sacral spinal root stimulation to induce colon contraction. The results have significant implications for design of a novel neuromodulation device to restore defecation function after spinal cord injury (SCI) and for optimizing sacral neuromodulation parameters to treat non-SCI people with chronic constipation.

cat; colon; sacral root; spinal cord injury; stimulation

INTRODUCTION

After spinal cord injury (SCI) severe constipation occurs with slow transit of feces in the colon and/or dyssynergic contractions between the colorectum and anal sphincter (1). Clinical management of neurogenic bowel dysfunction, which usually involves digital evacuation, stool softeners, and suppositories, is very challenging (1). A fully implantable stimulator has also been used clinically to restore defecation function after SCI by stimulating sacral anterior roots, after surgically transecting the sacral posterior roots, i.e., sacral posterior rhizotomy (2, 3). Sacral anterior root stimulation requires a major invasive spinal surgery to expose the sacral spinal roots for implantation of stimulation electrodes. Sacral posterior rhizotomy prevents the dyssynergic contraction of the anal sphincter, but it also eliminates spinal reflex defecation and sexual function such as penile erection (4). Therefore, a minimal invasive neuromodulation approach that does not require invasive spinal surgery and sacral posterior rhizotomy will be desirable for management of bowel dysfunction after SCI.

A minimally invasive surgical approach to access the sacral spinal roots for electrical stimulation has been used successfully for many years to treat spinal intact patients with an overactive bladder (5). It uses a foramen needle to insert a tined lead electrode percutaneously in proximity to the S3 spinal root under the guide of fluoroscopic imaging. This minimally invasive sacral neuromodulation approach using S3/S4 spinal root stimulation has also been evaluated in recent years as a treatment for chronic constipation but was ineffective (6–8). However, the stimulation parameters/pattern used for sacral neuromodulation to treat overactive bladder may not be suitable for modulation of colorectal motility to improve chronic constipation caused by slow transit of feces in the colon. Therefore, optimization of sacral neuromodulation parameters/pattern for improving colorectal motility or inducing defecation is needed so that this minimally invasive surgical approach could be used as an effective therapy for SCI or non-SCI people with chronic constipation.

In this study using spinal cord intact cats under anesthesia, we investigated the effects of electrical stimulation of S1–S3 roots on colon activity to determine which spinal root and

which stimulation parameters are effective in inducing colon contraction and/or defecation. This preclinical study is important to better understand the potential of stimulating a sacral spinal root using the minimally invasive surgical approach as used in current clinical application (5) to restore defecation function in SCI people or for treating chronic constipation in non-SCI people.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical Procedures

A total of nine cats (4 females and 5 males, 4.0 ± 0.3 kg; Liberty Research, Waverly, NY) were anesthetized with isoflurane (2%–5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg iv and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847 V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, one balloon catheter (G15766, Cook Urological, Spencer, IN) 1.4 cm in diameter and 5 cm in length was inserted into the proximal colon via a small incision at the proximal end of the colon (Fig. 1). The second balloon catheter of the same size was inserted into the distal colon via the anus. The two balloons were filled with water (5–7 mL) to a resting pressure of 10–15 cmH₂O to measure proximal and distal colon contractions in the first group of seven cats. In the second group of two cats, a large balloon catheter made of a condom (3.5 cm in diameter and 15 cm in length) was inserted via the small incision at the proximal end of the colon to occupy the entire colon so that

both proximal and distal colon contractions were recorded. The condom was filled with water (60–70 mL) to a resting pressure of 10–15 cmH₂O. In these two cats, 1–4 glass marbles (diameter 1.5 cm) were also inserted into the rectum via the anus so that evacuation of these marbles could be videotaped simultaneously with the colon pressure recording.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1–S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together. The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35°C–37°C) mineral oil. A heating pad was used to maintain the animal body temperature between 35°C and 37°C. Bipolar stainless steel hook electrodes (2–3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Experimental Protocol

In the first group of seven cats, stimulation (7 Hz frequency, 0.2 ms pulse width, and 1 min duration) was delivered via the hook electrode to individual sacral ventral roots (S1, S2, or S3) at different intensity (1–16 V) to determine the intensity threshold (T) for inducing an observable pressure increase in the proximal colon. Then, an intensity (1.5–3 T) that induced the maximal colon contraction was used to test colon responses to different stimulation frequencies (1–50 Hz). If no colon contraction was induced by stimulation of an individual ventral root, the maximal stimulation intensity that did not produce movement of the animal's lower body was used to test the frequency response. It was our intention not to fix the hip and lower spine, so that sacral root stimulation by the hook electrode could produce local movement of the tail or hindlimb. However, the maximal stimulation intensity was limited by any movement of the lower spine,

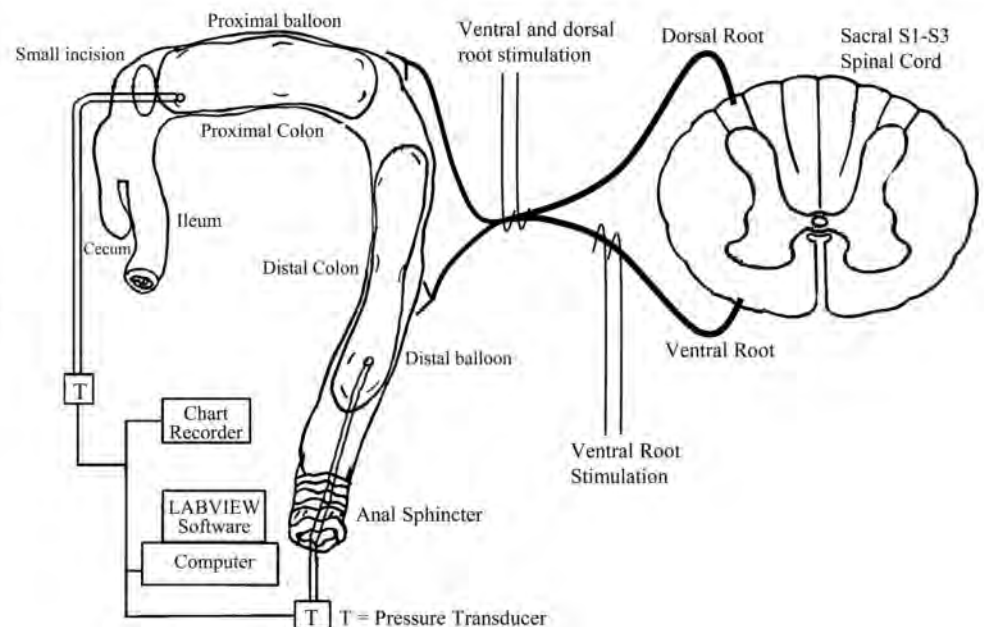


Figure 1. Experimental setup for electrical stimulation of sacral roots S1, S2, or S3 to induce colon contractions.

which would displace the hook electrode from the nerve. After stimulating each ventral root, the S2 ventral root, which elicited the largest colon contraction, was combined with the S2 dorsal root and the combination was stimulated with the most effective frequency (7 Hz.). The combined S2 root stimulation (1 min duration) was applied five times at 1-min intervals and then followed by a continuous 5-min stimulation to determine any fatigue in colon contraction.

In the second group of two cats, the combined S2 ventral and dorsal roots were stimulated (7 Hz) at an intensity that induced the largest colon contraction (distal or proximal colon) and for various durations (2.5–11 min) to determine if the stimulation could induce defecation, i.e., the evacuation of marbles that were inserted into the rectum. The defecation induced by S2 root stimulation was videotaped, and the time of evacuation of the marbles was marked on the colon pressure recording.

Data Analysis

To compare the colon contractions induced by stimulation of different ventral roots at different frequencies, the contraction amplitudes were always normalized to the maximal

contraction amplitude induced by stimulation in the same animal. The colon contractions induced by the repeated 1-min stimulations were normalized to the maximal contraction amplitude induced during the repeated stimulations to determine repeatability. The maximal contraction amplitude induced during the 5-min continuous stimulation, and the contraction amplitude at the end of the 5-min stimulation were compared to determine any fatigue during the continuous prolonged stimulation. The data from different animals were averaged and presented as means $\pm$ SE. Statistical significance ($P < 0.05$) was determined by paired t test or repeated-measures ANOVA followed by Dunnett multiple comparison (one-way) or Bonferroni multiple comparison (two-way).

RESULTS

Colon Contraction Induced by Sacral Ventral Root Stimulation is Dependent on Stimulation Frequency and the Spinal Segment Stimulated

Stimulation of the S2 ventral root induced larger colon contractions than stimulation of S1 or S3 ventral roots, and the most effective stimulation frequency was 7–10 Hz (Figs. 2 and

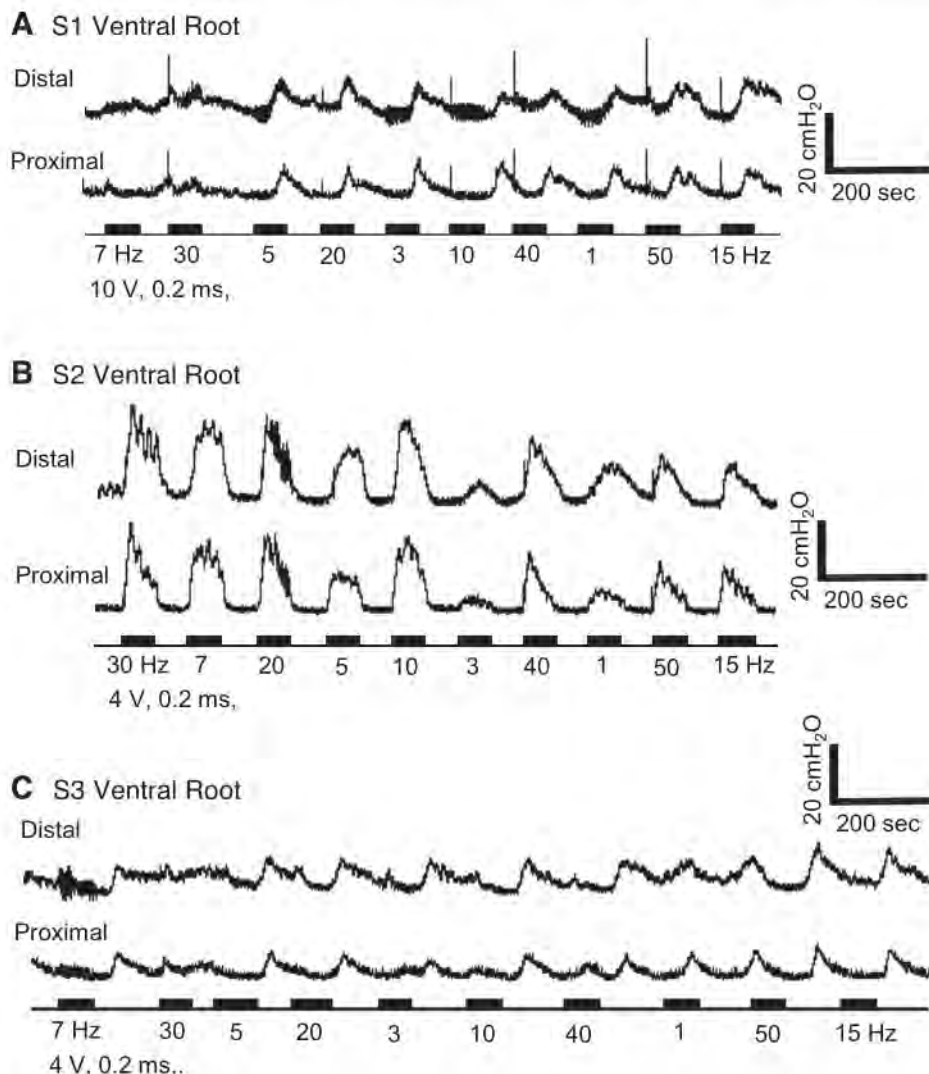


Figure 2. Distal and proximal colon responses to stimulation of sacral S1–S3 ventral roots at different frequencies (1–50 Hz). A: S1 ventral root. B: S2 ventral root. C: S3 ventral root. Data were obtained from the same animal.

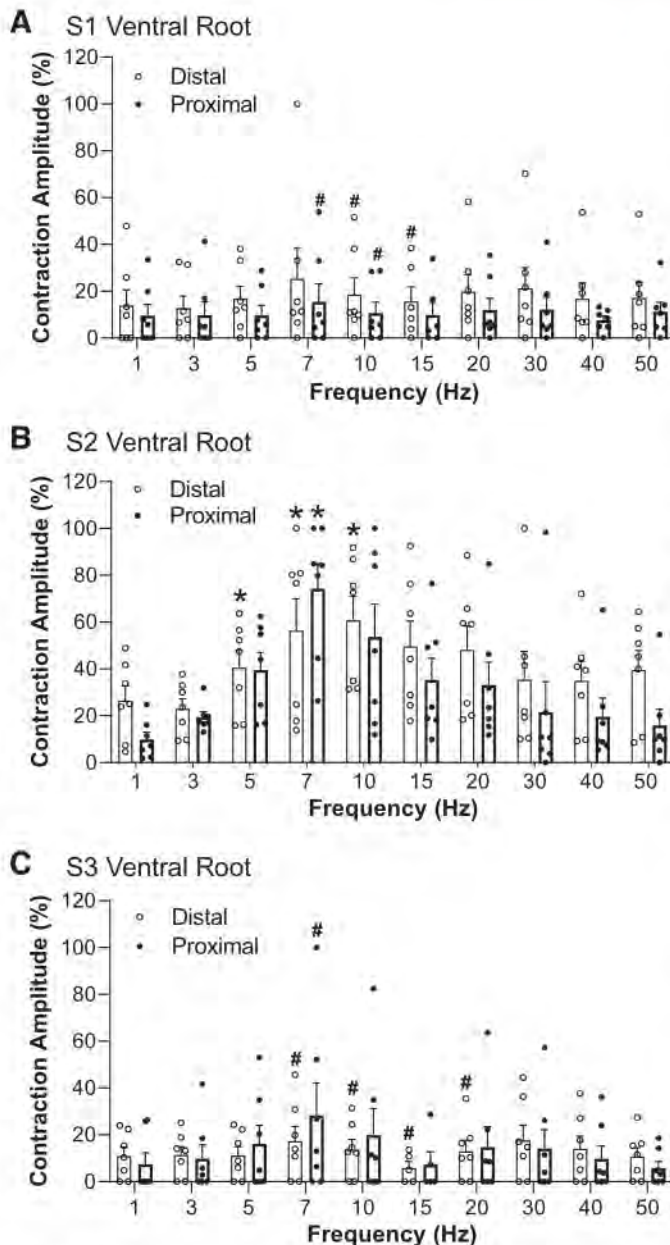


Figure 3. Distal and proximal colon responses to sacral ventral root stimulation are frequency and spinal segment dependent. A: S1 ventral root. B: S2 ventral root. C: S3 ventral root. The contraction amplitude induced by different stimulation frequencies in different sacral segments is normalized to the maximal response in the same animal. Stimulation intensity (4–16 V) for S2 ventral root was 1.5–3 times threshold intensity for 7 Hz stimulation to induce an observable contraction of proximal colon. When S1 and S3 ventral root stimulation did not induce a colon contraction, the maximal stimulation intensity (8–12 V) without causing lower body movement was used to test different frequencies. *Significantly different from 1 Hz (one-way ANOVA); #significantly different from S2 ventral root (two-way ANOVA). $n = 7$ cats.

3). Figure 2 shows the distal and proximal colon pressure traces recorded from the same cat. Stimulation of the S2 ventral root induced colon contractions >20 cmH₂O at frequencies 7–30 Hz with a similar contraction amplitude in the distal and proximal colon (Fig. 2B). Stimulation of the S1 or S3 ventral root did not induce colon contractions larger than the

small (<10 cmH₂O) rhythmic baseline colon contractions that occurred asynchronously during the periods of stimulation in a few experiments (Fig. 2, A and C). In this cat, the stimulation intensity used for S2 ventral root stimulation is two times the threshold intensity used during 7 Hz stimulation to induce an observable proximal colon contraction. Due to the weak colon response to S1 and S3 ventral root stimulation, the maximal stimulation intensity just below the threshold for inducing lower spine movement was applied to these roots. Hindlimb (mainly S1/S2 stimulation) and/or tail (mainly S2/S3 stimulation) movements were induced by stimulation of each of the three roots. Figure 3 summarizes the results from seven cats showing that S2 ventral root stimulation at 7–10 Hz was optimal for producing significantly ($P < 0.05$) larger colon contractions than those elicited by S1 or S3 ventral root stimulation. There was no significant difference between the contraction amplitudes of distal and proximal colon (Fig. 3).

Fatigue of Colon Contractions Induced by S2 Root Stimulation

Stimulation (7 Hz) of the S2 ventral and dorsal roots together induced large amplitude (>20 cmH₂O) contractions in both distal and proximal colon (Fig. 4). Figure 4A shows that intermittent 1-min stimulation at 1.5 times threshold intensity for inducing a proximal colon contraction produced a stronger contraction during the second period of stimulation and that the large amplitude contraction was maintained throughout the next three 1-min stimulations. However, the contraction amplitude gradually declined during a continuous 5-min stimulation, indicating a fatigue in the colon contractions (Fig. 4A). On average the contraction amplitude for both the distal and proximal colon was maintained during the five intermittent 1-min stimulations ($n = 4$ cats, Fig. 4B), but was significantly ($P < 0.05$) reduced from the mean maximal amplitude of 30 cmH₂O to ~ 18 cmH₂O at the end of the 5-min continuous stimulation ($n = 7$ cats, Fig. 4C).

Defecation Induced by S2 Root Stimulation

Stimulation of the S2 ventral and dorsal roots together induced a maximal amplitude colon contraction of 60 cmH₂O in cat 1 (Fig. 5A) and 40 cmH₂O in cat 2 (Fig. 5B). In cat 1 after three marbles were inserted into the rectum, S2 root stimulation (7 Hz, 6 V, 0.2 ms) induced a successful defecation that eliminated the first marble after 1 min of stimulation and the second marble after 2 min of stimulation (Fig. 5A). The elimination of the third marble, which started during the last 2 min of the 6-min stimulation, was incomplete (Fig. 5A). In cat 2 after one marble was inserted into the rectum, the S2 root stimulation (7 Hz, 6 V, 0.2 ms) eliminated the marble after 2.5 min of stimulation (Fig. 5B). However, after four marbles were inserted into the rectum, the stimulation eliminated only one of the four marbles after 4.5 min of stimulation with three marbles in the rectum at the end of 11 min of stimulation (Fig. 5B). Although stimulation of S2 ventral and dorsal roots together only eliminated 1 or 2 marbles in these two cats, stimulating the S2 ventral root alone without the dorsal root successfully eliminated all four marbles in both cats.

A S2 Ventral and Dorsal Roots

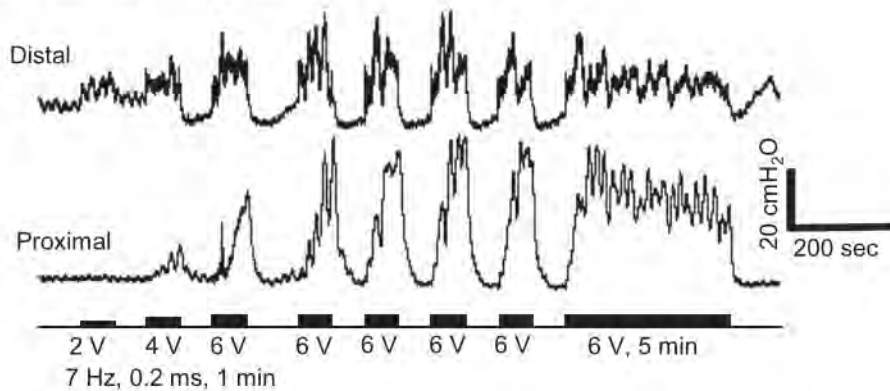
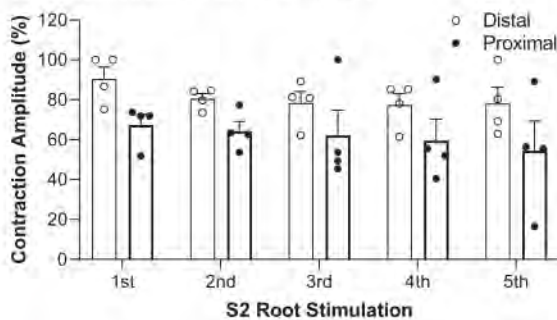
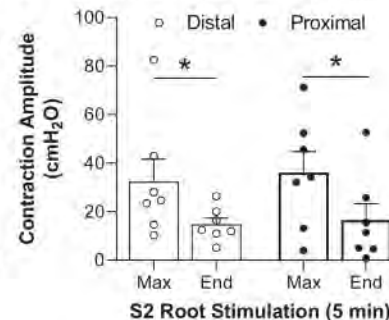


Figure 4. Distal and proximal colon responses to intermittent (5×1 min) or long duration (5 min) continuous stimulation of both S2 ventral and dorsal roots. **A:** pressure traces showing distal and proximal colon contractions. The intensity threshold (T) for proximal colon contraction was 4 V, and 6 V (1.5 T) was used to induce the intermittent or continuous contractions. **B:** summarized results ($n = 4$ cats) showing that the contraction amplitudes were maintained during intermittent stimulation (7 Hz, 0.2 ms, 1.5–4 T, $T = 2$ –4 V). **C:** summarized results ($n = 7$ cats) showing that the maximal (Max) contraction amplitude was significantly reduced from mean 30 cmH₂O to about 18 cmH₂O at the end of the continuous 5-min stimulation (7 Hz, 0.2 ms, 1.5–3 T, $T = 2$ –4 V). *Significantly different ($P < 0.05$, paired t test).

B Intermittent 1-min Stimulation



C 5-min Continuous Stimulation



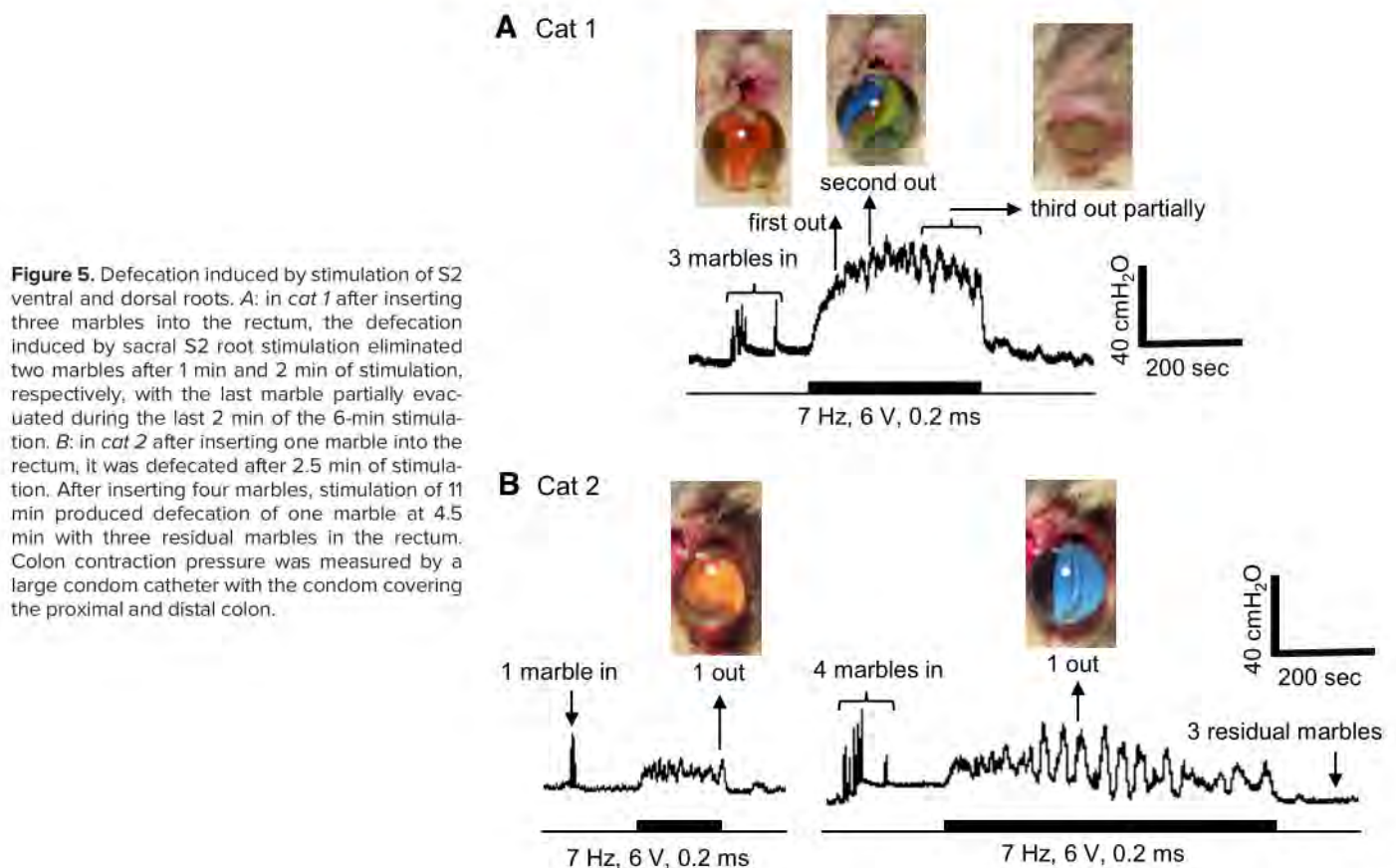
DISCUSSION

This study in anesthetized cats shows that the colon response to sacral root stimulation is dependent on stimulation frequency and the spinal segment stimulated. S2 ventral root stimulation at 7 Hz is optimal to induce both proximal and distal colon contractions (Figs. 2 and 3). Stimulation of the S2 ventral and dorsal roots together is also effective in inducing colon contraction (Fig. 4A), and intermittent stimulation (1 min on and 1 min off) is more fatigue resistant than continuous stimulation (Fig. 4, B and C). More importantly, defecation can be induced by S2 spinal root stimulation (Fig. 5).

These results have significant implications for developing a new neuromodulation device to restore defecation function in humans after SCI. Since stimulation of the entire S2 spinal root (ventral and dorsal) is as effective as stimulation of the ventral root alone (Fig. 3) and successfully induces defecation (Fig. 4), sacral posterior rhizotomy may not be necessary, and stimulation of sacral spinal roots instead of ventral roots could be effective in restoring defecation function after SCI in human. The major reason to perform sacral posterior rhizotomy is to eliminate the detrusor sphincter dyssynergia (DSD) that can generate high pressure in the bladder and cause kidney damage (2, 9). However, a high pressure in colon and/or rectum might not be as harmful as the high bladder pressure that can cause kidney failure. If colorectal pressure is high enough to overcome the dyssynergic anal sphincter contraction, feces should be evacuated. In addition, our recent studies in cats (10, 11) have developed an

effective method to transiently suppress sphincter activity by blocking pudendal nerve conduction using kHz electrical stimulation. This method is effective in treat DSD in spinal cord injured cats (10, 11) and could also be applied to treat dyssynergic defecation if a strong enough colorectal contraction could not be generated to overcome the dyssynergic anal sphincter contraction. Therefore, a novel neuromodulation device may stimulate the sacral spinal root instead of the ventral root and at the same time prevent DSD and/or dyssynergic defecation by blocking pudendal nerve conduction to restore bladder function as well as defecation function after SCI. The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to use a minimally invasive surgical approach for stimulation. S1–S3 spinal roots are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder (5).

S2 ventral root stimulation eliminated all four marbles, but stimulation of S2 ventral and dorsal roots together only eliminated 1 or 2 marbles (Fig. 5). This difference suggests that stimulating sensory nerves in the dorsal root might have induced additional reflexes to the anal sphincter, which caused dyssynergic defecation and prevented complete emptying of the rectum. This possibility was shown clearly in cat 1, where the evacuation of the third marble was incomplete due to the anal sphincter contraction holding the marble in place (Fig. 5A). If the pudendal nerve were blocked in this experiment, the external anal sphincter would have been relaxed and the third marble would have been fully



evacuated. However, pudendal nerve block will be ineffective if the dyssynergic defecation is caused by the contractions of internal anal sphincter (smooth muscle) instead of the external anal sphincter (striated muscle). The internal anal sphincter and rectal contraction pressures were not recorded in this study to insert marbles into the rectum to test defecation. A small movable balloon in the distal bowel is needed to measure pressure simultaneously as the balloon is eliminated during defecation (12). Thus, additional studies are warranted to better understand the rectum and anal sphincter coordination during the defecation induced by sacral root stimulation. It is worth noting that in cats some sensory fibers travel in the ventral roots to the spinal cord (13, 14). However, our results show that defecation induced by stimulating the ventral root alone is more efficacious than that induced by stimulating ventral and dorsal roots together. This difference indicates that the small population of sensory fibers traveling in the ventral root did not affect the responses induced by stimulation of the motor fibers in the ventral root as much as activation of the sensory fibers in the dorsal root.

In this study, the colon contraction and defecation induced by S2 root stimulation always required a stimulation intensity higher than the intensity to induce hindlimb and/or tail movement. This result is expected because in cats the afferent and efferent nerve fibers in the S2 spinal root innervating the colon are small unmyelinated C-fibers that have higher excitation threshold than the large motor fibers (12,

15). Since small C-fibers in the dorsal root are also involved in transmission of nociception, the stimulation intensity required for defecation could also generate painful sensation at the same time. This should not be a critical issue for restoring defecation function in people with a complete SCI, since they have no sensation below the level of the injury. However, it will be a problem to induce defecation for non-SCI people with chronic constipation if the extrinsic innervation of the human colon is similar to that in cats. Previous studies in humans (16, 17) have shown that colon contractions can be induced by sacral root stimulation at a suprasensory intensity without causing painful sensation, indicating that the sacral nerve fibers innervating the colon in humans might be different from those in cats. Therefore, it might be possible to induce defecation for non-SCI people with chronic constipation by sacral root stimulation without producing painful sensation.

Recent tests of sacral neuromodulation for treating non-SCI people with chronic constipation failed (6–8), which could be partially due to the use of a low stimulation intensity that only produces somatic sensations. This low stimulation intensity is probably not directly stimulating the nerve fibers in the sacral spinal root innervating the colon. However, it may modulate colon function indirectly by activating the large somatosensory fibers in the sacral root that can trigger central mechanisms to facilitate colon motility via the enteric nervous system (18). This central modulation mechanism could be sensitive to stimulation frequency, but

only a single stimulation frequency of 14 Hz was used in recent clinical trials to treat non-SCI people with chronic constipation (6–8). Our study shows that a lower frequency 7 Hz is optimal for sacral root stimulation to induce colon contractions, whereas other studies in rats and dogs (19, 20) found an effective frequency of 5 Hz or 10 Hz. Maximal colon contraction could also be induced in cats by directly stimulating the pelvic nerve at a frequency as low as 8 Hz (21). Therefore, it seems that a frequency lower than 14 Hz should be tested in clinical studies to treat non-SCI people with chronic constipation.

Our study also shows that continuous stimulation can cause fatigue of colon contractions, whereas intermittent stimulation is more fatigue resistant (Fig. 4). This indicates that the continuous stimulation of a sacral spinal root used in recent clinical trials (6–8) to treat chronic constipation may not be optimal and intermittent stimulation may be more effective in modulating colon motility to improve colon transit time. Recent animal studies (20, 22) have begun to focus on developing optimal stimulation parameters to facilitate colon motility and improve sacral neuromodulation therapy for non-SCI people with chronic constipation.

In summary, this study in cats optimized the stimulation parameters for sacral spinal root stimulation to induce colon contraction. The results have significant implications for design of a novel neuromodulation device to restore defecation function after SCI, and for optimizing sacral neuromodulation parameters to treat non-SCI people with chronic constipation.

GRANTS

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DISCLAIMERS

The views, opinions, and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

DISCLOSURES

Dr. Tai is the inventor of a patent related to this study. No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. conceived and designed research; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. performed experiments; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. analyzed data; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. interpreted results of experiments; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. prepared figures; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. drafted manuscript; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. edited and revised manuscript; J.W., Z.S., B.S., J.J., T.H., K.G., W.W., J.B., W.C.d.G., C.C., and C.T. approved final version of manuscript.

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Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats

Zhijun Shen ¹, Jicheng Wang ¹, Bing Shen ¹, Jianan Jian ¹, Khari Goosby ¹, William Wang ¹, Jonathan Beckel ², William C. de Groat ², Christopher Chermansky ¹, Changfeng Tai ^{1,2,3}

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

³ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Running Head: Penile Erection by Sacral Root Stimulation

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Conflict of Interest: Dr. Tai is the inventor of a patent related to this study. The other authors have no conflicts of interest.

Correspondence to:

Changfeng Tai, Ph.D.
Department of Urology
University of Pittsburgh
700 Kaufmann Building
Pittsburgh, PA 15213
Phone: 412-692-4142
Fax: 412-692-4380
Email: cftai@pitt.edu

ABSTRACT

Objective: This study aimed at determining if stimulation of sacral spinal roots can induce penile erection in cats.

Materials and Methods: In anesthetized cats, a 20-gauge catheter was inserted into the corpus cavernosum to measure the penile pressure. Stimulus pulses (5-80 Hz, 0.2 ms) were applied via bipolar hook electrodes to sacral ventral roots alone or to combined ventral and dorsal roots of a single S1-S3 segment to induce penile pressure increases and penile erection.

Results: Stimulation of the S1 or S2 ventral root at 30-40 Hz induced observable penile erection with rigidity and the largest increase (>150 cmH₂O) in penile pressure. Continuous stimulation (10 minutes) of afferent and efferent axons by simultaneous stimulation of the S1 or S2 dorsal and ventral roots at 30 Hz also produced a large (about 150 cmH₂O on average) increase in penile pressure that was sustainable during the entire stimulation period. After a complete spinal cord transection at the T9-T10 level, simultaneous stimulation of the S1 or S2 dorsal and ventral roots induced large (>100 cmH₂O) and sustainable increases in penile pressure.

Conclusion: This study indicates the possibility to develop a novel neuromodulation device to restore penile erection after spinal cord injury using a minimally invasive surgical approach to insert a lead electrode via the sacral foramen to stimulate a sacral spinal root.

Keywords: stimulation, sacral spinal root, erection, spinal cord injury, cat

INTRODUCTION

After spinal cord injury (SCI) erectile dysfunction presents as a short duration or an absence of penile erection, or poor penile rigidity that is insufficient for sexual intercourse (1,2). Although drug treatment is effective in restoring erectile function after SCI, it is not suitable for every SCI person due to the drug side effects and the health conditions of the SCI person (2). In addition to drug treatment, a fully implantable stimulator has also been used successfully in humans to restore penile erection after SCI by stimulating sacral anterior roots (3-5). However, sacral anterior root stimulation requires a major invasive spinal surgery to expose the sacral spinal roots for implantation of stimulation electrodes. Therefore, a minimally invasive neuromodulation approach that does not require invasive spinal surgery will be desirable for treatment of erectile dysfunction after SCI.

A minimally invasive surgical approach for stimulation of sacral spinal roots has been available for many years to treat patients with an overactive bladder (6). It employs a foramen needle to insert a tined lead electrode percutaneously under the guide of fluoroscopic imaging in proximity to the S3 spinal root that consists of both anterior (motor) and posterior (sensory) spinal roots. This minimally invasive sacral neuromodulation approach has also been evaluated in recent years to treat erectile dysfunction with a significant improvement in quality of sexual life for 20-40% subjects (7,8). However, the sacral neuromodulation stimulation parameters used in these studies to treat erectile dysfunction may not be optimal for improving penile erection because they were the same as those used to treat overactive bladder. Therefore, optimization of sacral neuromodulation parameters for treatment of erectile dysfunction is needed so that this minimally invasive surgical approach could be used as an effective therapy for SCI or non-SCI people with erectile dysfunction.

In this study using anesthetized cats, we investigated the penile response to electrical stimulation of an individual S1-S3 ventral root to determine the optimal stimulation frequency and the most effective spinal root in inducing penile erection. Then, combined stimulation of ventral and dorsal roots (termed spinal root stimulation) of the most effective spinal segment (S1 or S2) was used to induce sustainable (>10 minutes) penile erection. The information obtained in this preclinical study is important to design a human clinical trial to further evaluate sacral spinal root stimulation using the minimally invasive surgical approach as used in current clinical application (6) to treat erectile dysfunction in both SCI and non-SCI people.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical procedures

A total of 8 male cats (4.6 ± 0.2 kg) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder

via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened and each of the S1-S3 roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both dorsal and ventral roots together (see Fig.1). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2-3 mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord.

Experimental protocol

At the beginning of the experiment, stimulation (30 Hz frequency, 0.2 ms pulse width, and 1-2 min duration) was delivered via the hook electrode to sacral S1 ventral root at a minimal intensity (0.5 V). If no erection was observed, the stimulation intensity was increased and then tested again until a penile erection was observed. If S1 ventral root stimulation was not effective, then the stimulation electrode was moved to the S2 ventral root, and the test was repeated. A penile erection, which was evident as a full protrusion of the penis with rigidity resistant to bending, was always observed by stimulation of the individual S1 and S2 ventral roots. After the penile erection was observed, a 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (see

Fig.1). Since electrically evoked penile protrusion is very well correlated with increased corpus cavernosum pressure (9,10), the pressure recording was used as a quantitative measurement of penile erection in this study.

After placement of the penile catheter (N=8 cats), stimulation (30 or 40 Hz frequency, 0.2 ms pulse width, and 1 min duration) was delivered via the hook electrode to individual left or right sacral ventral roots (S1, S2, or S3) at a range of intensities (0.5-15 V) to determine the intensity threshold (T) for inducing an observable increase in penile pressure. Then, an intensity (1.5-3T) that induced the maximal penile pressure (>100 cmH₂O) was used to test penile responses to different stimulation frequencies (5-80 Hz). If no penile pressure >100 cmH₂O was induced by stimulation of an individual ventral root, the maximal stimulation intensity that did not produce movement of the animal's lower body was used to test the frequency response. It was our intention not to fix the hip and lower spine, so that sacral root stimulation by the hook electrode could produce local movement of the tail or hindlimb. However, the maximal stimulation intensity was limited by any movement of the lower spine, which would displace the hook electrode from the nerve. After testing each ventral root, the ventral root (S1 or S2) that elicited the largest penile pressure was combined with the dorsal root and the combination was stimulated continuously for 10 mins with the most effective frequency (30 Hz) and intensity to determine if the induced penile erection was sustainable during the entire stimulation period. Finally, the spinal cord was completely transected at the T9-T10 level (N=6 cats). Then, the continuous 10-minute stimulation of the same spinal root (ventral and dorsal roots together) was tested again to induce penile erection.

Data analysis

To compare the penile erection responses induced by stimulation of different ventral roots at different frequencies, the maximal amplitudes of the penile pressure induced by stimulation were measured and averaged across different animals for the same stimulation conditions. To determine the sustainability of the induced penile erection, the maximal penile pressure induced by the continuous 10-minute stimulations was compared to the pressure at the end of the 10-minute stimulation. To determine the effect of the spinal cord transection, the maximal penile pressures induced by the continuous 10-minute stimulation were compared before and after the spinal cord transection. The data from different animals are averaged and presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined by paired t-test or repeated-measures one-way ANOVA followed by Dunnett multiple comparison.

RESULTS

Penile erection dependent on stimulation frequency and the spinal segment stimulated.

Penile erection was observed as a full protrusion of the penis with rigidity in 6 cats by stimulation (30 Hz, 0.2 ms, 4-8 V) of S1 ventral root (S2 and S3 ineffective) and in 2 cats by stimulation of S2 ventral root (S1 and S3 ineffective). These responses which were quantified by penile pressure recordings showed that S1 or S2 ventral root stimulation in 6 cats and 2 cats, respectively, induced large increases in pressure (about 150 cmH₂O on average, Fig.2 A-C). The optimal stimulation frequency to induce penile erection by S1 or S2 ventral root stimulation was 30-40 Hz on average (Fig.2D), although lower frequencies (10-20 Hz, see Fig.2A) or higher frequencies (60-80 Hz, see Fig.2 A-B) could also induce large increases in penile pressure in

some cats. The stimulation intensity (0.6-12 V, mean 3.5 ± 1.4 V) for 30-40 Hz S1 or S2 ventral root stimulation to induce the largest increase in penile pressure was always above the motor threshold to induce leg muscle contraction (S1) or anal sphincter contraction (S2).

Sustainable penile erection induced by S1/S2 spinal root stimulation

Continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (i.e., ventral and dorsal roots together) induced a large increase (>150 cmH₂O) in penile pressure (Fig.3). The maximal penile pressure was either sustained (Fig.3A) or gradually developed (Fig.3B) during the 10-minute stimulation. On average (N=8 cats), the penile pressure at the end of 10-minute stimulation was not significantly ($p>0.05$) different from the maximal pressure developed during the stimulation (Fig.3C). The maximal penile pressure induced by stimulation of ventral and dorsal root together was also not significantly ($p>0.05$) different from the maximal pressure induced by stimulation of ventral root alone (Fig.2D and Fig.3C).

After a complete spinal cord transection at the T9-T10 level, continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (ventral and dorsal roots together) also induced a large increase (>100 cmH₂O) in penile pressure (Fig.4). As seen prior to spinal transection, the maximal penile pressure was also sustained or gradually developed (Fig.4B) during the 10-minute stimulation. However, on average (N=6 cats) the induced penile pressure was significantly ($p<0.05$) reduced to 113 ± 19 cmH₂O after spinal cord transection when compared to the penile response (186 ± 9 cmH₂O) before the spinal cord transection (Fig.4C). The blood pressure was also significantly ($p<0.05$) reduced from 229 ± 11 cmH₂O to 186 ± 7 cmH₂O by spinal cord transection, but it was not changed by sacral root stimulation.

DISCUSSION

This study in anesthetized cats shows that the penile erection induced by sacral root stimulation is dependent on stimulation frequency and the spinal segment stimulated. S1/S2 ventral root stimulation at 30-40 Hz is optimal to induce the largest increase (>150 cmH₂O) in penile pressure (Fig.2). Stimulation of the S1/S2 spinal root (ventral and dorsal roots together) is also effective in inducing a large penile pressure increase that is sustainable for the entire 10-minute stimulation (Fig.3). More importantly, large sustainable penile pressure increase (>100 cmH₂O) can also be induced by S1/S2 spinal root stimulation after an acute complete transection of the spinal cord (Fig.4). These results are important for the design of a new neuromodulation device to treat erectile dysfunction.

Previous studies have shown that penile erection can be induced by electrical stimulation of pelvic nerve, cavernous nerve, or dorsal penile nerve in mice, rats, cats, dogs, monkeys, or humans (11-15). However, the pelvic and cavernous nerves require invasive surgery for implantation of stimulation electrodes while the dorsal penile nerve is not a convenient location to attach a stimulation electrode during sexual intercourse. Therefore, these stimulation methods are not adopted for clinical application to treat erectile dysfunction. Sacral ventral root stimulation was also shown to be effective in inducing penile erection in this study and in previous studies using cats (16) and dogs (17). In addition, sacral anterior root stimulation was used successfully to restore bladder function and erectile function for SCI people (3-5,18). However, invasive spinal surgery is required to implant the stimulation electrodes on the sacral anterior roots, preventing this effective treatment to be used in SCI or non-SCI people for the sole purpose of treating erectile dysfunction. Our study in cats indicates that penile erection can be induced by stimulation of the sacral spinal roots. A previous study in dogs also showed a

similar effect (17). The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to employ a minimally invasive surgical approach for stimulation. Sacral spinal roots in humans are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder (6). Therefore, our study has significant implications for developing a new sacral neuromodulation therapy using a minimally invasive surgical approach to restore erectile function for both SCI and non-SCI people.

Previous studies using sacral neuromodulation to treat overactive bladder also evaluated the effects on penile erection in both SCI and non-SCI people (7,8). Questionnaires indicate that subjects with moderate-mild erectile dysfunction significantly improved their quality of sexual life by sacral neuromodulation therapy. However, these studies used stimulation parameters optimized to treat overactive bladder, i.e., stimulation frequency 20 Hz at the sensory threshold intensity. These stimulation parameters may not be optimal for the treatment of erectile dysfunction because our study indicates that a higher stimulation frequency (30-40 Hz) at intensities above sensory and motor thresholds can induce penile erection quickly once the stimulation is turned on and the erection can be sustained during the entire 10-minute stimulation. This type of on-demand erection should be much more satisfactory for the patient than the quality-of-life improvement produced by continuous (24 hours x 7 days) sacral neuromodulation at a lower frequency and weaker stimulation intensity. Therefore, it seems that a higher frequency (30-40 Hz) and a stronger stimulation intensity (above sensory and motor thresholds) should be tested in clinical studies to treat erectile dysfunction. Although leg movement will be induced by a stronger stimulation, the leg muscle contraction will be tonic and the movement will only occur at the beginning of the stimulation, which should not be a problem for successful

sexual intercourse. When anterior root stimulation was used to induce penile erection in SCI people, it also induced leg movement but did not prevent successful sexual intercourse (18).

Stimulation of the sacral spinal root (ventral and dorsal roots together) induced a large increase in penile pressure similar to that produced by stimulation of the ventral root alone (Fig.3). This result indicates that activation of additional sensory nerves in the dorsal root did not disrupt the penile erection induced by direct stimulation of the efferent nerves in the ventral root. A previous study in dogs (17) also reported no difference in penile erection elicited by ventral root stimulation alone or by stimulation of ventral and dorsal roots together. In human application under awake conditions, activation of sensory nerves may cause uncomfortable or painful sensation that can limit the stimulation intensity and therefore limiting the efficacy of the stimulation in treating erectile dysfunction. This potential issue can only be resolved by a clinical study in non-SCI human subjects. However, it should not be an issue for subjects with a complete SCI who have no sensation below the injury.

Our study shows that the penile erection induced by sacral root stimulation is sustainable or gradually develops during the 10-minute stimulation (Fig.3 A-B). This indicates that sacral root stimulation can produce long duration erections when it is applied for a longer time. It also indicates that stimulation of afferent nerves in the dorsal root did not reflexively activate the inhibitory sympathetic pathway that can suppress penile erection (19). Sacral root stimulation probably activated both parasympathetic and somatic pathways to produce the erection and penile rigidity (19, 20). However, after spinal cord transection the amplitude of the induced penile pressure was significantly reduced, possibly due to the spinal shock effect and the decrease in blood pressure after an acute spinal cord transection. Nevertheless, sacral root stimulation still induced a large (>100 cmH₂O on average, Fig.4) sustained increase in penile

pressure after acute spinal cord transection, indicating that this stimulation could be useful in restoring erectile function after SCI (21). Whether sacral root stimulation will be less effective in SCI people than in non-SCI people to induce penile erection is a question to be answered by human clinical studies. However, it is known that sacral anterior root stimulation is effective in inducing penile erection strong enough for sexual intercourse in SCI people (3-5).

In summary, this study in cats optimized the stimulation parameters for sacral spinal root stimulation to induce penile erection. The results have significant implications for design of a novel neuromodulation device to restore erectile function after SCI, and for optimizing sacral neuromodulation parameters to treat non-SCI people with erectile dysfunction.

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FIGURE CAPTION

Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

Fig.2: Penile pressure in the corpus cavernosum induced by electrical stimulation of sacral ventral roots at different frequencies and different spinal segments. A. In a cat, S1 ventral root

stimulation (10-80 Hz) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. B. In another cat, S2 ventral root stimulation (30-80 Hz) of short duration (1 minute) was most effective in inducing large increases (150 cmH₂O) in penile pressure. C. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. * indicates significantly different from S1 ventral root ($p < 0.05$, ANOVA). Stimulation: 30 Hz, 0.6-12 V, 0.2 ms. D. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root. N = 8 cats. * indicates significantly different from 5 Hz ($p < 0.05$, ANOVA). Stimulation: 0.6-12 V, 0.2 ms.

Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

Fig.4: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the 10 minutes of stimulation. B. In the same cat, 10 minutes after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure

during the first 3 minutes and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference ($p=0.0025$, paired t-test).

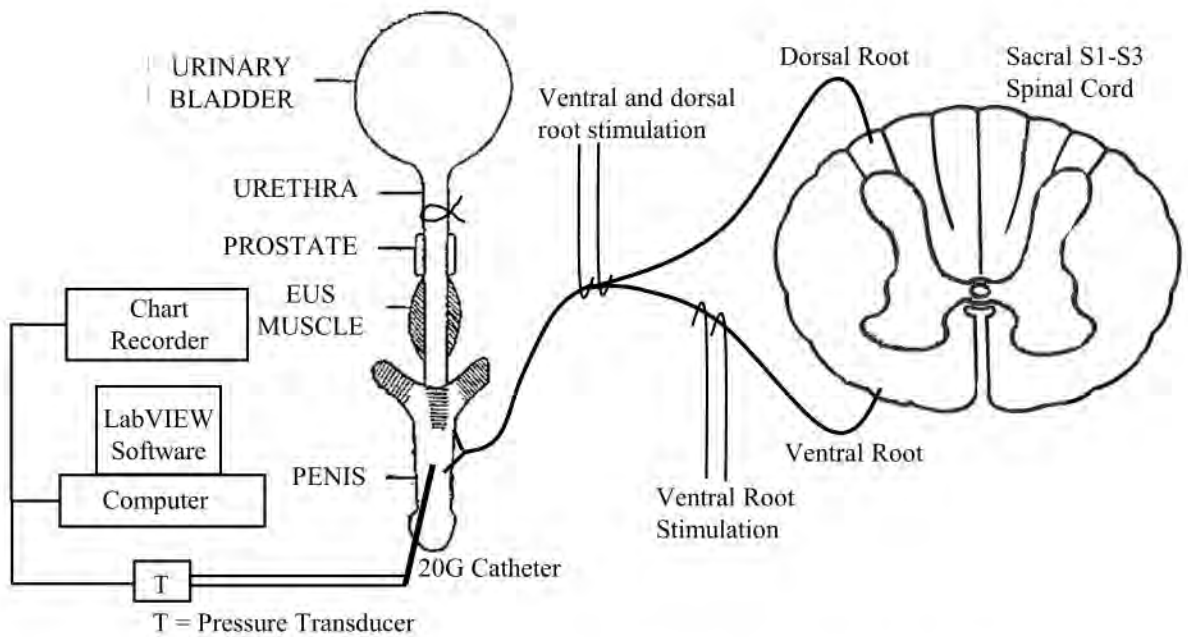
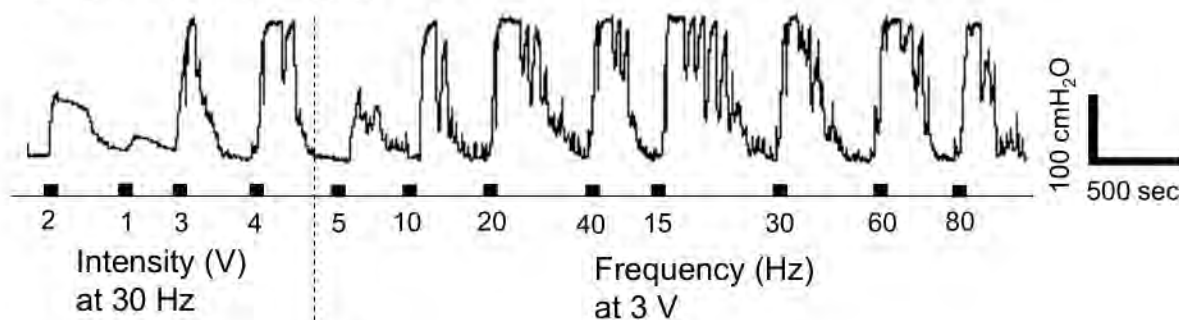
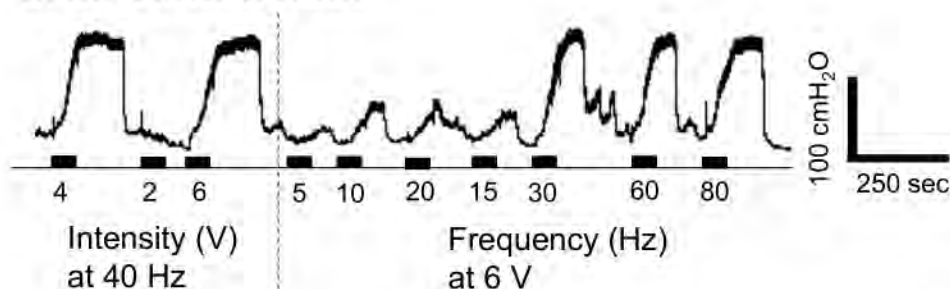


Fig.1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

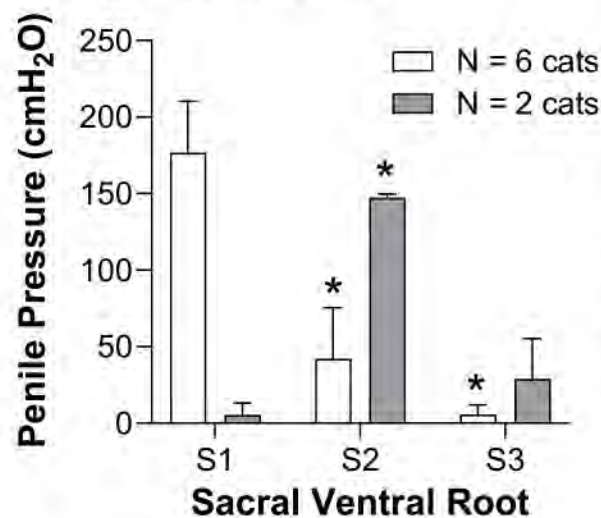
A. S1 Ventral Root



B. S2 Ventral Root



C. Segmental Effect



D. Frequency Effect

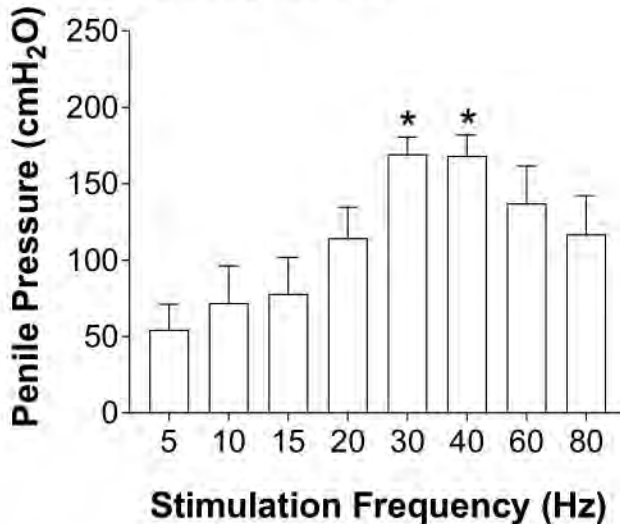
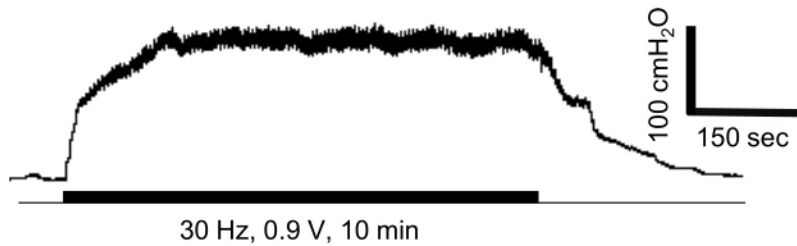
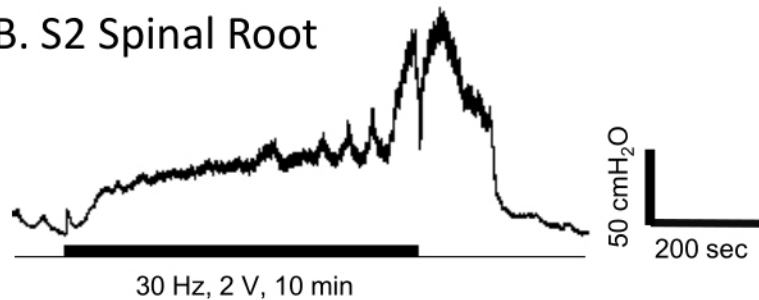


Fig.2: Penile pressure in the corpus cavernosum induced by electrical stimulation of sacral ventral roots at different frequencies and different spinal segments. A. In a cat, S1 ventral root stimulation (10-80 Hz) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. B. In another cat, S2 ventral root stimulation (30-80 Hz) of short duration (1 minute) was most effective in inducing large increases (150 cmH₂O) in penile pressure. C. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. * indicates significantly different from S1 ventral root ($p < 0.05$, ANOVA). Stimulation: 30 Hz, 0.6-12 V, 0.2 ms. D. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root. N = 8 cats. * indicates significantly different from 5 Hz ($p < 0.05$, ANOVA). Stimulation: 0.6-12 V, 0.2 ms.

A. S1 Spinal Root



B. S2 Spinal Root



C. Sustained Pressure

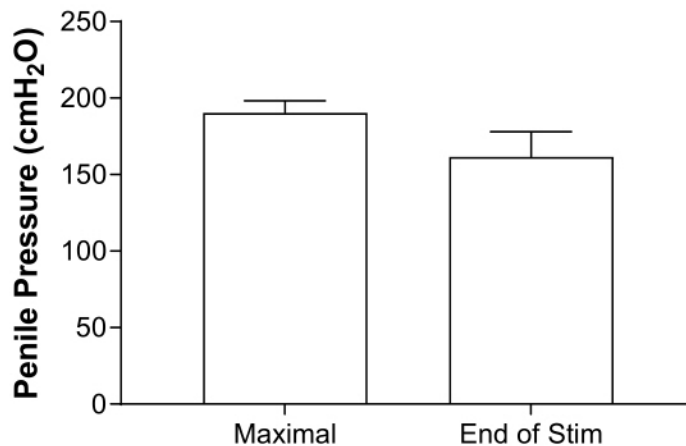
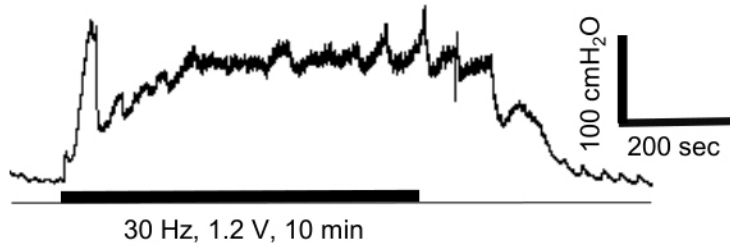
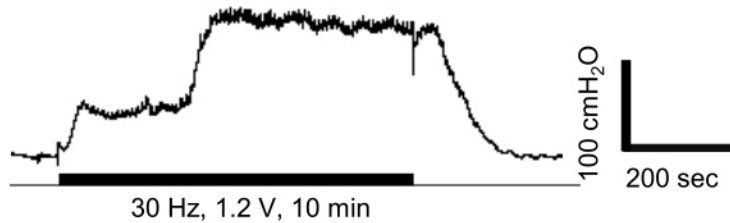


Fig.3: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). A. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. B. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation reaching a penile pressure of 140 cmH₂O at the end of the stimulation. C. On average (N=8 cats) the penile pressure at the end of 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation.

A. Before Spinal Transection



B. After Spinal Transection



C. Maximal Pressure

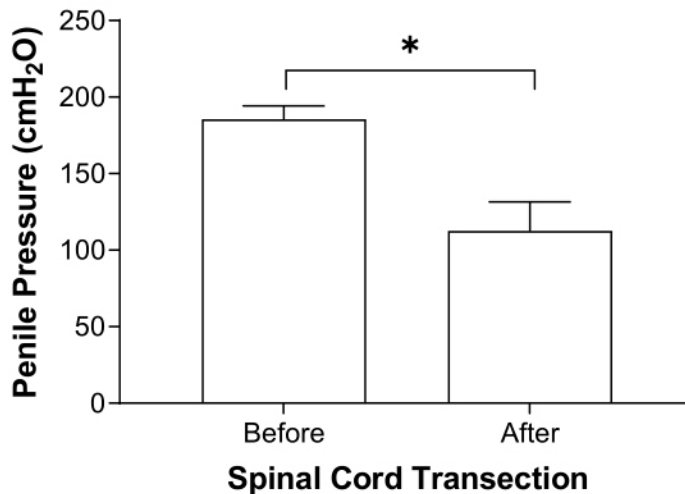


Fig.4: Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. A. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and then maintained a high pressure during the 10 minutes of stimulation. B. In the same cat, 10 minutes after spinal cord transection S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure during the first 3 minutes and then triggered a fast pressure increase reaching a maximal penile pressure of 150 cmH₂O. C. On average (N=6 cats) the maximal penile pressure induced by sacral S1/S2 root stimulation was significantly reduced by the acute spinal cord transection. * indicates a significant difference (p=0.0025, paired t-test).

Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats

Jialiang Chen ^{1,2}, Yihua Zhong ^{1,3}, Jicheng Wang ¹, Bing Shen ¹, Zhijun Shen ¹,
Jonathan Beckel ⁴, William C. de Groat ⁴, Christopher Chermansky ¹, Changfeng Tai ^{1,4,5}

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Urology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, P.R. China

³ School of Biomedical Engineering, Capital Medical University, Beijing, P.R. China

⁴ Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

⁵ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Running Head: Vaginal Lubrication by Pudendal Nerve Stimulation

Abstract: 307 words

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Correspondence to:

Changfeng Tai, Ph.D.
Department of Urology
University of Pittsburgh
700 Kaufmann Building
Pittsburgh, PA 15213
Phone: 412-692-4142
Fax: 412-692-4380
Email: cftai@pitt.edu

ABSTRACT

Background: Vaginal lubrication and contractions are among the top difficulties affecting sexual intercourse in women after spinal cord injury (SCI).

Aim: This study aimed at determining if pudendal nerve stimulation (PNS) can improve vaginal lubrication and induce increases in vaginal pressure.

Methods: In anesthetized cats, a small piece of cotton was inserted into the vagina for 10 minutes with or without PNS to measure vaginal wetness by the weight increase of the vaginal cotton. Then, a small balloon catheter was inserted into the vagina to measure the pressure increase induced by PNS. Intensity response of the vagina to PNS (30 Hz, 0.2 ms, 5 secs) was determined at 1-4 times of intensity threshold (T) for PNS to induce an observable vaginal pressure increase. Frequency response was determined at 2T intensity in a range of PNS frequencies (5-50 Hz). Finally, fatigue in vaginal pressure was determined by applying PNS (30 Hz, 2T) either continuously or intermittently (5 secs on and 5 secs off) for 4 minutes.

Outcomes: The effectiveness of PNS in increasing vaginal wetness and pressure is evaluated.

Results: PNS significantly ($p=0.0327$) increased the measurement of vaginal wetness from 15.8 ± 3.8 mg during control without stimulation to 32.4 ± 4.7 mg after stimulation. Vaginal pressure increased as PNS intensity or frequency increased. PNS (30 Hz, 2T) induced vaginal pressure increase $\geq 80\%$ of the maximal response. Intermittent PNS induced significantly ($p=0.0354$) smaller fatigue ($45.6\pm3.7\%$) in vaginal pressure than continuous PNS ($69.1\pm3.0\%$) during the 4-minute stimulation.

Clinical Translation: This study indicates the possibility to develop a novel pudendal neuromodulation device to improve both female sexual function and bladder function after SCI.

Strengths & Limitations: This study provides preclinical data supporting the development of a novel pudendal neuromodulation device. The limitation includes the lack of chemical analysis of the vaginal secretion.

Conclusion: PNS can improve vaginal lubrication and induce increases in vaginal pressure.

Keywords: stimulation, pudendal nerve, vagina, spinal cord injury, cat

INTRODUCTION

Spinal cord injury (SCI) impairs sexual function in women both psychologically and physically.¹⁻³ About 65% of SCI women complain about vaginal lubrication that is one of the top three difficulties affecting sexual intercourse.^{1,4,5} In addition, urinary incontinence during sexual intercourse, which reduces desire for sexual activity with a partner, is also a significant issue for SCI women.¹ An implantable neuromodulation device has been developed to restore urinary bladder function and has also been used to induce penile erection for SCI men.⁶ However, a neuromodulation device to restore both bladder and sexual functions for SCI women is still not available.

Our recent preclinical studies in cats^{7,8} have shown that pudendal nerve block and stimulation could be a very promising new approach to restore urinary bladder function after SCI. Since the pudendal nerve also innervates the female sexual organ, it might be possible to improve female sexual function as well as bladder function after SCI by pudendal nerve stimulation (PNS). To explore this opportunity, in this study we investigated the effects of PNS

on vaginal lubrication and vaginal pressure increase in anesthetized cats. If successful, the potential clinical promise is that a novel pudendal neuromodulation device could be developed to empty the bladder first to prevent urinary incontinence during sexual intercourse, and then produce vaginal lubrication and induce increase/change in vaginal pressure during the sexual intercourse for women with SCI.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical procedures

Four female cats (2.7-3.4 kg) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. The left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via urethra to drain the bladder during the experiment and the urethra was tied with a suture. The pudendal nerve on the left side was dissected through an incision in the region of sciatic notch and a bipolar cuff electrode (NC223pt, MicroProble, Gaithersburg, MD) was implanted on the nerve

for electrical stimulation. After the surgery, all incisions were closed with sutures. A heating pad was used to maintain the animal body temperature between 35 and 37 °C during the experiment.

Experimental protocol

At the beginning of the experiment, a small piece of dry cotton was weighed (about 30 mg) and inserted into the vagina 2-3 cm from the external orifice for 10 minutes without stimulation. After the 10 minutes, the cotton was removed and weighed again to determine the increase in weight that was used as the control value to indicate the vaginal wetness. Then, the same 10-minute cotton test was repeated using another piece of dry cotton (weight about 30 mg) while PNS (30 Hz, 0.2 ms) at the 2 times intensity threshold for inducing anal twitching was also applied intermittently (5 secs on and 5 secs off) during the 10-minute period to increase the vaginal wetness.

After testing the vaginal wetness, a small catheter (7 French) with a balloon at the tip was inserted into the vagina with the balloon slightly distended by 0.2 ml air to measure vaginal pressure increase. PNS (30 Hz, 0.2 ms) was applied for 5 secs each time at five intensities increasing from 1T to 4T to determine the intensity response. Then, PNS at an intensity of 2 times the threshold (T) for inducing a small observable vaginal pressure increase was applied at different frequencies (5-50 Hz) to determine the frequency response. Finally, PNS (30 Hz, 0.2 ms, 2T) was applied for 4 minutes either continuously or intermittently (5 secs on and 5 secs off) in a random order to determine fatigue in vaginal pressure during the 4-minute-long stimulation. A time interval of 5-10 minutes was inserted between the two 4-minute PNS to avoid any carry-over fatigue effect.

Data analysis

Vaginal wetness was measured by the weight increase of the cotton inserted in the vagina under control condition or during PNS. The vaginal pressure increases induced by PNS of different intensities or frequencies was normalized to the maximal response to determine the effect of PNS intensity or frequency. Vaginal fatigue was measured as the vaginal pressure reduction at the end of the 4-minute PNS divided by the maximal vaginal pressure increase during the PNS. The data from different animals are averaged and presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined by paired t-test or repeated-measures one-way ANOVA followed by Dunnett multiple comparison.

RESULTS

PNS applied intermittently (5 secs on and 5 secs off) for 10 minutes at the intensity of 2 times threshold for inducing anal twitching significantly increased vaginal wetness (Fig.1). Without PNS, the vaginal wetness as indicated by the increase in the vaginal cotton weight during a 10-minute period was 15.8 ± 3.8 mg (control in Fig.1). After 10-minute PNS, the vaginal wetness was significantly ($p=0.0327$) increased to 32.4 ± 4.7 mg (PNS in Fig.1).

The vaginal pressure was significantly ($p < 0.05$) increased as the PNS intensity increased (Fig.2). PNS (30 Hz) at an intensity of $\geq 2T$ induced a vaginal pressure increase equal to 90% of the maximal response (Fig.2B). Meanwhile, at intensity of $2T$ the PNS induced a significantly ($p < 0.05$) larger increase in vaginal pressure at frequencies ≥ 20 Hz than ≤ 10 Hz (Fig.3). When PNS (30 Hz, $2T$) was applied for 4 minutes, it produced less fatigue when applied intermittently (Fig.4A) than continuously (Fig.4B). On average ($N=4$), intermittent PNS induced $45.6 \pm 3.7\%$

fatigue that is significantly ($p=0.0354$) smaller than the fatigue induced by continuous PNS ($69.1\pm3.0\%$, Fig.4C).

DISCUSSION

This study in anesthetized female cats shows that PNS can improve vagina lubrication by increasing the wetness of the vagina (Fig.1). PNS induced large increase in vaginal pressure (Fig.2 and Fig.3) that is more fatigue resistant during intermittent than during continuous stimulation (Fig.4). These results suggest that PNS might be useful to improve female sexual function after SCI. Since our recent studies in cats have shown that PNS can also enhance urine storage and voiding after SCI,^{7,8} a novel pudendal neuromodulation device could be developed to benefit SCI women by enhancing vaginal lubrication and inducing vaginal pressure increase/change as well as preventing urinary incontinence during sexual intercourse by emptying the bladder before sexual activity.

Previous animal studies in mice, rats, rabbits, and dogs have shown that vaginal blood flow and vaginal lubrication could be increased by mechanical stimulation of the vagina or electrical stimulation of the pelvic nerve or clitoral nerve.⁹⁻¹³ Our current study in cats shows that PNS can also increase vaginal lubrication. Since the clitoral nerve is a branch of the pudendal nerve, PNS might have activated clitoral afferent input to the central nervous system to trigger a reflex output via the pelvic nerve to increase the blood flow and lubrication of the vagina.¹⁴⁻¹⁶ In addition, since the pudendal motor axons innervate the striated muscle surrounding the vagina,^{17,18} anal sphincter, and pelvic floor muscles, PNS can produce contraction of these muscles to increase vaginal pressure and further facilitate vaginal secretion.¹⁴ Periodic or pulsed

contractions of this groups of pelvic muscles also occur during orgasm.^{14,19} Therefore, in addition to increasing vaginal lubrication PNS may also induce or facilitate orgasmic sensations for women with incomplete SCI who still have some sensation from the vagina or pelvic area. Only continuous and intermittent PNS were tested in this study (Fig.4), but other stimulation patterns that more closely mimic those during orgasm could also be induced by programing the PNS stimulation intensity and duration.

Since the urethra was ligated by a suture, it is not possible for bladder to leak any urine into the vagina to change the vaginal cotton wetness. Therefore, the increase in the vaginal cotton weight must be due to fluid accumulation in the vagina during the 10-minute period. Vagina lubrication during sexual arousal is mainly due to the increased blood flow in the vagina producing transudate on the surface of the vaginal wall.²⁰ In addition, vaginal fluid could also contain secretions from female prostate or Bartholin's glands.²⁰ Therefore, it is highly likely that the fluid absorbed by the cotton in this study contains both the transudate from vaginal blood vessels and secretions from female sexual glands. However, the vaginal fluid collected by the cotton in the vagina was not analyzed for the chemical composition in this study.

In summary, this study in cats shows that PNS can improve vaginal lubrication and induce vaginal pressure increase/change. These results indicate that it is possible to develop a pudendal neuromodulation device to restore or improve female sexual function after SCI. Since our recent studies in cats (7,8) have shown that pudendal nerve stimulation/block can successfully restore bladder function after SCI, this current study indicates a possibility to develop a novel implantable pudendal neuromodulation device to restore both bladder and sexual function for women with SCI.

ACKNOWLEDGEMENT

This study is funded by the Bridging the Gap Plus (BG+) program of the Defense Advanced Research Projects Agency (DARPA) under the contract N66001-20-C-4050. The views, opinions and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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FIGURE CAPTION

Fig.1. Pudendal nerve stimulation (PNS) increased vaginal wetness. PNS: 30 Hz, 0.2 ms, 1.5T = 0.15-0.45 V, 5 secs on and 5 secs off for 10 minutes. T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p=0.0327$). N = 4 cats.

Fig.2. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation intensities. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 30 Hz, 0.2 ms, 5 secs, T = 0.16 V. T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 30 Hz, 0.2 ms, 5 secs, T = 0.16-0.5 V. * indicates significantly different from 1T (one-way ANOVA). N = 4 cats.

Fig.3. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation frequencies. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 2T = 0.32 V, 0.2 ms, 5 secs. T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 2T = 0.32-1 V, 0.2 ms, 5 secs. * indicates significantly different from 5 Hz (one-way ANOVA). N = 4 cats.

Fig.4. Fatigue of vaginal pressure increases induced by prolonged (4 minutes) pudendal nerve stimulation (PNS). A. Vaginal pressure responses induced by intermittent PNS. B. Vaginal pressure responses induced by continuous PNS. Black bars under the pressure trace indicate the PNS duration. C. Fatigue of vaginal pressure responses. The reduction in vaginal pressure responses at the end of 4-minutes PNS was normalized to the maximal response to indicate the muscle fatigue. PNS: 30 Hz, 0.2 ms, 2T = 0.32-1 V, 4 minutes. T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p = 0.0354$). N = 4 cats.

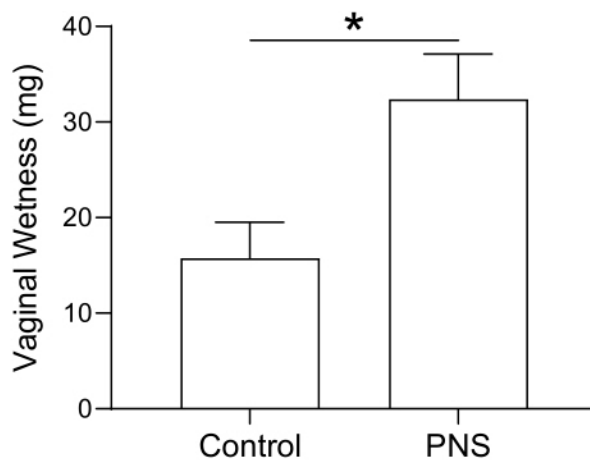
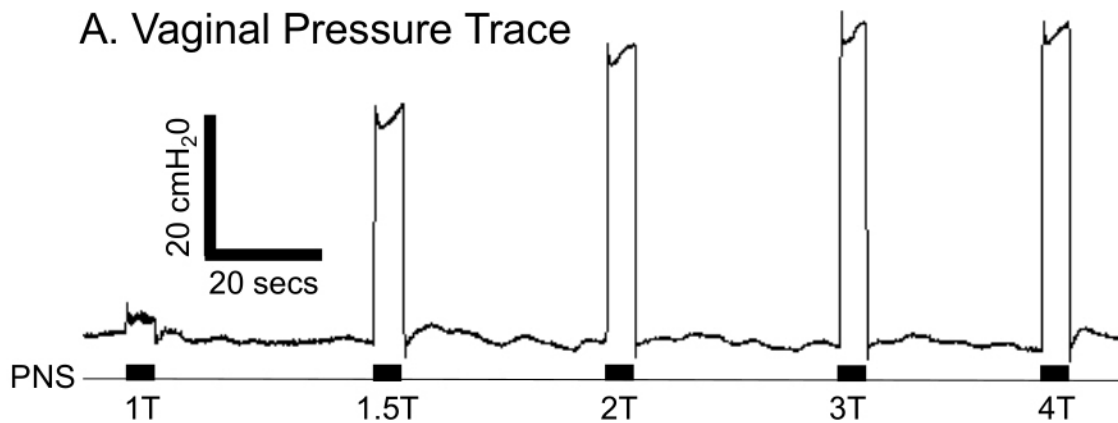


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B. Normalized Vaginal Pressure

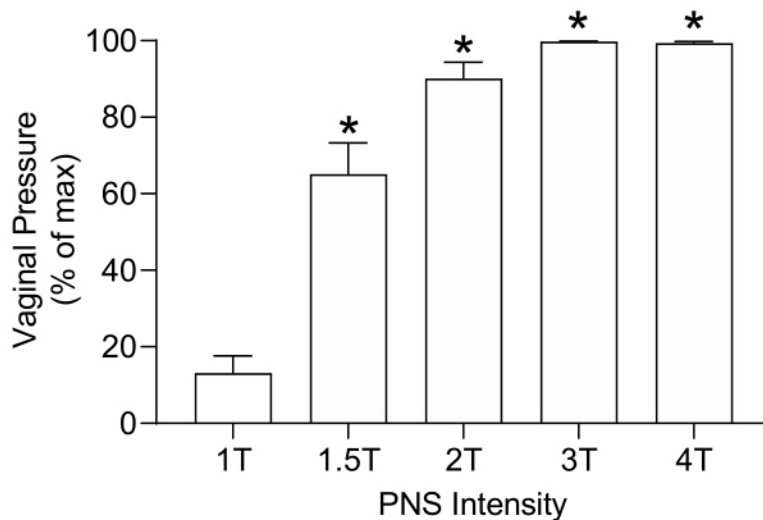
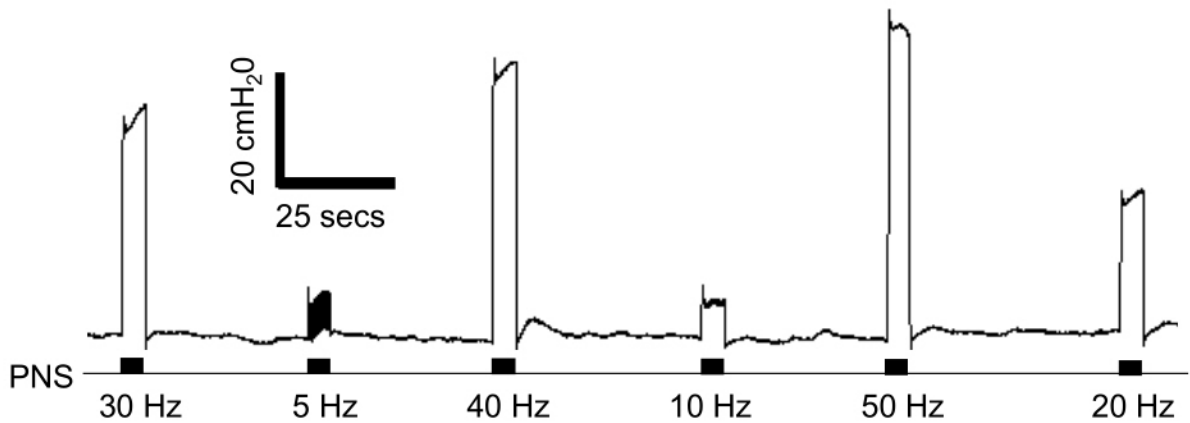


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A. Vaginal Pressure Trace



B. Normalized Vaginal Pressure

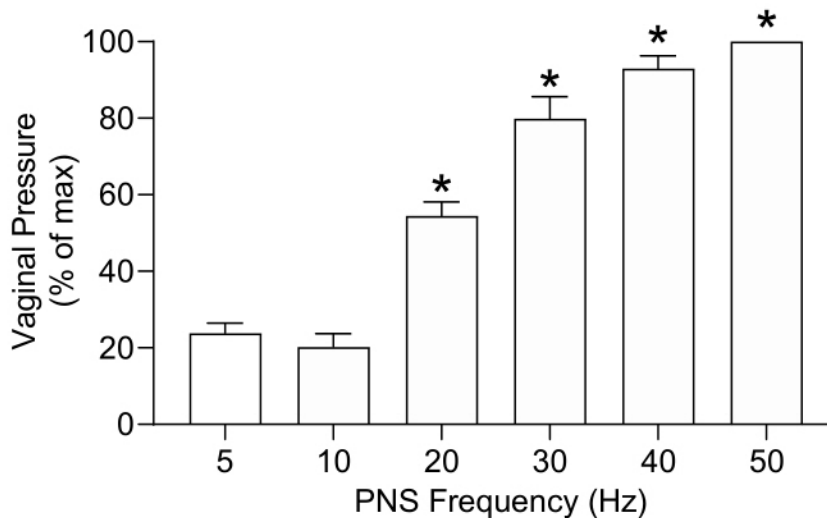


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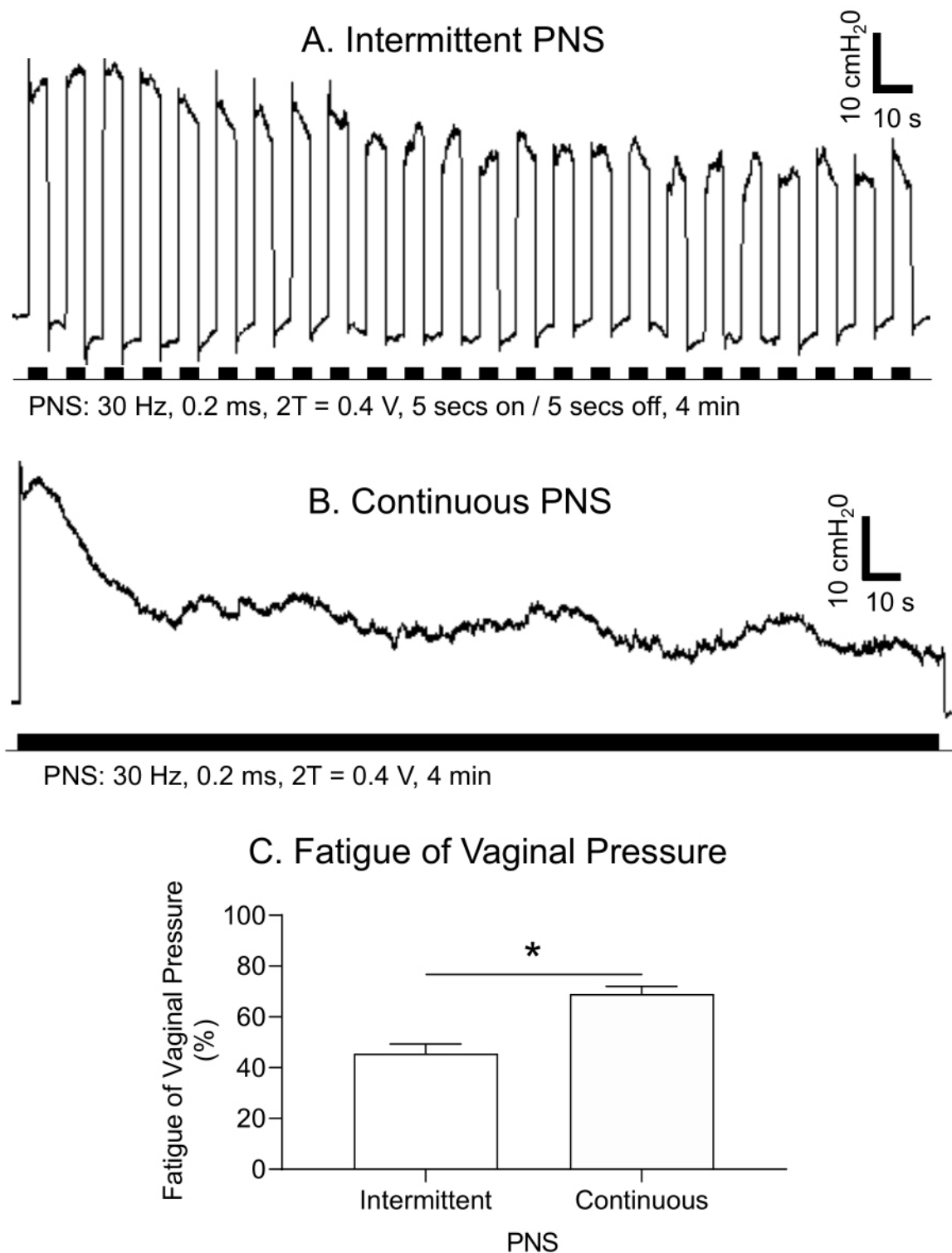


Fig.4. Fatigue of vaginal pressure increases induced by prolonged (4 minutes) pudendal nerve stimulation (PNS). A. Vaginal pressure responses induced by intermittent PNS. B. Vaginal pressure responses induced by continuous PNS. Black bars under the pressure trace indicate the PNS duration. C. Fatigue of vaginal pressure responses. The reduction in vaginal pressure responses at the end of 4-minutes PNS was normalized to the maximal response to indicate the muscle fatigue. PNS: 30 Hz, 0.2 ms, 2T = 0.32-1 V, 4 minutes. T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p = 0.0354$). N = 4 cats.

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University of Pittsburgh

Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: February 1, 2022 through April 30, 2022

(b)(4)

Date of Report: May 31, 2022

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: \$10,701,439.00

Subcontractors: (b)(4) and Thermaquil, Inc.

Program Manager: (b)(4), (b)(6)

Submitted by:

(b)(4), (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) we have made major progresses in the following areas:

1. Scientific progress:

- We have successfully demonstrated in 4 anesthetized female cats that pudendal nerve stimulation at (b)(4) is most effective to induce vaginal lubrication and contractions. This success indicates that our pudendal stimulation/block device can also be used to restore female sexual function after SCI in addition to restoring bladder function. We have finished processing and analyzing the data/results from this animal study and the manuscript has been submitted to The Journal of Sexual Medicine for publication.
- We have successfully demonstrated in 4 anesthetized cats that the nerve activity recorded by (b)(4)

(b)(4)

- Our third paper titled “Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats” has been reviewed by Neuromodulation with a revision decision for publication.
- Our fourth paper titled “Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats” has been submitted to The Journal of Sexual Medicine for publication (see attachment).
- We also submitted the early feasibility human study protocol for review by the IRB at the University of Pittsburgh.

2. Engineering progress:

- (b)(4) development is completed with FDA IDE approval.
- The (b)(4) were tested and are ready to be shipped out for the IDE study.
- The (b)(4) circuit board design is completed. Part placement was also completed, with routing of the PCB better than half done prior to seeking quotes from PCBA manufacturing suppliers.
 - (b)(4)
- (b)(4)
- The (b)(4) manufacturing process continues to be improved upon, inspected, and tested.
 - (b)(4)
 -
 -
- The first prototypes of (b)(4) are being created to be verified in cat study.
- The (b)(4) continues to be developed. The design of the (b)(4) was completed, and fabrication of the (b)(4) is currently in process.
- Operational qualifications were completed for the (b)(4)
- Process qualifications have begun for the (b)(4)
- Biocompatibility quotes for (b)(4) have been received and the biocompatibility effort continues amongst suppliers, consultants, and test laboratories.
 - Exact surface areas of (b)(4) were calculated for biological evaluation.
 - The team has been verifying quantities needed for testing. With those numbers, parts are currently being ordered in preparation.

- (b)(4) hired consultants for biocompatibility to prepare an overall test evaluation plan and optimal steps forward.
 - Verification testing for the human (b)(4) patient controller is underway. Modifications were made to the GUI (b)(4)
 - An IFU for the (b)(4) was delivered as part of the IDE documents.
3. Regulatory progress:
- Revised our draft early feasibility human clinical protocol for the (b)(4)
 - Submitted the FDA IDE and the IDE was unconditionally approved
 - Drafted and submitted Pitt IRB clinical protocol
4. Administrative progress:
- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.
 - Weekly meetings were hold between University of Pittsburgh, (b)(4) and Thermoquil to coordinate the project activity.
 - Monthly meeting was hold with DARPA to report our progress.
 - (b)(4)

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.3 Restore sexual function (b)(4)

During this quarter and previous quarter, a total of 4 female cats were used to study the female sexual function by stimulation of sacral roots or pudendal nerve. The animals were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via urethra to drain the bladder during the experiment and the urethra was tied with a suture. Then, the abdominal incision was closed with sutures.

At the beginning of the experiment, a small piece of dry cotton was weighted and inserted into the vagina for 10 minutes without any stimulation. After the 10 minutes, the cotton was pulled out and weighted again to determine the increase in the cotton's weight that is used as the control value to indicate the vaginal wetness. Then, the same 10-minute cotton test was repeated using another piece of dry cotton while pudendal nerve stimulation (PNS: 30 Hz, 0.2 ms) at the 2 times intensity threshold for inducing anal twitching was also applied intermittently (5 secs on and 5 secs off) during the 10-minute period to increase the vaginal wetness.

After testing the vaginal wetness, a small catheter (b)(4) was inserted into the vagina with the balloon slightly distended by 0.2 ml air to measure vaginal contraction. PNS (b)(4) was applied for 5 secs each time at different intensities to determine the intensity response. Then, PNS at an intensity of 2 times the threshold (T) for inducing a small observable vaginal contraction was applied at different frequencies (b)(4) to determine the frequency response. Finally, PNS (b)(4) was applied for 4 minutes either continuously or intermittently (5 secs on and 5 secs off) in a random order to determine vaginal fatigue during the 4-minute-long contraction. A time interval of 5-10 minutes was inserted between the two 4-minute PNS to avoid any carry-over fatigue effect.

PNS applied intermittently (5 secs on and 5 secs off) for 10 minutes significantly increased vaginal wetness (Fig.1). Without PNS, the vaginal wetness as indicated by the increase in the vaginal cotton weight during a 10-minute period is 15.8 ± 3.8 mg (control in Fig.1). After 10-minute PNS, the vaginal wetness was significantly ($p=0.0327$) increased to 32.4 ± 4.7 mg (PNS in Fig.1).

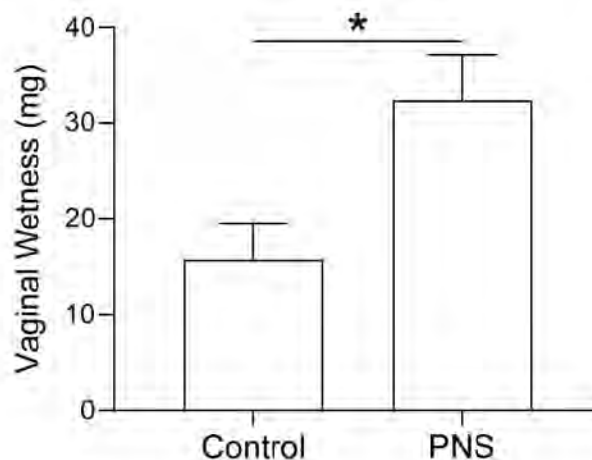


Fig.1. Pudendal nerve stimulation (PNS) increased vaginal wetness. PNS: (b)(4)
(b)(4) T = threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p=0.0327$). N = 4 cats.

Vaginal contraction was significantly ($p < 0.05$) increased as the PNS intensity increased (Fig.2). PNS (b)(4) at an intensity (b)(4) induced vaginal contraction stronger than 90% of the maximal contraction (Fig.2). Meanwhile, at intensity of 2T the PNS induced significantly ($p < 0.05$) large vaginal contraction at frequencies (b)(4) (Fig.3). At frequencies (b)(4) PNS induced vaginal contraction larger than 80% of the maximal contraction (Fig.3). When PNS (b)(4) was applied for 4 minutes, it produced less fatigue when applied intermittently than continuously (Fig.4). On average ($N=3$), intermittent PNS induced fatigue that is significantly ($p=0.0354$) smaller than continuous PNS Fig.4).

In summary, our studies in anesthetized female cats have shown successfully that sexual functions could be restore after SCI by pudendal nerve stimulation.

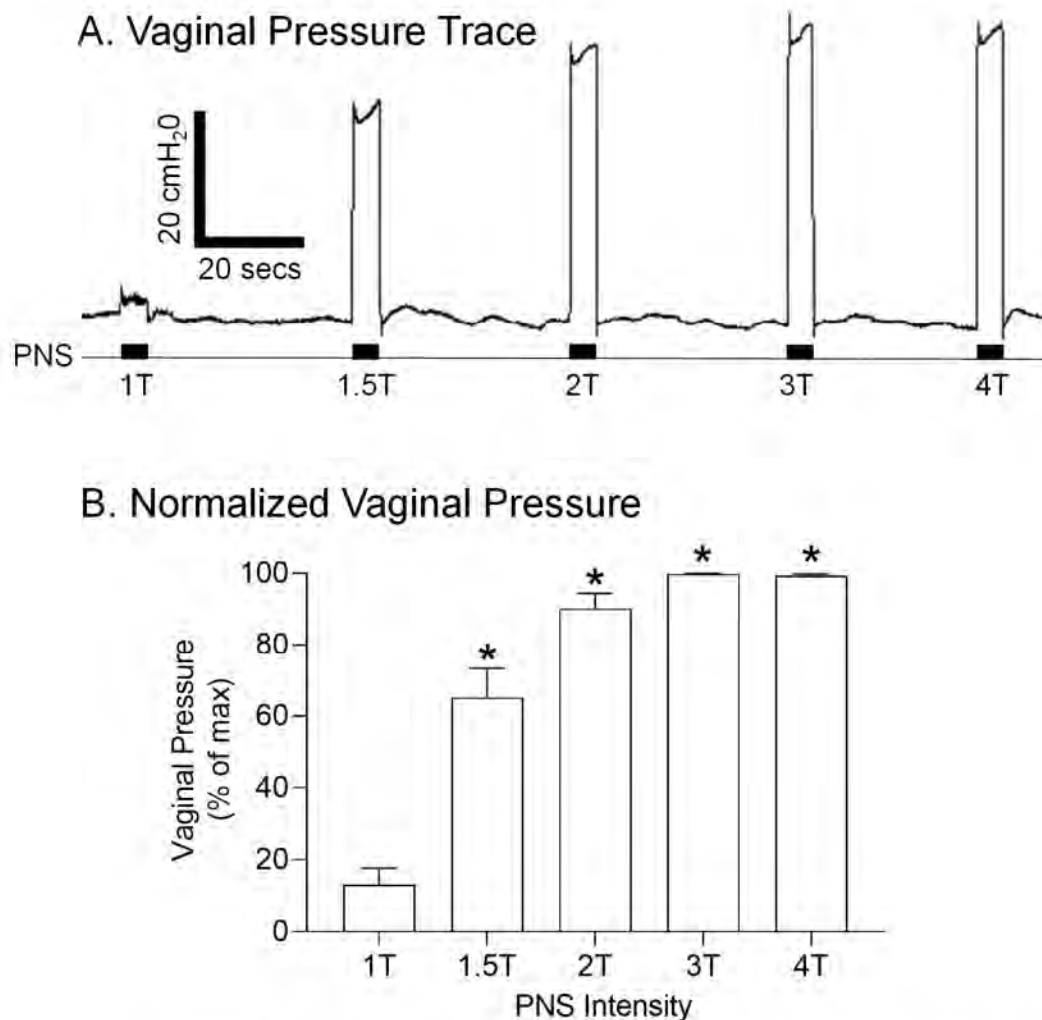
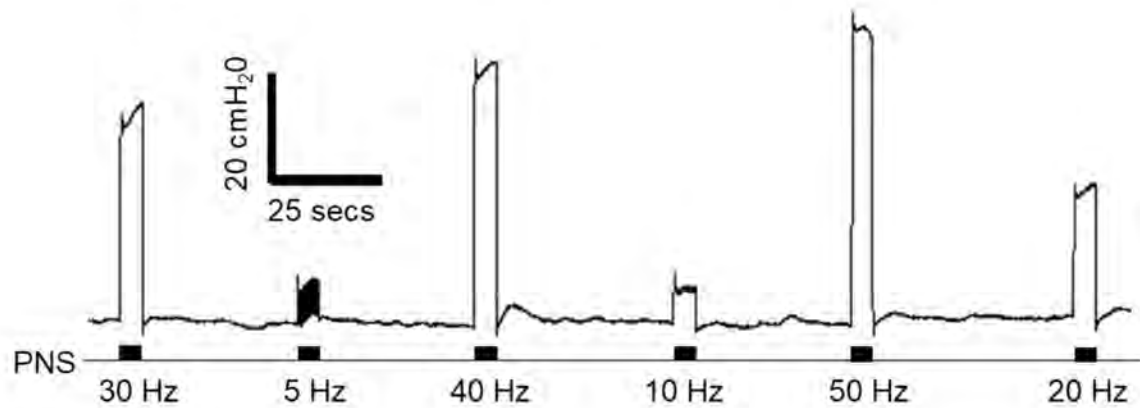


Fig.2. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation intensities. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: (b)(4) T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: (b)(4) (b)(4) * indicates significantly different from 1T (one-way ANOVA). N =4 cats.

A. Vaginal Pressure Trace



B. Normalized Vaginal Pressure

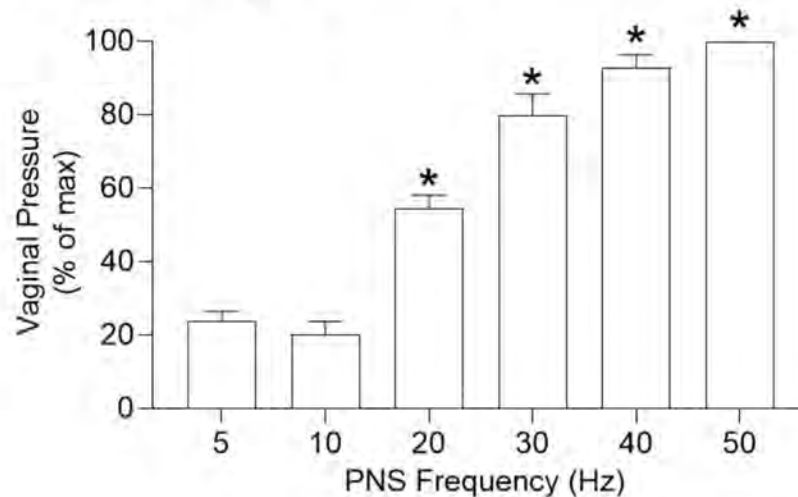


Fig.3. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation frequencies. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 2T = (b)(4) T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 2T = (b)(4) (b)(4) * indicates significantly different from 5 Hz (one-way ANOVA). N =4 cats.

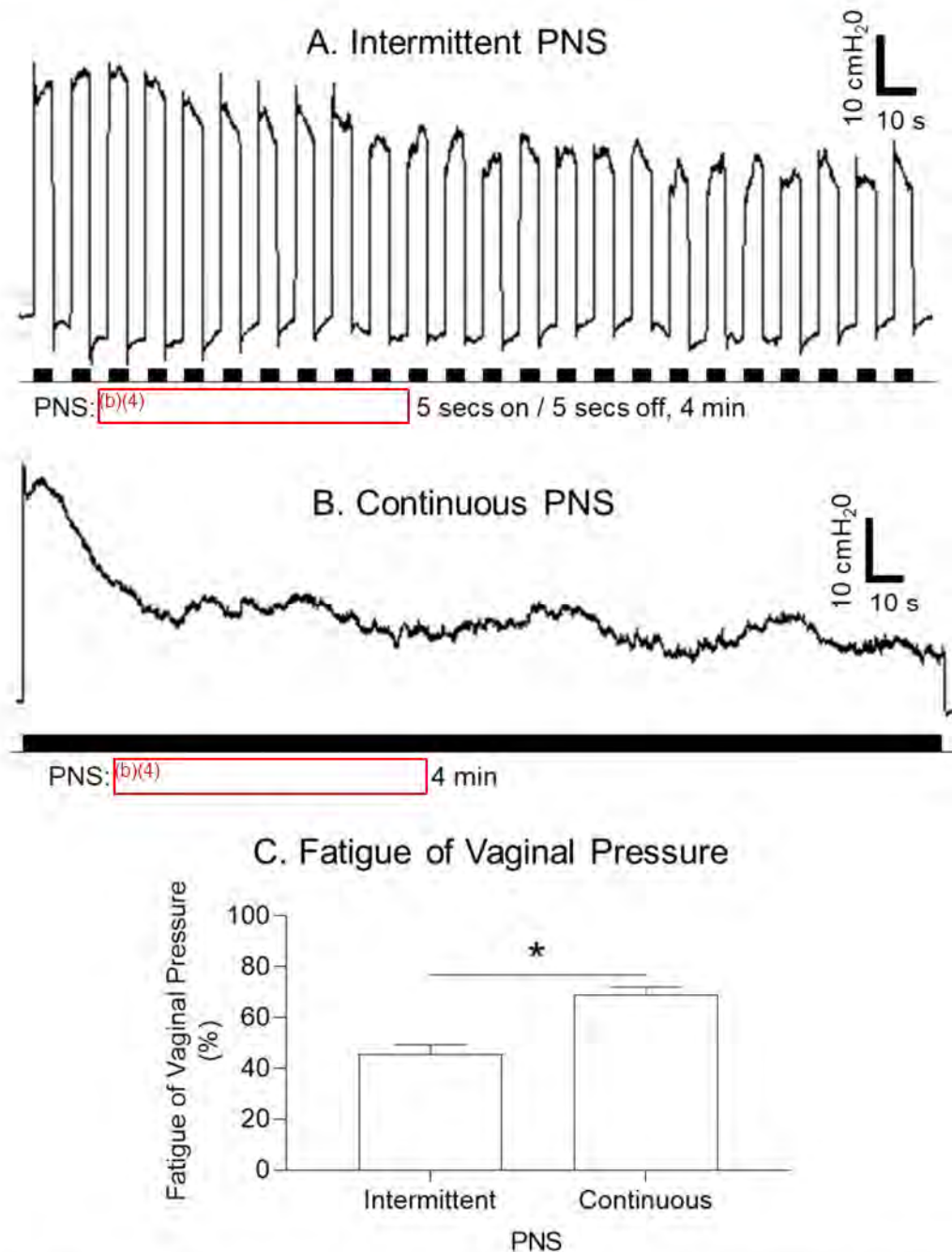


Fig.4. Fatigue of vaginal pressure increases induced by prolonged (4 minutes) pudendal nerve stimulation (PNS). A. Vaginal pressure responses induced by intermittent PNS. B. Vaginal pressure responses induced by continuous PNS. Black bars under the pressure trace indicate the PNS duration. C. Fatigue of vaginal pressure responses. The reduction in vaginal pressure responses at the end of 4-minutes PNS was normalized to the maximal response to indicate the muscle fatigue. PNS: (b)(4) (b)(4) T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, p = 0.0354). N = 4 cats.

Sub-Task 1.2.4 Measure fullness of bladder and bowel (b)(4)

In 4 anesthetized cats, the pudendal nerve and sacral dorsal roots (S1-S3) were surgically exposed to implant (b)(4) on the nerves for recording nerve activity induced by bladder or colon distention (Fig.5). The bladder was distended by raising a saline reservoir that was connected to the bladder via a tube through the bladder dome. The colon was distended by balloon catheters placed in the distal or proximal colon. The purpose of this study is to determine the feasibility to detect bladder/bowel fullness by recording nerve activity from the pudendal nerve or sacral roots. If it is possible, the nerve activity could be used as feedback control signal to trigger either micturition or defecation.

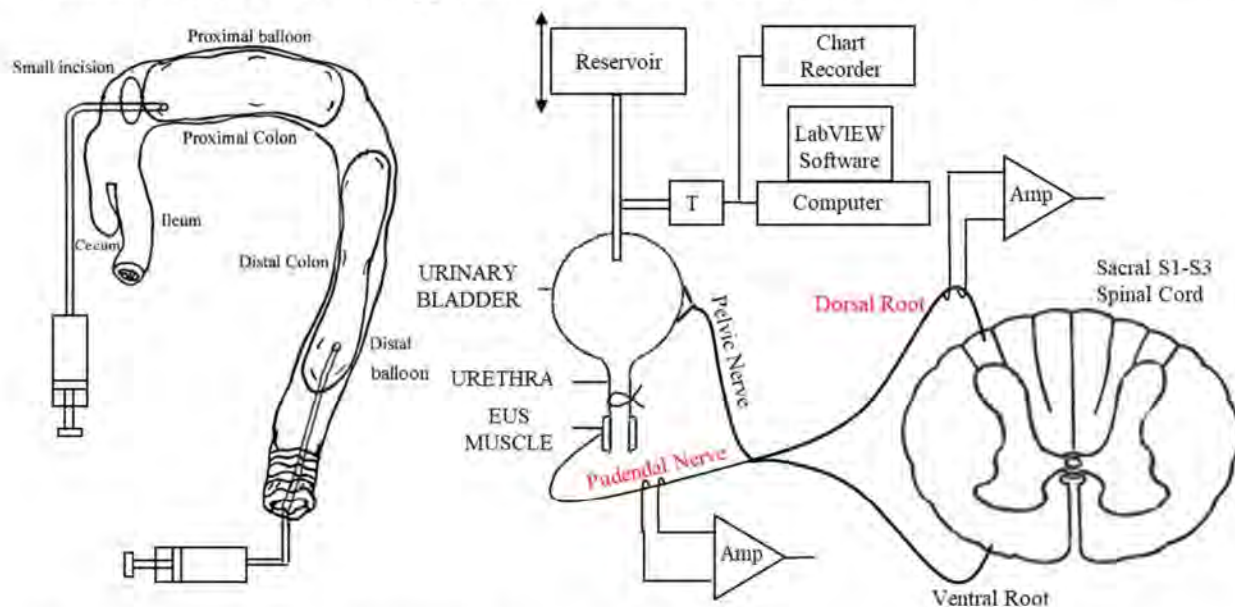


Fig.5. Experimental setup to detect bladder/bowel fullness by recording nerve activity from sacral S1, S2 or S3 dorsal root, or pudendal nerve. Bladder is distended by raising a saline reservoir and colon is distended by infusing saline into the colon balloons. Cuff electrode is used to record nerve activity.

Fig.6 shows the pudendal nerve activities induced by bladder distention in a cat. The saline reservoir was at the same level as the bladder initially (0 cmH₂O pressure) and then was raised to (b)(4) than the bladder (b)(4) to slowly infuse the bladder with saline. During the slow infusion, large reflex bladder contractions could be induced as the bladder volume became large enough. Then, the reservoir was lowered to the bladder level again to allow the bladder contractions to end by gradually emptying the bladder. The firing rate of pudendal nerve activity recorded by the cuff electrode could be increased (Fig.6A), decreased (Fig.6B), or not changed (Fig.6C) during a large bladder contraction in the same cat at different recording times. These results indicate a complex physiological relationship between bladder pressure and pudendal nerve activity recorded by a cuff electrode.

(b)(4)

Fig.7 shows the nerve activity recorded from sacral S1, S2 and S3 dorsal roots during bladder contractions. In this cat the firing rate of sacral root nerve activity was not changed by the pressure increase in the bladder, indicating that recording nerve activity from the sacral dorsal roots might not be an effective method to detect bladder contractions. However, in another cat the nerve activity from S2 and S3 dorsal roots did change with the bladder pressure (Fig.8). Unfortunately, the S2/S3 nerve root activity was decreased (not increased) during bladder contractions (Fig.8). This results further indicate a complex physiological relationship between the recorded nerve activity and the bladder pressure.

Fig.8 also shows that the sacral S2 or S3 nerve root activity could also be reduced/inhibited by colon distention (see the bottom 2 traces in Fig.8 A and B). The distal colon was distended by infusing 40 ml saline into the balloon catheter as shown in Fig.5. In this cat shown in Fig.8, nerve activity from S1 dorsal root was not sensitive to bladder or colon distention.

(b)(4)

(b)(4)

Our studies in cats show that the nerve activity recorded by a (b)(4)

(b)(4)

(b)(4)

Task 1.4 Implantable pulse generator with custom (b)(4)

(b)(4)

1.4.5 Full integration and test completed for initial prototype (b)(4)

The complete integration of the (b)(4) circuit into an IPG for animal implantation is currently underway. (b)(4)

(b)(4) The IPG circuit layout is complete with all parts placed and tracing (b)(4)

(b)(4) (Fig.9 and Fig.10). The last quarter has been focused on (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4) samples were delivered to (b)(4) from the machinist that will produce the IPG enclosures with (b)(4). These came in two different finishes and the team approved and ordered (b)(4) enclosures for production (Fig.11). These were also received during (b)(4) and validated that will meet specifications. More enclosures will be ordered in preparation for design verification testing (b)(4).

Task 1.7 (b)(4) electrodes for human use (b)(4)

Sub-Task 1.7.3 First prototypes (b)(4) delivered for test (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.7.4 Finalize design and (b)(4) production (b)(4)

(b)(4)

Sub-Task 1.7.5 Biocompatibility test is completed. Documentation for FDA submission

(b)(4)

Biocompatibility quotes from three test houses for the (b)(4) were received. The biocompatibility discussions with the test houses are ongoing while we prepare the materials and assemblies to be tested. Surface areas (b)(4) were calculated. A consultant was brought on to create an overall evaluation test plan to help guide the team in the right direction. This evaluation plan should be completed by the end of (b)(4). Once the team has the evaluation plan, (b)(4) will start being made and then will be provided to the test houses to start all the testing.

Task 1.9 Patient controller (b)(4)

Sub-Task 1.9.2 Verification testing completed (b)(4)

The verification testing for the human (b)(4) patient controller is paired with the investigator controller. The patient controller user interface has been developed and will be updated as needed based on simplified features of the investigator controller. This GUI is a simplified version of (b)(4) (Fig.14 and Fig.15). This task is completed since (b)(4) (b)(4) (Fig.16).

(b)(4)

(b)(4)

Task 1.10 Software development kit (SDK) (b)(4)

Sub-Task 1.10.2 At the completion of the development, the SDK is complete with the documentation on how to access the devices via the software API (b)(4)

While the SDK has progressed significantly (b)(4) focus is now shifted to the (b)(4) delivery. With a completion target day attained, the SDK package will regain traction and effort thru completion. The initial IFU for in human IDE for (b)(4) is complete, elements of which to be used for (b)(4)

Task 1.11 GLP animal study for (b)(4)

The purpose of the GLP safety study is to provide support for the safe initiation of an Early Feasibility IDE Study of (b)(4) system in humans. This was originally intended to support

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.13 Early feasibility human clinical protocol for (b)(4)

Thermaquil drafted the early feasibility clinical study for (b)(4) with input from clinicians during (b)(4). This clinical protocol is needed for the FDA IDE submission and was submitted for FDA (b)(4). We received specific feedback

(b)(4)

We updated our protocol by changing (b)(4)

(b)(4)

(b)(4)

Task 1.16 FDA IDE Submission (b)(4)

Task 1.16.1 Prepare and submit FDA IDE application (b)(4)

(b)(4)

Thermaquil has submitted our IDE submission on (b)(4) FDA confirmed that the IDE was received on (b)(4) (Fig.19). (b)(4) (Fig.20-28). FDA approved the IDE unconditionally (Fig.29) and thanked Thermaquil for quick and comprehensive responses to the interactive requests (Fig.30).

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

(b)(4), (b)(6)

Task 2.2 IRB approval and NIWC HRPO approval for human studies for (b)(4)

(b)(4)

Thermaquil submitted the IDE protocol to the University of Pittsburgh's IRB on (b)(4)
(Fig.31). The University requested (b)(4)
(b)(4) (Fig.32) which was submitted.

(b)(4)

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Weekly meetings with the team members from University of Pittsburgh, (b)(4) and Thermaquil were hold regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. (b)(4)

(b)(4) University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, (b)(4) and Thermaquil provided a solid foundation for a successful translation of the technology developed by this

DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Peer-Reviewed Journal Papers

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"

Experimental Neurology, 2021; 346:113860.

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".

American Journal of Physiology - Gastrointestinal and Liver Physiology, 2021; 321: G735-G742

Zhijun Shen, Jicheng Wang, Bing Shen, Jianan Jian, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

"Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats"

Neuromodulation, 2022. Submitted for review and publication.

Jialiang Chen, Yihua Zhong, Jicheng Wang, Bing Shen, Zhijun Shen, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats"

Journal of Sexual Medicine, 2022. Submitted for review and publication.

Meeting Abstracts

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)		(b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Urologist	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.3 Restore sexual function (b)(4)	(b)(4)					Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.2.4 Measure fullness of bladder and bowel (b)(4)	(b)(4)					Milestone 1.2.4(1-3) – Deliverable 1.2.4	
2.2 IRB approval and NIWC HRPO approval for human studies (b)(4)							
2.2.1 Prepare and submit IRB protocols for human studies (b)(4)	(b)(4)						
1.4 Implantable pulse generator with custom (b)(4)							
Full integration and test	(b)(4)						
1.4.5 completed for initial prototype (b)(4)	(b)(4)					Milestone 1.4.5 – Deliverable 1.4	
1.7 Leads and cuff electrodes for human use (b)(4)							
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)					Milestone 1.7.3 – Deliverable 1.7	
1.7.4 Finalize design and production (b)(4)	(b)(4)					Milestone 1.7.4 – Deliverable 1.7	
Biocompatibility test is completed. Documentations for FDA submission (b)(4)	(b)(4)					Milestone 1.7.5 – Deliverable 1.7	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.9 Patient controller for human	(b)(4)						(b)(4)
1.9.2 Verification testing completed	(b)(4)					Milestone 1.9.2 – Deliverable 1.9	
1.10 Software Development Kit (SDK)	(b)(4)						
At the completion of the development, the SDK is							
1.10. complete with the	(b)(4)					Milestone 1.10.2 – Deliverable 1.10	
2 documentation on how to access the devices via the software API	(b)(4)						
1.16 FDA IDE Submission	(b)(4)						
1.16. FDA IDE approval for the	(b)(4)					Milestone 1.16.2 – Deliverable 1.16.2	
2	(b)(4)						
1.13 Early feasibility human clinical protocol for	(b)(4)						
1.13 Early feasibility human clinical protocol for	(b)(4)					Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study	(b)(4)						
1.11 GLP animal study	(b)(4)					Milestone 1.11 – Deliverable 1.11	
2.11 Early feasibility human clinical protocol for	(b)(4)						
2.11 Early feasibility human clinical protocol for	(b)(4)					Milestone 2.11 – Deliverable 2.11.1	
2.12 FDA	(b)(4)						
2.12. FDA IDE for	(b)(4)					Milestone 2.13 – Deliverable 2.13.1	
1	(b)(4)						

Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats

Jialiang Chen ^{1,2}, Yihua Zhong ^{1,3}, Jicheng Wang ¹, Bing Shen ¹, Zhijun Shen ¹,
Jonathan Beckel ⁴, William C. de Groat ⁴, Christopher Chermansky ¹, Changfeng Tai ^{1,4,5}

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Urology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, P.R. China

³ School of Biomedical Engineering, Capital Medical University, Beijing, P.R. China

⁴ Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

⁵ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Running Head: Vaginal Lubrication by Pudendal Nerve Stimulation

Abstract: 307 words

Text: 1854 words

Correspondence to:

Changfeng Tai, Ph.D.
Department of Urology
University of Pittsburgh
700 Kaufmann Building
Pittsburgh, PA 15213
Phone: 412-692-4142
Fax: 412-692-4380
Email: cftai@pitt.edu

ABSTRACT

Background: Vaginal lubrication and contractions are among the top difficulties affecting sexual intercourse in women after spinal cord injury (SCI).

Aim: This study aimed at determining if pudendal nerve stimulation (PNS) can improve vaginal lubrication and induce increases in vaginal pressure.

Methods: In anesthetized cats, a small piece of cotton was inserted into the vagina for 10 minutes with or without PNS to measure vaginal wetness by the weight increase of the vaginal cotton. Then, a small balloon catheter was inserted into the vagina to measure the pressure increase induced by PNS. Intensity response of the vagina to PNS (30 Hz, 0.2 ms, 5 secs) was determined at 1-4 times of intensity threshold (T) for PNS to induce an observable vaginal pressure increase. Frequency response was determined at 2T intensity in a range of PNS frequencies (5-50 Hz). Finally, fatigue in vaginal pressure was determined by applying PNS (30 Hz, 2T) either continuously or intermittently (5 secs on and 5 secs off) for 4 minutes.

Outcomes: The effectiveness of PNS in increasing vaginal wetness and pressure is evaluated.

Results: PNS significantly ($p=0.0327$) increased the measurement of vaginal wetness from 15.8 ± 3.8 mg during control without stimulation to 32.4 ± 4.7 mg after stimulation. Vaginal pressure increased as PNS intensity or frequency increased. PNS (30 Hz, 2T) induced vaginal pressure increase $\geq 80\%$ of the maximal response. Intermittent PNS induced significantly ($p=0.0354$) smaller fatigue ($45.6\pm3.7\%$) in vaginal pressure than continuous PNS ($69.1\pm3.0\%$) during the 4-minute stimulation.

Clinical Translation: This study indicates the possibility to develop a novel pudendal neuromodulation device to improve both female sexual function and bladder function after SCI.

Strengths & Limitations: This study provides preclinical data supporting the development of a novel pudendal neuromodulation device. The limitation includes the lack of chemical analysis of the vaginal secretion.

Conclusion: PNS can improve vaginal lubrication and induce increases in vaginal pressure.

Keywords: stimulation, pudendal nerve, vagina, spinal cord injury, cat

INTRODUCTION

Spinal cord injury (SCI) impairs sexual function in women both psychologically and physically.¹⁻³ About 65% of SCI women complain about vaginal lubrication that is one of the top three difficulties affecting sexual intercourse.^{1,4,5} In addition, urinary incontinence during sexual intercourse, which reduces desire for sexual activity with a partner, is also a significant issue for SCI women.¹ An implantable neuromodulation device has been developed to restore urinary bladder function and has also been used to induce penile erection for SCI men.⁶ However, a neuromodulation device to restore both bladder and sexual functions for SCI women is still not available.

Our recent preclinical studies in cats^{7,8} have shown that pudendal nerve block and stimulation could be a very promising new approach to restore urinary bladder function after SCI. Since the pudendal nerve also innervates the female sexual organ, it might be possible to improve female sexual function as well as bladder function after SCI by pudendal nerve stimulation (PNS). To explore this opportunity, in this study we investigated the effects of PNS

on vaginal lubrication and vaginal pressure increase in anesthetized cats. If successful, the potential clinical promise is that a novel pudendal neuromodulation device could be developed to empty the bladder first to prevent urinary incontinence during sexual intercourse, and then produce vaginal lubrication and induce increase/change in vaginal pressure during the sexual intercourse for women with SCI.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical procedures

Four female cats (2.7-3.4 kg) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. The left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via urethra to drain the bladder during the experiment and the urethra was tied with a suture. The pudendal nerve on the left side was dissected through an incision in the region of sciatic notch and a bipolar cuff electrode (NC223pt, MicroProble, Gaithersburg, MD) was implanted on the nerve

for electrical stimulation. After the surgery, all incisions were closed with sutures. A heating pad was used to maintain the animal body temperature between 35 and 37 °C during the experiment.

Experimental protocol

At the beginning of the experiment, a small piece of dry cotton was weighed (about 30 mg) and inserted into the vagina 2-3 cm from the external orifice for 10 minutes without stimulation. After the 10 minutes, the cotton was removed and weighed again to determine the increase in weight that was used as the control value to indicate the vaginal wetness. Then, the same 10-minute cotton test was repeated using another piece of dry cotton (weight about 30 mg) while PNS (30 Hz, 0.2 ms) at the 2 times intensity threshold for inducing anal twitching was also applied intermittently (5 secs on and 5 secs off) during the 10-minute period to increase the vaginal wetness.

After testing the vaginal wetness, a small catheter (7 French) with a balloon at the tip was inserted into the vagina with the balloon slightly distended by 0.2 ml air to measure vaginal pressure increase. PNS (30 Hz, 0.2 ms) was applied for 5 secs each time at five intensities increasing from 1T to 4T to determine the intensity response. Then, PNS at an intensity of 2 times the threshold (T) for inducing a small observable vaginal pressure increase was applied at different frequencies (5-50 Hz) to determine the frequency response. Finally, PNS (30 Hz, 0.2 ms, 2T) was applied for 4 minutes either continuously or intermittently (5 secs on and 5 secs off) in a random order to determine fatigue in vaginal pressure during the 4-minute-long stimulation. A time interval of 5-10 minutes was inserted between the two 4-minute PNS to avoid any carry-over fatigue effect.

Data analysis

Vaginal wetness was measured by the weight increase of the cotton inserted in the vagina under control condition or during PNS. The vaginal pressure increases induced by PNS of different intensities or frequencies was normalized to the maximal response to determine the effect of PNS intensity or frequency. Vaginal fatigue was measured as the vaginal pressure reduction at the end of the 4-minute PNS divided by the maximal vaginal pressure increase during the PNS. The data from different animals are averaged and presented as means $\pm$ SE. Statistical significance ($p < 0.05$) was determined by paired t-test or repeated-measures one-way ANOVA followed by Dunnett multiple comparison.

RESULTS

PNS applied intermittently (5 secs on and 5 secs off) for 10 minutes at the intensity of 2 times threshold for inducing anal twitching significantly increased vaginal wetness (Fig.1). Without PNS, the vaginal wetness as indicated by the increase in the vaginal cotton weight during a 10-minute period was 15.8 ± 3.8 mg (control in Fig.1). After 10-minute PNS, the vaginal wetness was significantly ($p=0.0327$) increased to 32.4 ± 4.7 mg (PNS in Fig.1).

The vaginal pressure was significantly ($p < 0.05$) increased as the PNS intensity increased (Fig.2). PNS (30 Hz) at an intensity of $\geq 2T$ induced a vaginal pressure increase equal to 90% of the maximal response (Fig.2B). Meanwhile, at intensity of $2T$ the PNS induced a significantly ($p < 0.05$) larger increase in vaginal pressure at frequencies ≥ 20 Hz than ≤ 10 Hz (Fig.3). When PNS (30 Hz, $2T$) was applied for 4 minutes, it produced less fatigue when applied intermittently (Fig.4A) than continuously (Fig.4B). On average ($N=4$), intermittent PNS induced $45.6 \pm 3.7\%$

fatigue that is significantly ($p=0.0354$) smaller than the fatigue induced by continuous PNS ($69.1\pm3.0\%$, Fig.4C).

DISCUSSION

This study in anesthetized female cats shows that PNS can improve vagina lubrication by increasing the wetness of the vagina (Fig.1). PNS induced large increase in vaginal pressure (Fig.2 and Fig.3) that is more fatigue resistant during intermittent than during continuous stimulation (Fig.4). These results suggest that PNS might be useful to improve female sexual function after SCI. Since our recent studies in cats have shown that PNS can also enhance urine storage and voiding after SCI,^{7,8} a novel pudendal neuromodulation device could be developed to benefit SCI women by enhancing vaginal lubrication and inducing vaginal pressure increase/change as well as preventing urinary incontinence during sexual intercourse by emptying the bladder before sexual activity.

Previous animal studies in mice, rats, rabbits, and dogs have shown that vaginal blood flow and vaginal lubrication could be increased by mechanical stimulation of the vagina or electrical stimulation of the pelvic nerve or clitoral nerve.⁹⁻¹³ Our current study in cats shows that PNS can also increase vaginal lubrication. Since the clitoral nerve is a branch of the pudendal nerve, PNS might have activated clitoral afferent input to the central nervous system to trigger a reflex output via the pelvic nerve to increase the blood flow and lubrication of the vagina.¹⁴⁻¹⁶ In addition, since the pudendal motor axons innervate the striated muscle surrounding the vagina,^{17,18} anal sphincter, and pelvic floor muscles, PNS can produce contraction of these muscles to increase vaginal pressure and further facilitate vaginal secretion.¹⁴ Periodic or pulsed

contractions of this groups of pelvic muscles also occur during orgasm.^{14,19} Therefore, in addition to increasing vaginal lubrication PNS may also induce or facilitate orgasmic sensations for women with incomplete SCI who still have some sensation from the vagina or pelvic area. Only continuous and intermittent PNS were tested in this study (Fig.4), but other stimulation patterns that more closely mimic those during orgasm could also be induced by programing the PNS stimulation intensity and duration.

Since the urethra was ligated by a suture, it is not possible for bladder to leak any urine into the vagina to change the vaginal cotton wetness. Therefore, the increase in the vaginal cotton weight must be due to fluid accumulation in the vagina during the 10-minute period. Vagina lubrication during sexual arousal is mainly due to the increased blood flow in the vagina producing transudate on the surface of the vaginal wall.²⁰ In addition, vaginal fluid could also contain secretions from female prostate or Bartholin's glands.²⁰ Therefore, it is highly likely that the fluid absorbed by the cotton in this study contains both the transudate from vaginal blood vessels and secretions from female sexual glands. However, the vaginal fluid collected by the cotton in the vagina was not analyzed for the chemical composition in this study.

In summary, this study in cats shows that PNS can improve vaginal lubrication and induce vaginal pressure increase/change. These results indicate that it is possible to develop a pudendal neuromodulation device to restore or improve female sexual function after SCI. Since our recent studies in cats^{7,8} have shown that pudendal nerve stimulation/block can successfully restore bladder function after SCI, this current study indicates a possibility to develop a novel implantable pudendal neuromodulation device to restore both bladder and sexual function for women with SCI.

ACKNOWLEDGEMENT

This study is funded by the Bridging the Gap Plus (BG+) program of the Defense Advanced Research Projects Agency (DARPA) under the contract N66001-20-C-4050. The views, opinions and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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FIGURE CAPTION

Fig.1. Pudendal nerve stimulation (PNS) increased vaginal wetness. PNS: 30 Hz, 0.2 ms, 1.5T = 0.15-0.45 V, 5 secs on and 5 secs off for 10 minutes. T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p=0.0327$). N = 4 cats.

Fig.2. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation intensities. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 30 Hz, 0.2 ms, 5 secs, T = 0.16 V. T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 30 Hz, 0.2 ms, 5 secs, T = 0.16-0.5 V. * indicates significantly different from 1T (one-way ANOVA). N = 4 cats.

Fig.3. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation frequencies. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 2T = 0.32 V, 0.2 ms, 5 secs. T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 2T = 0.32-1 V, 0.2 ms, 5 secs. * indicates significantly different from 5 Hz (one-way ANOVA). N = 4 cats.

Fig.4. Fatigue of vaginal pressure increases induced by prolonged (4 minutes) pudendal nerve stimulation (PNS). A. Vaginal pressure responses induced by intermittent PNS. B. Vaginal pressure responses induced by continuous PNS. Black bars under the pressure trace indicate the PNS duration. C. Fatigue of vaginal pressure responses. The reduction in vaginal pressure responses at the end of 4-minutes PNS was normalized to the maximal response to indicate the muscle fatigue. PNS: 30 Hz, 0.2 ms, 2T = 0.32-1 V, 4 minutes. T – threshold intensity for inducing anal twitch. * indicates significantly different (paired t-test, $p = 0.0354$). N = 4 cats.

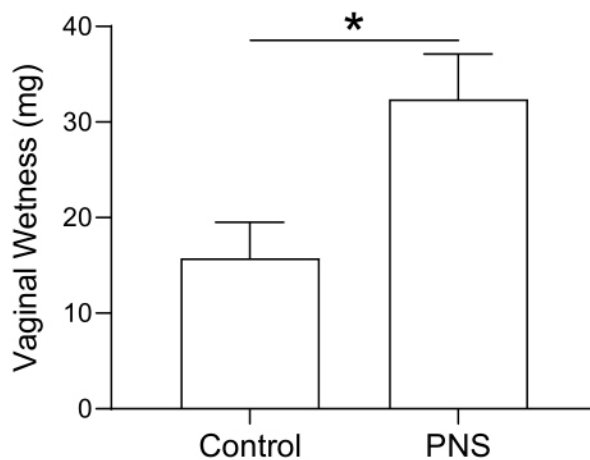
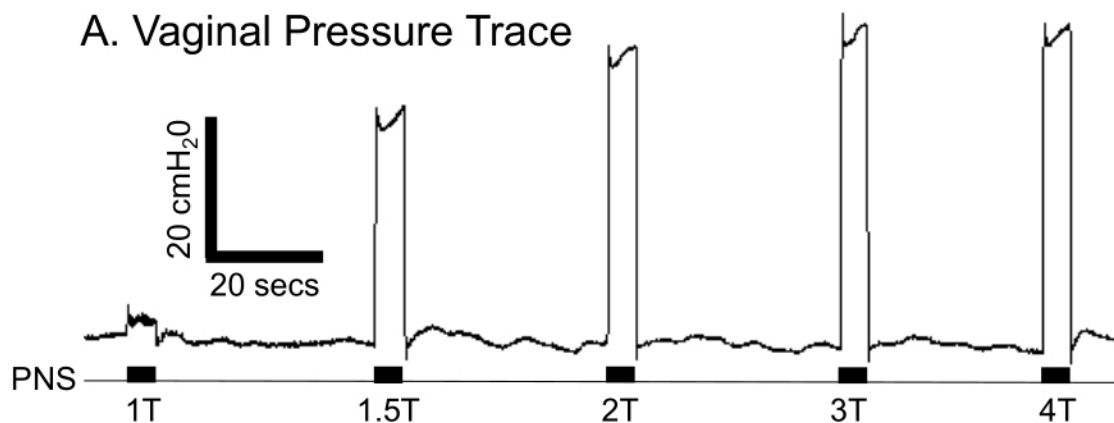


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B. Normalized Vaginal Pressure

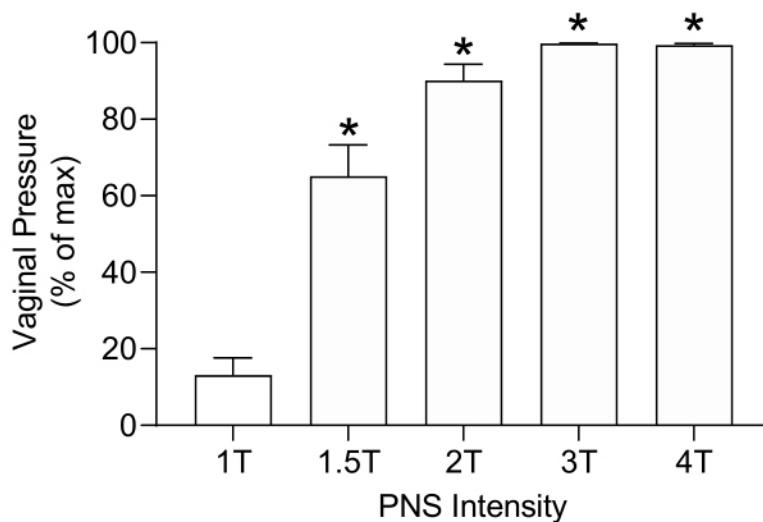
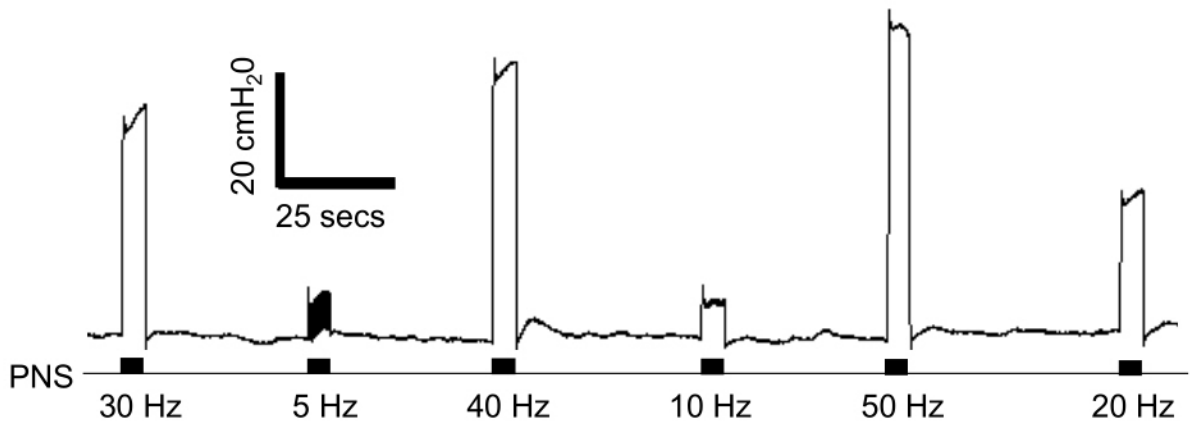


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A. Vaginal Pressure Trace



B. Normalized Vaginal Pressure

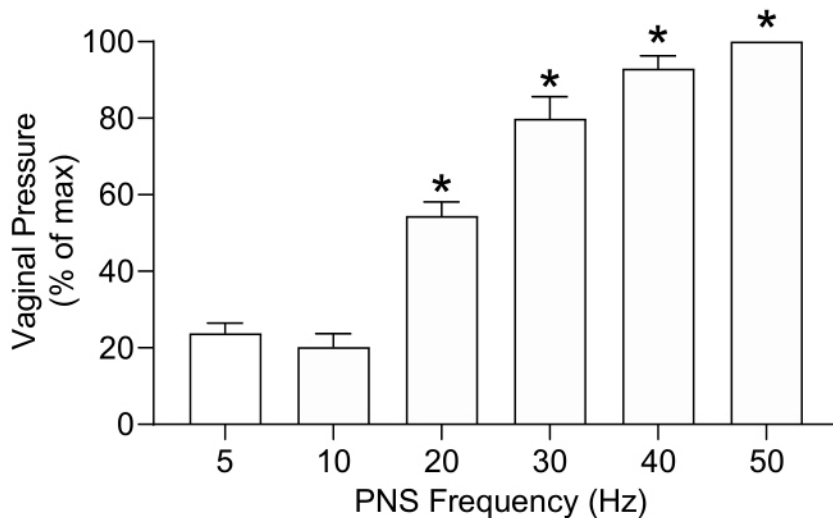


Fig.3. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation frequencies. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 2T = 0.32 V, 0.2 ms, 5 secs. T – threshold intensity for inducing anal twitch. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 2T = 0.32-1 V, 0.2 ms, 5 secs. * indicates significantly different from 5 Hz (one-way ANOVA). N =4 cats.

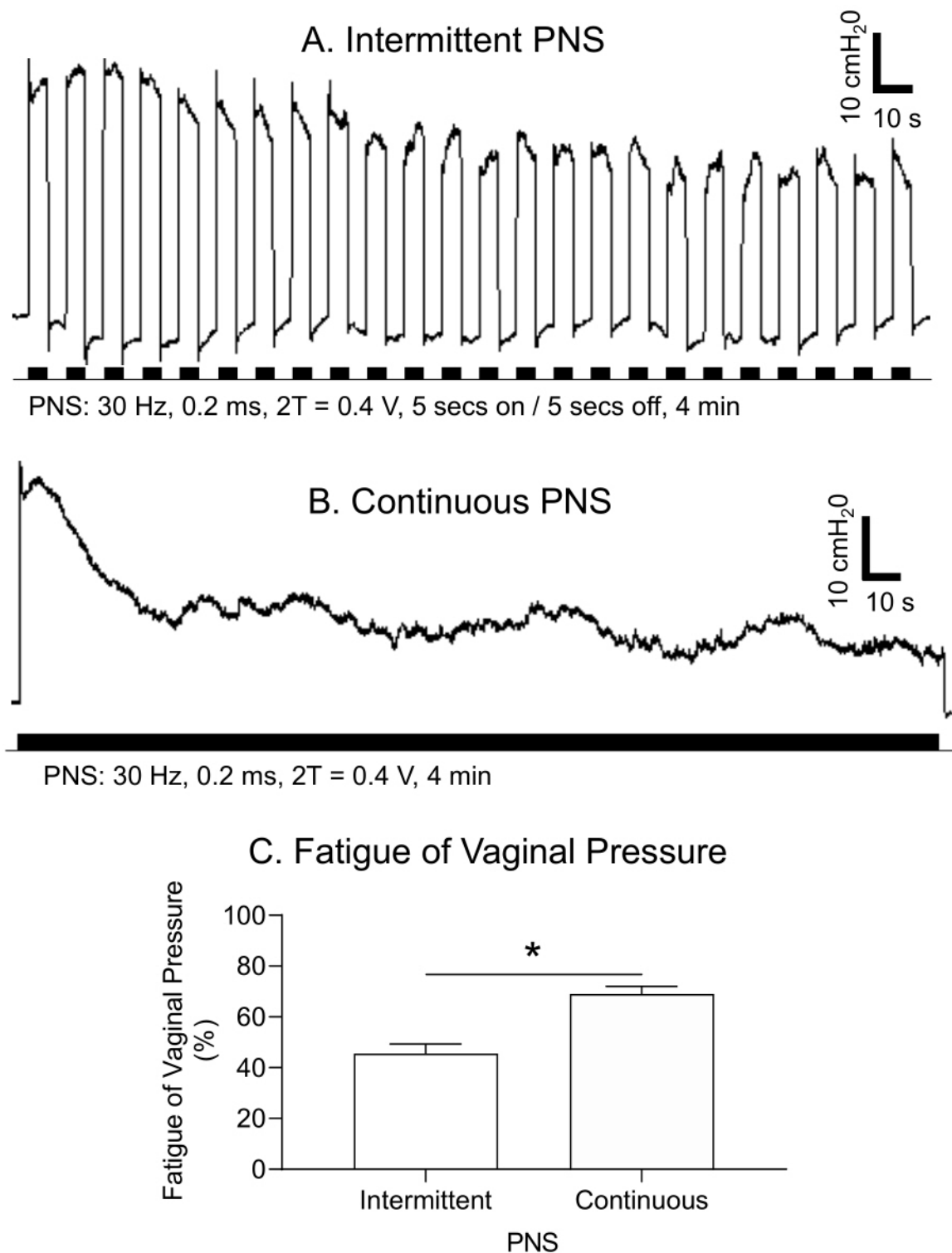


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University of Pittsburgh Bridging the GAP Plus (BG+) Quarterly Technical/ R&D Status Report

Period Covered by the Report: May 1, 2022 through July 31, 2022

(b)(4)

Date of Report: August 31, 2022

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: (b)(4)

Subcontractors: (b)(4) LLC and Thermaquil, Inc.

Program Manager: (b)(4); (b)(6)

Submitted by:

(b)(4), (b)(6)

Telephone: (b)(4), (b)(6)

Email: (b)(4), (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) quarter (MAC20-MAC22), we have made major progresses in the following areas:

1. Scientific progress:

- We have submitted the human study protocol to the IRB of University of Pittsburgh.

(b)(4)

- Our third paper titled "Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats" has been accepted for publication by Neuromodulation, and now is in production stage at the publisher (The proof is attached).
- Our fourth paper titled "Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats" has been accepted for publication by The Journal of Sexual Medicine (The proof is attached).

2. Engineering progress:

- Components for (b)(4) Long lead items were replaced with components that would be acceptable. At the end of (b)(4) there were only (b)(4) active components less to purchase.
- Parts to build both (b)(4) have been received at (b)(4)
- The (b)(4) PCBs are in process of being populated.
- The (b)(4) firmware is in the process of being updated to be more independent of (b)(4)
- (b)(4)
-
-
-
-
-
-
- Process qualifications are in progress and will be completed once (b)(4)
- (b)(4) were assembled and evaluated during (b)(4) were sent to UPitt for testing. (b)(4) were able to block the pudendal nerve, relax the external urethral sphincter, and stimulate bladder contraction during the block.
- (b)(4) has progressed significantly.
- The biocompatibility evaluation test plan was reviewed by an expert and the evaluation has been defined – (b)(4)
 - These will begin being built during (b)(4)

3. Regulatory progress:

- Began drafting (b)(4)
- Began drafting human Early Feasibility Study protocol for (b)(4)
- Responded to Pitt IRB questions. Drafted IRB submission to external IRB for the Early Feasibility Study (b)(4)

4. Administrative progress:

- Monthly payments to the subcontractors were processed regularly by University of Pittsburgh.

- Weekly meetings were held between University of Pittsburgh, (b)(4) and Thermaquil to coordinate the project activity.
- Monthly meeting was held with DARPA to report our progress.

2 Schedule: Milestones and Deliverables

See the attached Gantt chart table for the summary of milestones and deliverables.

3 Tasks: Progress, Accomplishments, and Plans

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.3 Restore sexual function (b)(4)

During this quarter, we have 2 papers accepted for publication. One is about restoring male sexual function and the other is about restoring female sexual function. Fig.1 and Fig.2 show the journal editor's acceptance letters. The titles of these two papers are listed below:

Zhijun Shen, Jicheng Wang, Bing Shen, Jianan Jian, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

"Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats"

Neuromodulation, 2022. Accepted for publication on June 21, 2022.

Jialiang Chen, Yihua Zhong, Jicheng Wang, Bing Shen, Zhijun Shen, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats"

Journal of Sexual Medicine, 2022. Accepted for publication on July 31, 2022.

Together with the other 2 papers for restoring bladder and bowel functions, we have published a total of 4 peer-reviewed journal papers since the initiation of this DARPA BG+ project. These published studies indicate the feasibility of restoring bladder, bowel, and sexual functions after SCI (b)(4)

(b)(4), (b)(6)

Neuromodulation: Technology at the Neural Interface <em@editorialmanager.com>
Tue 6/21/2022 12:47 PM
To: (b)(4), (b)(6)
Manuscript Number: NEUROM-D-22-00211R1
Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats
Dear (b)(6),
Thank you for submitting your manuscript to Neuromodulation: Technology at the Neural Interface.
I am pleased to inform you that your manuscript has been accepted for publication.
We look forward to your continued participation in our journal, and we hope you will consider us again for future submissions.
Kind regards,
(b)(6)
Neuromodulation: Technology at the Neural Interface

Fig.1: Acceptance letter from the Editor-in-Chief of Neuromodulation.

(b)(4), (b)(6)

Journal of Sexual Medicine - Decision on Manuscript JSM-22-200.R1
Journal of Sexual Medicine <onbehalf@manuscriptcentral.com>
Sun 7/31/2022 2:32 AM
(b)(4), (b)(6)
Dear (b)(6),
congratulations!!!!It is a pleasure to accept your manuscript entitled "Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats" in its current form for publication in the Journal of Sexual Medicine. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.
(b)(6)
(b)(6) Journal of Sexual Medicine
jsmeditorialstaff@gmail.com

Fig.2. Acceptance letter from Deputy Editor in Chief of Journal of Sexual Medicine.

Task 2.2 IRB approval and NIWC HRPO approval for human studies (b)(4)
(b)(4)

Sub-Task 2.2.1 Prepare and submit IRB protocols for human studies (b)(4)

We have submitted the human study protocol to the IRB of University of Pittsburgh and responded every review comments (Fig.3). During the IRB review, the University (b)(4)
(b)(4)

(b)(4)

(b)(4); (b)(6)

(b)(4), (b)(6)

(b)(4)

MASTER (b)(4) SERVICES AGREEMENT

This Master (b)(4) Services Agreement (“Agreement”) is entered into as of the date of the last signature below (“Effective Date”) by and between (b)(4) (b)(4) a Washington limited liability company, and (b)(4) (“Company”), a Delaware Corporation. (b)(4) and Company may hereinafter be referred to collectively as the “Parties” or individually as a “Party.”

WHEREAS, (b)(4) is a clinical research organization engaged in the business of providing (b)(4) and

WHEREAS, Company wishes to engage (b)(4) from time to time to provide various (b)(4) (b)(4) services in connection with certain (b)(4) projects Company may conduct, as more fully set forth in various statements of work to be attached to this Agreement; and

Fig.5. Draft contract with the CRO (b)(4)

Task 1.4 Implantable (b)(4)

(b)(4)

1.4.5 Full integration and test completed for initial prototype (b)(4)

The focus of this quarter is part acquisition and (b)(4) buildout. (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.7 Leads and cuff electrodes for human use (b)(4)

Sub-Task 1.7.3 First prototypes of (b)(4) delivered for test (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4) were sent to (b)(4) for testing. The feedback received is that the (b)(4)

(b)(4)

The team has also been working on process improvement for manufacturing (b)(4)

(b)(4)

(b)(4)

(b)(4)

Sub-Task 1.7.4 Finalize design and (b)(4) production (b)(4)

Process qualifications are still underway. These qualifications can be continued once the (b)(4) is finalized. The (b)(4) was completed during this quarter. The first (b)(4) were produced via (b)(4). The material had

(b)(4)

The (b)(4) design was reviewed and approved by the team. There will be (b)(4)

(b)(4) as seen in Figure 8 – the (b)(4)

(b)(4) The ranges allow for less (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.7.5 Biocompatibility test is completed. Documentation for FDA submission
(b)(4)

The biocompatibility evaluation plan was written and reviewed by an expert. It was determined that (b)(4) systems need to be produced as soon as manufacturing processes are finalized, and components arrive which will begin (b)(4). Once biocompatibility testing starts, the required tests will be completed (b)(4)

(b)(4)

(b)(4)

Task 1.10 Software development kit (SDK) (b)(4)

Sub-Task 1.10.2 At the completion of the development, the SDK is complete with the documentation on how to access the devices via the software API (b)(4)

During (b)(4) firmware process was made. The (b)(4) firmware was updated and tested to be (b)(4) The firmware previously (b)(4)

(b)(4) The firmware is much more intelligent now and does not require as much information or battery drain when receiving commands. Additionally, (b)(4) features have been incorporated and tested. The (b)(4) is important for chronic studies, DVT, and for evaluating (b)(4) in-vitro and in-vivo.

Task 2.10 (b)(4)

(b)(4)

(b)(4)

Task 2.12 FDA IDE (b)(4)

Task 2.12.1 FDA IDE (b)(4)

(b)(4)

Task 2.11 Early feasibility human clinical protocol for (b)(4)

Thermaquil is drafting an early feasibility clinical study for the (b)(4) with input from clinicians (Fig.11). This protocol is needed for the FDA IDE submission. We will submit the

protocol for FDA feedback (b)(4)

(b)(4)

(b)(4)

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

Weekly meetings with the team members from University of Pittsburgh, Med-Ally, and Thermoquil were held regularly to coordinate this project.

4.2 Dissemination and Translation (if applicable)

Thermaquil is the regulatory sponsor of the devices developed by this DARPA BG + project and is responsible for commercialization of the technology. Med-Ally is the manufacturer of the devices. University of Pittsburgh is the clinical study site to test the FDA IDE approved devices. This collaboration between University of Pittsburgh, Med-Ally, and Thermaquil provided a solid foundation for a successful translation of the technology developed by this DARPA BG+ project into clinical application and eventually for a successful commercialization to benefit people suffering from spinal cord injury.

5 Publications and Presentations

Peer-Reviewed Journal Papers

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"

Experimental Neurology, 2021; 346:113860.

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.
"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".

American Journal of Physiology - Gastrointestinal and Liver Physiology, 2021; 321: G735-G742

Zhijun Shen, Jicheng Wang, Bing Shen, Jianan Jian, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.

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(b)(4)

Meeting Abstracts

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"

American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

6 Team Contact Information

Prime Team Members and Contact Information: University of Pittsburgh

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)		(b)(6)
Clinical PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Senior Scientific Consultant	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	
Postdoc	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Thermaquil

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(4); (b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Urologist	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Subcontract Team Members and Contact Information: Med-Ally

Title	Full Name	Contact Information (phone and email)	Role(s)
PI	(b)(4); (b)(6)	(b)(4); (b)(6) (b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	(b)(6)
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
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Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	
Co-I	(b)(4); (b)(6)	(b)(4); (b)(6)	

7 Financial Summary / Animal and Human Use

The "Quarterly Financial Summary" spreadsheet is attached.



Milestones and Deliverables Summary

University of Pittsburgh – Quarterly Report (b)(4)

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
2.2 IRB approval and NIWC HRPO approval for human studies	(b)(4)	(b)(4)	(b)(4)				(b)(4)
2.2.1 Prepare and submit IRB protocols for human studies	(b)(4)						
1.4 Implantable pulse generator with	(b)(4)						
Full integration and test							
1.4.5 completed for initial prototype	(b)(4)					Milestone 1.4.5 – Deliverable 1.4	
(b)(4)							
1.7 Leads and cuff electrodes for human use	(b)(4)						
1.7.3 First prototypes of delivered for test	(b)(4)	(b)(4)				Milestone 1.7.3 – Deliverable 1.7	
1.7.4 Finalize design and lead production	(b)(4)					Milestone 1.7.4 – Deliverable 1.7	
Biocompatibility test is							
1.7.5 completed. Documentations for FDA submission	(b)(4)					Milestone 1.7.5 – Deliverable 1.7	
1.10 Software Development Kit (SDK)	(b)(4)						
At the completion of the development, the SDK is							
1.10. complete with the	(b)(4)						
2 documentation on how to access the devices via the software API	(b)(4)					Milestone 1.10.2 – Deliverable 1.10	



Milestones and Deliverables Summary

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
(b)(4)							(b)(4)
(b)(4)						Milestone 2.10 – Deliverable 2.10.1	
2.11 Early feasibility human clinical protocol for (b)(4)							
2.11 Early feasibility human clinical protocol for (b)(4)						Milestone 2.11 – Deliverable 2.11.1	
2.12 FDA IDE (b)(4)							
2.12. FDA (b)(4)							
1 (b)(4)						Milestone 2.13 – Deliverable 2.13.1	

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Penile Erection Induced by Stimulation of Sacral S1/S2 Spinal Root in Cats

Zhijun Shen, PhD¹; Jicheng Wang, PhD¹; Bing Shen, DVM¹; Jianan Jian, PhD¹;
 Khari Goosby, BS¹; William Wang, BS¹; Jonathan Beckel, PhD²;
 William C. de Groat, PhD²; Christopher Chermansky, MD¹;
 Changfeng Tai, PhD^{1,2,3} 

ABSTRACT

Objective: This study aimed at determining whether stimulation of sacral spinal roots can induce penile erection in cats.

Materials and Methods: In anesthetized cats, a 20-gauge catheter was inserted into the corpus cavernosum to measure the penile pressure. Stimulus pulses (5–80 Hz, 0.2 ms) were applied through bipolar hook electrodes to sacral ventral roots alone or to combined ventral and dorsal roots of a single S1–S3 segment to induce penile pressure increases and penile erection.

Results: Stimulation of the S1 or S2 ventral root at 30 to 40 Hz induced observable penile erection with rigidity and the largest increase (169 ± 11 cmH₂O) in penile pressure. Continuous stimulation (10 minutes) of afferent and efferent axons by simultaneous stimulation of the S1 or S2 dorsal and ventral roots at 30 Hz also produced a large increase (190 ± 8 cmH₂O) in penile pressure that was sustainable during the entire stimulation period. After a complete spinal cord transection at the T9–T10 level, simultaneous stimulation of the S1 or S2 dorsal and ventral roots induced large (186 ± 9 cmH₂O) and sustainable increases in penile pressure.

Conclusion: This study indicates the possibility to develop a novel neuromodulation device to restore penile erection after spinal cord injury using a minimally invasive surgical approach to insert a lead electrode through the sacral foramen to stimulate a sacral spinal root.

Keywords: Cat, erection, sacral spinal root, spinal cord injury, stimulation

Conflict of Interest: Changfeng Tai is the inventor of a patent related to this study. The remaining authors reported no conflict of interest.

INTRODUCTION

After spinal cord injury (SCI), erectile dysfunction presents as a short duration or an absence of penile erection, or poor penile rigidity that is insufficient for sexual intercourse.^{1,2} Although drug treatment is effective in restoring erectile function after SCI, it is not suitable for every person with SCI because of the drug adverse effects and the health conditions of the person with SCI.² In addition to drug treatment, a fully implantable stimulator has been used successfully in humans to restore penile erection after SCI by stimulating the sacral anterior roots.^{3–5} However, sacral anterior root stimulation requires major invasive spinal surgery to expose the sacral spinal roots for the implantation of stimulation electrodes. Therefore, a minimally invasive neuromodulation approach that does not require invasive spinal surgery would be desirable for treatment of erectile dysfunction after SCI.

A minimally invasive surgical approach for stimulation of the sacral spinal roots has been available for many years to treat patients with an overactive bladder.⁶ It employs a foramen needle to insert a tined lead electrode percutaneously under the guide of fluoroscopic imaging near the S3 spinal root that consists of both anterior (motor) and posterior (sensory) spinal roots. In recent years, this minimally invasive sacral neuromodulation approach

also has been evaluated to treat erectile dysfunction, with a significant improvement in quality of sexual life for 20% to 40% of patients.^{7,8} However, the sacral neuromodulation stimulation parameters used in these studies to treat erectile dysfunction may not be optimal for improving penile erection because they were the same as those used to treat overactive bladder. Therefore, the optimization of sacral neuromodulation parameters for the treatment of erectile dysfunction is needed so that this minimally

Address correspondence to: Changfeng Tai, PhD, Department of Urology, University of Pittsburgh, 700 Kaufmann Bldg, Pittsburgh, PA 15213, USA. Email: cftai@pitt.edu

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA;

² Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA; and

³ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

For more information on author guidelines, an explanation of our peer review process, and conflict of interest informed consent policies, please see the journal's *Guide for Authors*.

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invasive surgical approach could be used as an effective therapy for people with or without SCI who have erectile dysfunction.

In this study using anesthetized cats, we investigated the penile response to electrical stimulation of an individual S1–S3 ventral root to determine the optimal stimulation frequency and the most effective spinal root in inducing penile erection. Next, combined stimulation of ventral and dorsal roots (termed spinal root stimulation) of the most effective spinal segment was used to induce sustainable (>10 minutes) penile erection. The effective stimulation parameters obtained in this preclinical study could be used to guide the design of a human clinical trial to further evaluate sacral spinal root stimulation using the minimally invasive surgical approach as used in the current clinical application⁶ to treat erectile dysfunction in both people with SCI and people without SCI.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical Procedures

A total of eight male cats (4.6 ± 0.2 kg, domestic shorthair) were anesthetized with isoflurane (2%–5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg intravenously and supplemented as needed) during data collection. The left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into the right carotid artery to monitor systemic blood pressure through a pressure transducer (BLPR2, WPI, Sarasota, FL) connected to an amplifier (TBM4M, WPI). Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder through the urethra to drain the bladder during the experiment, and the urethra was tied with a suture. Next, the abdominal incision was closed with sutures.

The spinal cord was exposed from lumbar L7 to sacral S3 segments by a dorsal laminectomy. The dura mater was opened, and each of the S1–S3 roots was identified. The dorsal and ventral roots

were separated to stimulate either the ventral root individually or both dorsal and ventral roots together (Fig. 1). The animals were in a prone position during the experiment, with a surgical retractor keeping the spinal incision open to form a pool that was filled with warm (35–37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (2–3-mm distance between the electrodes) were used to deliver the stimulation to each sacral spinal root by slightly lifting the root above the spinal cord. The stimulation was generated by a stimulator (S88, Grass Instruments, West Warwick, RI) and delivered by means of a constant voltage stimulus isolator (SIU5A, Grass Instruments).

Experimental Protocol

At the beginning of the experiment, stimulation (30-Hz frequency, 0.2-ms pulse width, and 1–2-minute duration) was delivered through the hook electrode to the sacral S1 ventral root at a minimal intensity (0.5 V). If no erection was observed, the stimulation intensity was increased and tested again until a penile erection was observed. If S1 ventral root stimulation was not effective, the stimulation electrode was moved to the S2 ventral root, and the test was repeated. A penile erection, which was evident as a full protrusion of the penis with rigidity resistant to bending, was always observed by stimulation of the individual S1 and S2 ventral roots. After the penile erection was observed, a 20-gauge catheter was inserted into the corpus cavernosum through a small incision at the tip of the penis to record penile pressure during the erection (Fig. 1), using a pressure transducer (BLPR2, WPI) connected to an amplifier (TBM4M, WPI). The pressure data were recorded on a chart recorder (TA4000, Gould, Chandler, AZ) and digitized by a computer running a LabView program (National Instruments, Austin, TX). Because electrically evoked penile protrusion is very well correlated with increased corpus cavernosum pressure,^{9,10} the pressure recording was used as a quantitative measurement of penile erection in this study.

After placement of the penile catheter ($N = 8$ cats), stimulation (30- or 40-Hz frequency, 0.2-ms pulse width, and 1-minute duration) was delivered through the hook electrode to the individual left or right sacral ventral roots (S1, S2, or S3) at a range of

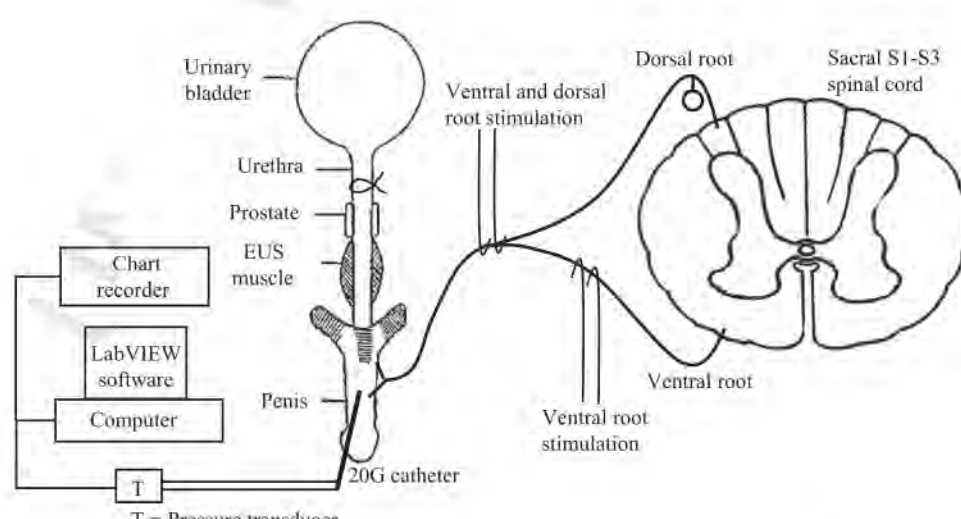


Figure 1. Experimental setup for electrical stimulation of sacral roots S1, S2, or S3 to induce penile erection. EUS, XXX.

intensities (0.5–15 V) to determine the intensity threshold (T) for inducing an observable increase in penile pressure. Next, an intensity (1.5–3T) that induced the maximal penile pressure (>100 cmH₂O) was used to test penile responses to different stimulation frequencies (b)(4). If no penile pressure > 100 cmH₂O was induced by stimulation of an individual ventral root, the maximal stimulation intensity that did not produce movement of the animal's lower body was used to test the frequency response. It was our intention not to fix the hip and lower spine so that sacral root stimulation by the hook electrode could produce local movement of the tail or hindlimb. However, the maximal stimulation intensity was limited by any movement of the lower spine that would displace the hook electrode from the nerve. After each ventral root was tested, the ventral root (S1 or S2) that elicited the largest penile pressure was combined with the dorsal root, and the combination was stimulated continuously for 10 minutes with the most effective frequency (30 Hz) and intensity to determine if the induced penile erection was sustainable during the entire stimulation period. Finally, the spinal cord was completely transected at the T9–T10 level ($N = 6$ cats). Approximately 10 minutes after the spinal cord transection, the continuous 10-minute stimulation of the same spinal root (ventral and dorsal roots together) was tested again to induce penile erection. No drug was used to treat the blood pressure change caused by the spinal transection.

Data Analysis

To compare the penile erection responses induced by stimulation of different ventral roots at different frequencies, the maximal amplitudes of the penile pressure induced by stimulation were measured and averaged across different animals for the same stimulation conditions. To determine the sustainability of the induced penile erection, the maximal penile pressure induced by the continuous 10-minute stimulations was compared with the pressure at the end of the 10-minute stimulation. To determine the effect of the spinal cord transection, the maximal penile pressures induced by the continuous 10-minute stimulation were compared before and after the spinal cord transection. The data from different animals are averaged and presented as means $\pm$ SE. Paired *t*-test or repeated-measures Friedman test followed by Dunnett's multiple comparison were performed for statistical analysis using a software package (Prism 9.3.1, GraphPad Software, San Diego, CA). The statistical significance was defined as $p < 0.05$.

RESULTS

Penile Erection Dependent on Stimulation Frequency and the Spinal Segment Stimulated

Penile erection was observed as a full protrusion of the penis with rigidity in six cats by stimulation of the S1 ventral root (S2 and S3 ineffective) and in two cats by stimulation of the S2 ventral root (S1 and S3 ineffective). The stimulation parameters were 30-Hz frequency, 0.2-ms pulse width, and intensity 4.3 ± 1.0 V (0.5–8 V). These responses, which were quantified by penile pressure recordings, showed that S1 or S2 ventral root stimulation in six cats and two cats, respectively, induced a large increase in penile pressure from the baseline pressure of 25 ± 1 cmH₂O to 177 ± 14 cmH₂O (S1) or 147 ± 2 cmH₂O (S2) (Fig. 2c). The optimal stimulation frequency to induce penile erection by S1 or S2 ventral root stimulation was 30 to 40 Hz (Fig. 2d), although lower frequencies (10–20 Hz; Fig. 2a) or higher frequencies (60–80 Hz; Fig. 2a,b) also could induce large increases in penile pressure in some cats. The stimulation intensity

for 30- to 40-Hz S1 or S2 ventral root stimulation to induce the largest increase in penile pressure was 3.5 ± 1.4 V (0.6–12 V), which was always above the motor threshold to induce leg muscle contraction (S1) or anal sphincter contraction (S2).

Sustainable Penile Erection Induced by S1/S2 Spinal Root Stimulation

The continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (ie, ventral and dorsal roots together) induced a large increase in penile pressure (190 ± 8 cmH₂O) (Fig. 3). The maximal penile pressure was either sustained (Fig. 3a) or gradually developed (Fig. 3b) during the 10-minute stimulation. The penile pressure at the end of the 10-minute stimulation was not significantly different from the maximal pressure developed during the stimulation (Fig. 3c). The maximal penile pressure induced by stimulation of the ventral and dorsal root together also was not significantly different from the maximal pressure induced by stimulation of a ventral root alone (Figs. 2d and 3c).

Sustainable Penile Erection Induced by S1/S2 Spinal Root Stimulation After Spinal Cord Transection

After a complete spinal cord transection at the T9–T10 level, the continuous 10-minute stimulation (30 Hz) of the S1 or S2 spinal root (ventral and dorsal roots together) also induced a large increase (186 ± 9 cmH₂O) in penile pressure (Fig. 4). As seen before spinal transection, the maximal penile pressure also was sustained or gradually developed (Fig. 4b) during the 10-minute stimulation. However, the induced penile pressure was significantly ($p = 0.0025$) reduced to 113 ± 19 cmH₂O after spinal cord transection compared with the penile response (186 ± 9 cmH₂O) before the spinal cord transection (Fig. 4c). The blood pressure also was significantly ($p = 0.0027$, $N = 6$) reduced from 229 ± 11 cmH₂O to 186 ± 7 cmH₂O by spinal cord transection, but it was not changed by sacral root stimulation.

DISCUSSION

This study of anesthetized cats shows that the penile erection induced by sacral root stimulation is dependent on stimulation frequency and the spinal segment stimulated. In each animal, one spinal root, most often S1, which contains a minor component of the parasympathetic efferent outflow to the pelvic viscera in the cat,^{11,12} elicited the most prominent response. The largest increases in penile pressure occurred at 30- to 40-Hz stimulation (Fig. 2), were sustainable during prolonged (10 minutes) stimulation (Fig. 3), and persisted after an acute complete transection of the spinal cord (Fig. 4). These data suggest that the efficacy of sacral neuromodulation in improving penile erectile function in people with SCI will depend on the selection of the appropriate spinal root and the optimal stimulation frequency.

Previous studies have shown that penile erection can be induced by electrical stimulation of the pelvic nerve, cavernous nerve, or dorsal penile nerve in mice, rats, cats, dogs, monkeys, or humans.^{13–17} However, the pelvic and cavernous nerves require invasive surgery for the implantation of stimulation electrodes, whereas the dorsal penile nerve is not a convenient location to attach a stimulation electrode during sexual intercourse. Therefore, these stimulation methods are not broadly adopted for clinical application to treat erectile dysfunction. Sacral ventral root stimulation also was shown to be effective in inducing penile erection in this study and in previous studies using cats¹⁸ and dogs.¹⁹ In

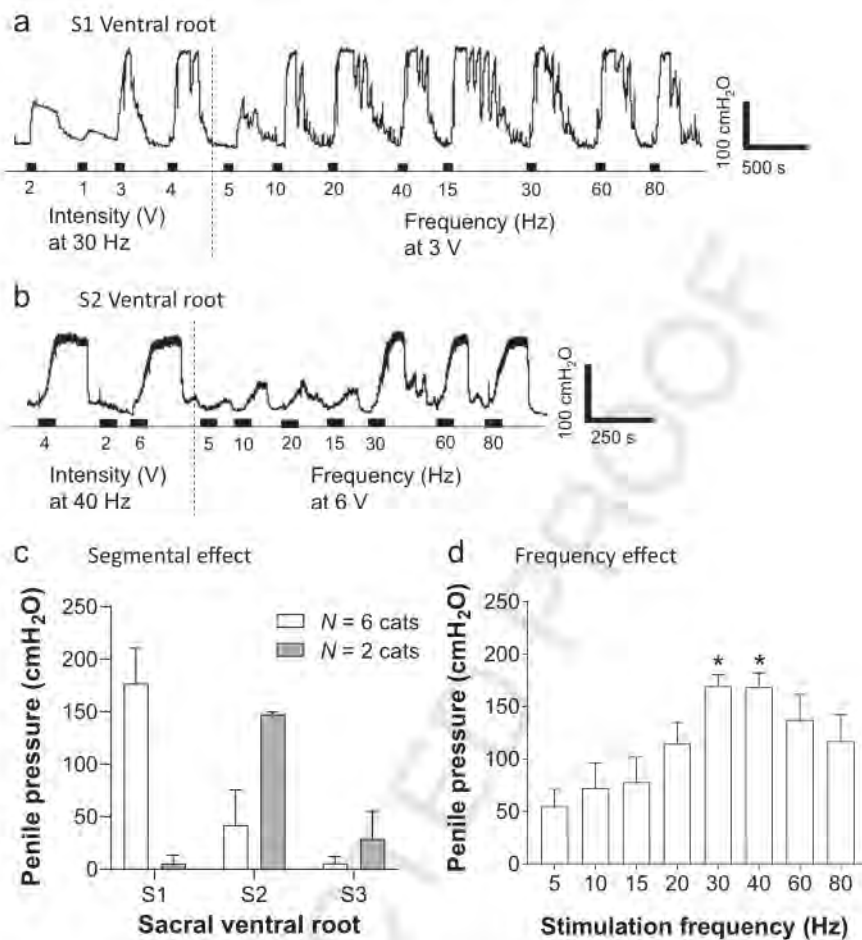


Figure 2. Penile pressure in the corpus cavernosum induced by electrical stimulation of the sacral ventral roots at different frequencies and different spinal segments. a. In a cat, S1 ventral root stimulation (10–80 Hz) of short duration (1 minute) induced a large increase (200 cmH₂O) in penile pressure lasting several minutes even after termination of the stimulation. b. In another cat, S2 ventral root stimulation (30–80 Hz) of short duration (1 minute) was most effective in inducing large increases (150 cmH₂O) in penile pressure. c. Spinal segmental effect on penile pressure induced by electrical stimulation of the ventral roots. Stimulation: 30 Hz, 0.2 ms, 3.5 ± 1.7 V (0.6–12 V) for S1 and 3.5 ± 2.5 V (1–6 V) for S2. d. Frequency effect on penile pressure induced by stimulation of S1 or S2 ventral root, $N = 8$ cats. *Significantly different from 5 Hz ($p = 0.0003$ for 30 Hz and $p = 0.0025$ for 40 Hz, Friedman test). Stimulation: 3.5 ± 1.4 V (0.6–12 V), 0.2 ms.

addition, sacral anterior root stimulation was used to restore bladder function and erectile function for some people with SCI.^{3–5,20} However, invasive spinal surgery is required to implant the stimulation electrodes on the sacral anterior roots, preventing this effective treatment from being used in people with or without SCI for the sole purpose of treating erectile dysfunction. This study of cats indicates that penile erection can be induced by stimulation of the sacral spinal roots. A previous study of dogs also showed a similar effect.¹⁹ The significance of stimulating the sacral spinal root instead of the ventral root lies in the potential to employ a minimally invasive surgical approach for stimulation. Sacral spinal roots in humans are accessible by inserting a foramen needle percutaneously to deploy a stimulating lead electrode. This surgical approach has been routinely used in sacral neuromodulation therapy for overactive bladder.⁶ Therefore, this study has significant implications for developing a new sacral neuromodulation therapy using a minimally invasive surgical approach to restore erectile function for both people with SCI and people without SCI.

Previous studies using sacral neuromodulation to treat overactive bladder also evaluated the effects on penile erection in both people with SCI and people without SCI.^{7,8} Questionnaires indicate

that participants with moderate-to-mild erectile dysfunction significantly improved their quality of sexual life by sacral neuromodulation therapy. However, these studies used stimulation parameters optimized to treat overactive bladder, ie, a stimulation frequency of 20 Hz at the sensory threshold intensity. These stimulation parameters may not be optimal for the treatment of erectile dysfunction because this study indicates that a higher stimulation frequency (30–40 Hz) at intensities above sensory and motor thresholds can induce penile erection quickly once the stimulation is turned on, and the erection can be sustained during the entire 10-minute stimulation. This type of on-demand erection should be much more satisfactory for the patient than the quality-of-life improvement produced by continuous (24 hours $\times$ seven days) sacral neuromodulation at a lower frequency and weaker stimulation intensity. Therefore, it seems that a higher frequency (30–40 Hz) and a stronger stimulation intensity (above sensory and motor thresholds) should be tested in clinical studies to treat erectile dysfunction. Although leg movement will be induced by a stronger stimulation, the leg muscle contraction will be tonic, and the movement will occur only at the beginning of the stimulation, which should not be a problem for successful sexual intercourse.

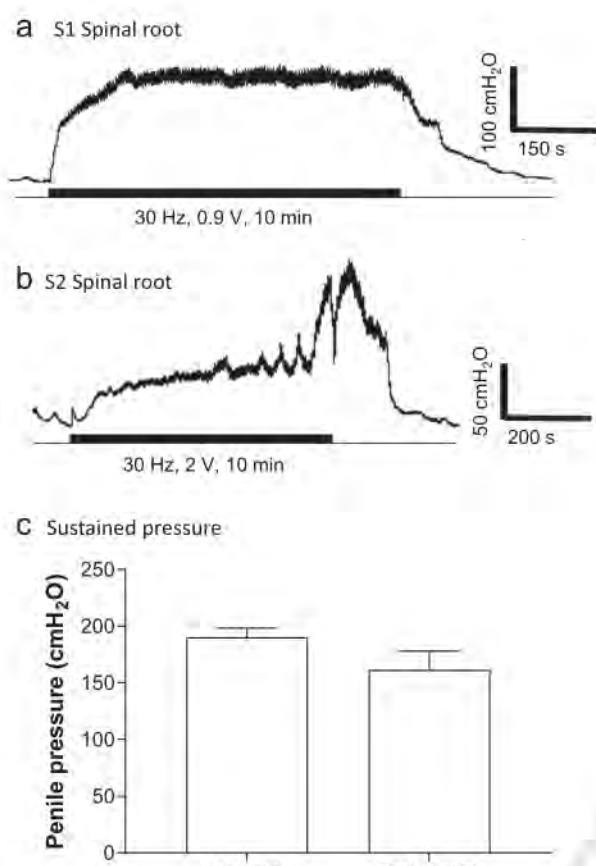


Figure 3. Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of a sacral spinal root (both ventral and dorsal roots). a. In a cat, S1 spinal root stimulation induced a large increase (160 cmH₂O) in penile pressure that was maintained during the entire 10 minutes of stimulation. b. In another cat, S2 spinal root stimulation gradually increased the penile pressure during the first 9 minutes and then triggered a fast increase in pressure during the last 1 minute of stimulation, reaching a penile pressure of 140 cmH₂O at the end of the stimulation. c. On average ($N = 8$ cats), the penile pressure at the end of the 10-minute stimulation was maintained at the same level of the maximal pressure generated during the stimulation. Stim, stimulation.

When anterior root stimulation was used to induce penile erection in people with SCI, it also induced leg movement but did not prevent successful sexual intercourse.²⁰

Stimulation of the sacral spinal root (ventral and dorsal roots together) induced a large increase in penile pressure similar to that produced by stimulation of the ventral root alone (Fig. 3). This result indicates that the activation of additional sensory nerves in the dorsal root did not disrupt the penile erection induced by direct stimulation of the efferent nerves in the ventral root. A previous study of dogs¹⁹ also reported no difference in penile erection elicited by ventral root stimulation alone or by stimulation of ventral and dorsal roots together. The threshold intensity for inducing leg movement or anal sphincter contraction was not measured in this study, which makes it difficult to estimate what types of nerve fibers were activated in the sacral roots when penile erection was induced. In human application under awake conditions, the activation of sensory nerves may cause uncomfortable or painful sensation that can limit the stimulation intensity and therefore limit the efficacy of the stimulation in treating erectile dysfunction. This potential issue

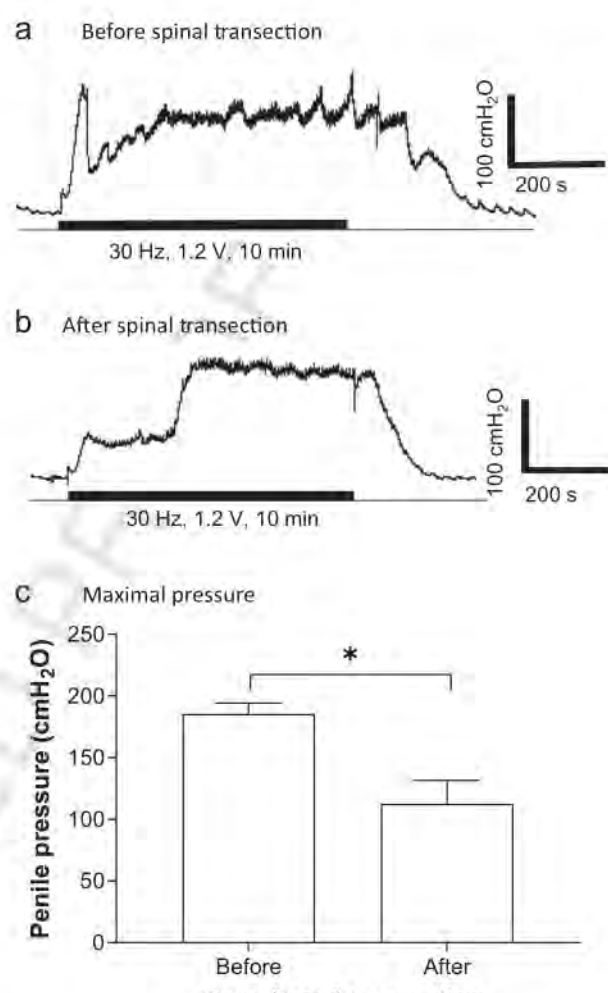


Figure 4. Penile pressure in the corpus cavernosum induced by prolonged (10 minutes) electrical stimulation of the sacral spinal root (both ventral and dorsal roots) before and after a complete spinal cord transection at the T9-T10 level. a. Before spinal transection, S1 spinal root stimulation in a cat induced a maximal 175 cmH₂O increase in penile pressure and maintained a high pressure during the 10 minutes of stimulation. b. In the same cat, 10 minutes after spinal cord transection, S1 spinal root stimulation induced a small (30 cmH₂O) increase in penile pressure during the first 3 minutes and then triggered a fast pressure increase, reaching a maximal penile pressure of 150 cmH₂O. c. On average ($N = 6$ cats), the maximal penile pressure induced by sacral S1/S2 stimulation was significantly reduced by the acute spinal cord transection. *Significant difference ($p = 0.0025$, paired t -test).

can be resolved only by a clinical study of human participants without SCI. However, it should not be an issue for participants with a complete SCI who have no sensation below the injury.

This study shows that the penile erection induced by sacral root stimulation is sustainable or gradually develops during the 10-minute stimulation (Fig. 3a,b). This indicates that sacral root stimulation can produce long-duration erections when it is applied for a longer time. It also indicates that the stimulation of afferent nerves in the dorsal root did not reflexively activate the inhibitory sympathetic pathway that can suppress penile erection.²¹ Sacral root stimulation probably activated both parasympathetic and somatic pathways to produce the erection and penile rigidity.^{21,22} However, after spinal cord transection, the amplitude of the induced penile pressure was significantly reduced, possibly owing to the spinal shock effect and

the decrease in blood pressure after an acute spinal cord transection. Nevertheless, sacral root stimulation still induced a large, sustained increase (>100 cmH₂O; Fig. 4) in penile pressure after acute spinal cord transection, indicating that this stimulation could be useful in restoring erectile function after SCI.²³ Whether sacral root stimulation will be less effective in people with SCI than in people without SCI to induce penile erection is a question to be answered by human clinical studies. However, it is known that sacral anterior root stimulation is effective in inducing penile erection strong enough for sexual intercourse in people with SCI.³⁻⁵

In summary, this study of cats optimized the stimulation parameters for sacral spinal root stimulation to induce penile erection. The results have significant implications for design of a novel neuromodulation device to restore erectile function after SCI, and for optimizing sacral neuromodulation parameters to treat people without SCI who have erectile dysfunction.

Authorship Statements

All authors designed and conducted the study, including data collection and data analysis. Changfeng Tai and William C. de Groat prepared the manuscript draft. Changfeng Tai had complete access to the study data. All authors approved the final manuscript.

How to Cite This Article

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COMMENT

The authors investigated the effect of neurostimulation of sacral roots. It appears in this animal study that stimulation of S1 or S2 makes it possible to recover erectile function by stimulating a parasympathetic component involved in penile erection. Nevertheless, it should be considered that this study should make it possible to better explore parasympathetic stimulation that is related to the functional component of erection under the direction of the autonomic nervous system. As in any animal study, this article imposes a large number of limitations. It is still difficult to be able to transpose pre-clinical studies directly to humans. Nevertheless, it seems that this study can give us indications on erectile functioning and also the possibility of helping patients suffering from spinal cord injury.

Damien Haton-Pietrin, MD, PhD
Angers, France

BASIC SCIENCE

Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats

Q2 Jialiang Chen, PhD,^{1,2} Yihua Zhong, MS,^{1,3} Jicheng Wang, PhD,¹ Bing Shen, DVM,¹ Zhijun Shen, PhD,¹ Jonathan Beckel, PhD,⁴ William-C. de-Groat, PhD,⁴ Christopher Chermansky, MD,¹ and Changfeng Tai, PhD^{1,4,5}

ABSTRACT

Background: Vaginal lubrication and contractions are among the top difficulties affecting sexual intercourse in women after spinal cord injury.

Aim: This study aimed at determining if pudendal nerve stimulation (PNS) can improve vaginal lubrication and induce increases in vaginal pressure.

Methods: In anesthetized cats, a small piece of cotton was inserted into the vagina for 10 minutes with or without PNS to measure vaginal wetness by the weight increase of the vaginal cotton. Then, a small balloon catheter was inserted into the vagina to measure the pressure increase induced by PNS. Intensity response of the vagina to PNS (30 Hz, 0.2 ms, 5 seconds) was determined at 1–4 times of intensity threshold (T) for PNS to induce an observable vaginal pressure increase. Frequency response was determined at 2T intensity in a range of PNS frequencies (5–50 Hz). Finally, fatigue in vaginal pressure was determined by applying PNS (30 Hz, 2T) either continuously or intermittently (5 seconds on and 5 seconds off) for 4 minutes.

Outcomes: The effectiveness of PNS in increasing vaginal wetness and pressure is evaluated.

Results: PNS significantly ($P = .0327$) increased the measurement of vaginal wetness from 15.8 ± 3.8 mg during control without stimulation to 32.4 ± 4.7 mg after stimulation. Vaginal pressure increased as PNS intensity or frequency increased. PNS (30 Hz, 2T) induced vaginal pressure increase $\geq 80\%$ of the maximal response. Intermittent PNS induced significantly ($P = .0354$) smaller fatigue ($45.6 \pm 3.7\%$) in vaginal pressure than continuous PNS ($69.1 \pm 3.0\%$) during the 4-minute stimulation.

Clinical Translation: This study raises the possibility of developing a novel pudendal neuromodulation device to improve female sexual function after spinal cord injury.

Strengths & Limitations: This study provides preclinical data supporting the development of a novel pudendal neuromodulation device. The limitation includes the lack of chemical analysis of the vaginal secretion.

Conclusion: PNS can improve vaginal lubrication and induce increases in vaginal pressure. **Chen J, Zhong Y, Wang J, et al. Vaginal Lubrication and Pressure Increase Induced by Pudendal Nerve Stimulation in Cats. J Sex Med 2022;XX:XXX–XXX.**

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Key Words: Stimulation; Pudendal Nerve; Vagina; Spinal Cord Injury; Cat

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¹Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA;

²Department of Urology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, PR China;

³School of Biomedical Engineering, Capital Medical University, Beijing, PR China;

⁴Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA;

⁵Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

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INTRODUCTION

Spinal cord injury (SCI) impairs sexual function in women both psychologically and physically.^{1–3} About 65% of SCI women complain about vaginal lubrication that is one of the top 3 difficulties affecting sexual intercourse.^{1,4,5} In addition, urinary incontinence during sexual intercourse, which reduces desire for sexual activity with a partner, is also a significant issue for SCI women.¹ An implantable neuromodulation device has been developed to restore urinary bladder function and has also been used to induce penile erection for SCI men.⁶ However, a neuromodulation device to restore both bladder and sexual functions for SCI women is still not available.

Preclinical studies in rats, cats and dogs^{7–12} have shown that pudendal nerve block and stimulation could be a very promising new approach to restore urinary bladder function after SCI. Since the pudendal nerve also innervates the female sexual organs including the striated muscles surrounding the vagina (vaginal vestibule) and the clitoris,^{13,14} it might be possible to improve female sexual function as well as bladder function after SCI by pudendal nerve stimulation (PNS). In addition to stimulating pudendal motor axons that contract the vaginal vestibule, PNS also stimulates sensory axons innervating the clitoris that could reflexively activate pelvic nerve efferent pathways to increase blood flow and lubrication of the vagina.^{15–17} To explore this opportunity, in this study we investigated the effects of PNS on vaginal lubrication and vaginal pressure increase in anesthetized spinal cord intact cats. If successful, the potential clinical promise is that a novel pudendal neuromodulation device could be developed to empty the bladder first to prevent urinary incontinence during sexual intercourse, and then produce vaginal lubrication and induce an increase/change in vaginal pressure during the sexual intercourse for women with SCI.

MATERIALS AND METHODS

The experimental protocol and animal use in this study were approved by the Institutional Animal Care and Use Committee at University of Pittsburgh.

Surgical Procedures

4 female cats (2.7–3.4 kg, Marshall BioResources, North Rose, NY, USA) were anesthetized with isoflurane (2–5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented 10–15 mg/kg as needed) during data collection. Cats were used in this study because we are currently also using cats to develop a pudendal neuromodulation device for restoring bladder function after SCI.^{7,8} The left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN, USA) attached to the tongue. Through an abdominal incision, a catheter was inserted into the bladder via the urethra and remained in place to drain the bladder during the experiment. The urethra was tied with a suture to prevent urine leakage into the urethra. The pudendal nerve on the left side was dissected through a 3–4 cm long incision in the region of sciatic notch between the base of the tail and the hip bone (iliac crest). A bipolar cuff electrode (NC223pt, MicroProbe, Gaithersburg, MD, USA) was implanted on the nerve and connected to external stimulator (S88, Grass Instruments, West Warwick, RI, USA) via a constant voltage stimulus isolator (SIU5A, Grass Instruments, West Warwick, RI, USA) for electrical stimulation. After the surgery, all incisions were closed with

sutures. A heating pad was used to maintain the animal body temperature between 35 and 37°C during the experiment.

Experimental Protocol

At the beginning of the experiment, a small piece of dry cotton was weighed (about 30 mg) and inserted into the vagina 2–3 cm from the external orifice for 10 minutes without stimulation. After the 10 minutes, the cotton was removed and weighed again to determine the increase in weight that was used as the control value to indicate the vaginal wetness. Similar method using a cotton swab to measure vaginal lubrication was also used in a previous animal study.¹⁸ Then, the same 10-minute cotton test was repeated using another piece of dry cotton (weight about 30 mg) while PNS (30 Hz, 0.2 ms) at the 2 times intensity threshold for inducing observable anal twitching was also applied intermittently (5 seconds on and 5 seconds off) during the 10-minute period to increase the vaginal wetness.

After testing the vaginal wetness, a small catheter (7 French, Cook Medical LLC, Bloomington, IN, USA) with a balloon at the tip was inserted 15–20 mm into the vagina. The balloon (10 mm in length) was slightly distended by 0.2 mL air to measure the vaginal pressure increase via a pressure transducer (BLPR2, WPI, Sarasota, FL, USA) connected to an amplifier (TBM4M, WPI, Sarasota, FL, USA). The catheter was secured in place using surgical tape and sutures. PNS (30 Hz, 0.2 ms) was applied for 5 seconds each time at 5 intensities increasing from the intensity threshold (T) for inducing a small observable vaginal pressure increase to 4T to determine the intensity response. Then, PNS of 2T intensity was applied at different frequencies (5–50 Hz) to determine the frequency response. Finally, PNS (30 Hz, 0.2 ms, 2T) was applied for 4 minutes either continuously or intermittently (5 seconds on and 5 seconds off) in a random order to determine fatigue in vaginal pressure during the 4-minute-long stimulation. A time interval of 5–10 minutes was inserted between the 2 4-minute PNS to avoid any carry-over fatigue effect.

Data Analysis

Vaginal wetness was measured by the weight increase of the cotton inserted in the vagina under control condition or during PNS. The vaginal pressure increases induced by PNS of different intensities or frequencies was normalized to the maximal response to determine the effect of PNS intensity or frequency. Vaginal fatigue was measured as the vaginal pressure reduction at the end of the 4-minute PNS divided by the maximal vaginal pressure increase during the PNS. The data from different animals are averaged and presented as means $\pm$ SE. Statistical significance ($P < .05$) was determined by paired *t*-test or repeated-measures one-way ANOVA followed by Dunnett multiple comparison.

RESULTS

PNS applied intermittently (5 seconds on and 5 seconds off) for 10 minutes at the intensity of 2 times threshold for inducing

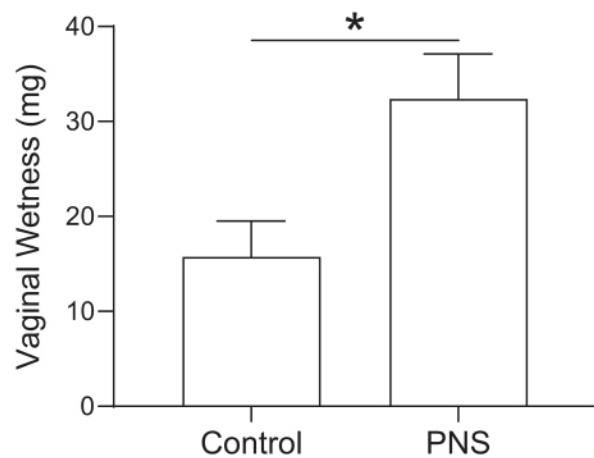


Figure 1. Pudendal nerve stimulation (PNS) increased vaginal wetness. PNS: 30 Hz, 0.2 ms, 1.5T = 0.15–0.45 V, 5 seconds on and 5 seconds off for 10 minutes. T – threshold intensity for inducing anal twitch. * indicates significantly different (paired *t*-test, $P = .0327$). The data are shown as mean + SE. $N = 4$ cats.

anal twitching significantly increased vaginal wetness (Figure 1). Without PNS, the vaginal wetness as indicated by the increase in the vaginal cotton weight during a 10-minute period was 15.8 ± 3.8 mg (control in Figure 1). After 10-minute PNS, the vaginal

wetness was significantly ($P = .0327$) increased to 32.4 ± 4.7 mg (PNS in Figure 1).

The vaginal pressure was significantly ($P < .05$) increased as the PNS intensity increased (Figure 2). PNS (30 Hz) at an intensity of $\geq 2T$ induced a vaginal pressure increase equal to 90% of the maximal response (Figure 2B). Meanwhile, at intensity of 2T the PNS induced a significantly ($P < .05$) larger increase in vaginal pressure at frequencies ≥ 20 Hz compared to frequencies ≤ 10 Hz (Figure 3). When PNS (30 Hz, 2T) was applied for 4 minutes, it produced less fatigue when applied intermittently (Figure 4A) than continuously (Figure 4B). On average ($N = 4$), intermittent PNS induced $45.6 \pm 3.7\%$ fatigue that is significantly ($P = .0354$) smaller than the fatigue induced by continuous PNS ($69.1 \pm 3.0\%$, Figure 4C).

DISCUSSION

This study in anesthetized spinal intact female cats shows that PNS can improve vaginal lubrication by increasing the wetness of the vagina (Figure 1). PNS induced a large increase in vaginal pressure (Figures 2 and 3) that is more fatigue resistant during intermittent than during continuous stimulation (Figure 4).

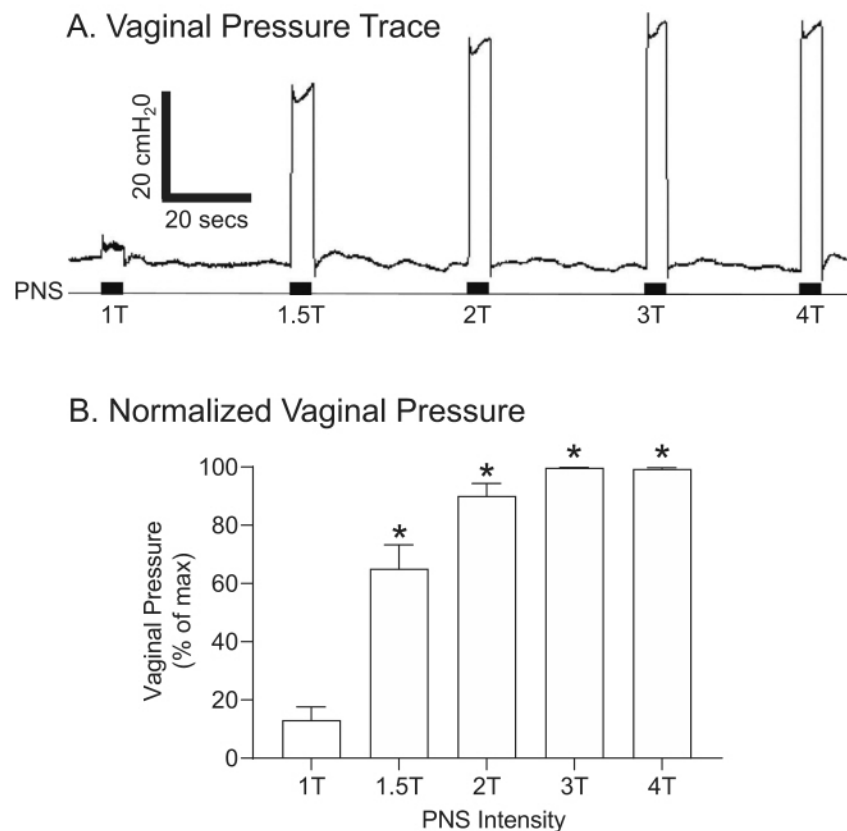


Figure 2. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation intensities. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 30 Hz, 0.2 ms, 5 seconds, $T = 0.16$ V. T – threshold intensity for inducing a small observable vaginal pressure increase. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 30 Hz, 0.2 ms, 5 seconds, $T = 0.16$ –0.5 V. * indicates significantly different from 1T (one-way ANOVA followed by Dunnett multiple comparison). The data are shown as mean + SE. $N = 4$ cats.

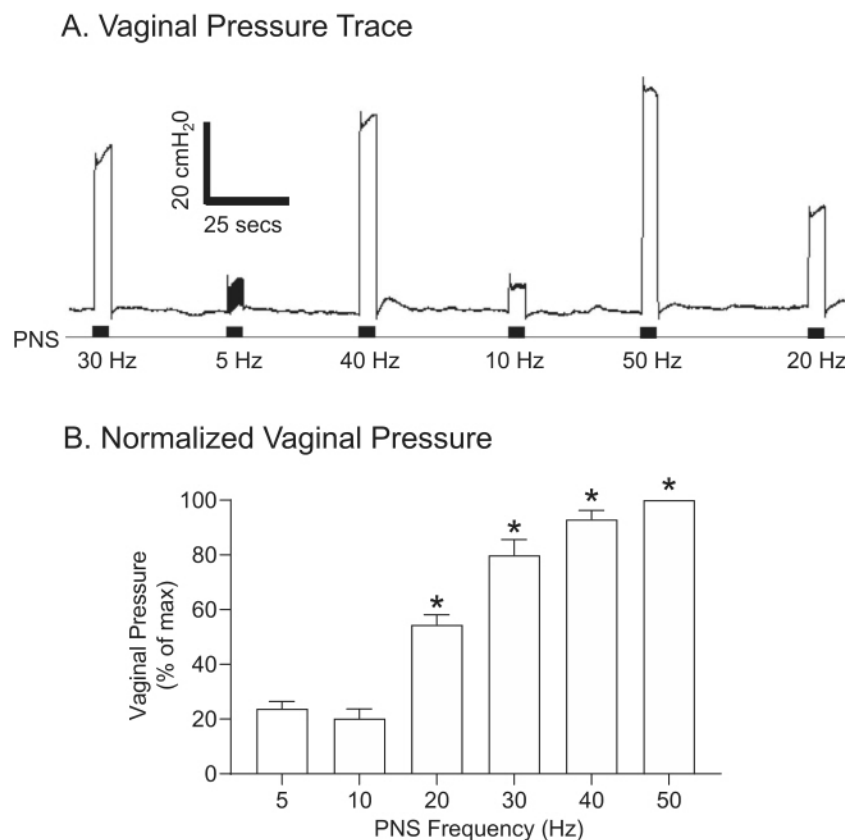


Figure 3. Vaginal pressure increase induced by pudendal nerve stimulation (PNS) at different stimulation frequencies. A. Vaginal pressure trace. Black bar under the pressure trace indicates the PNS duration. PNS: 2T = 0.32 V, 0.2 ms, 5 seconds. T – threshold intensity for inducing a small observable vaginal pressure increase. B. Vaginal pressure normalized to the maximal response in each cat. PNS: 2T = 0.32 V, 0.2 ms, 5 seconds. * indicates significantly different from 5 Hz (one-way ANOVA followed by Dunnett multiple comparison). The data are shown as mean + SE. N = 4 cats.

These results suggest that PNS might be useful to improve female sexual function after SCI. Since our recent studies have shown that PNS can also enhance urine storage and voiding in both anesthetized spinal intact cats and awake cats with a chronic complete spinal cord transection at T9/T10 level,^{7,8} a novel pudendal neuromodulation device could be developed to benefit SCI women by enhancing vaginal lubrication and inducing a vaginal pressure increase/change as well as preventing urinary incontinence during sexual intercourse by emptying the bladder before sexual activity.

Previous animal studies in mice, rats, rabbits, and dogs have shown that vaginal blood flow and vaginal lubrication could be increased by mechanical stimulation of the vagina or electrical stimulation of the pelvic nerve or clitoral/pudendal nerve.^{19–24} Our current study in cats shows that PNS can also increase vaginal lubrication in cats. Since the clitoral nerve is a branch of the pudendal nerve, PNS might have activated clitoral afferent input to the central nervous system to trigger a reflex output via the pelvic nerve to increase the blood flow and lubrication of the vagina.^{15–17} In addition, since the pudendal motor axons innervate the striated muscle surrounding the vagina,^{13,14} anal sphincter, and pelvic floor muscles, PNS can produce contraction of

these muscles to increase vaginal pressure and further facilitate vaginal secretion.¹⁵ Periodic or pulsed contractions of this group of pelvic muscles also occur during orgasm.^{15,25} Therefore, in addition to increasing vaginal lubrication PNS may also induce or facilitate orgasmic sensations for women with incomplete SCI who still have some sensation from the vagina or pelvic area.

The advantages of PNS over pelvic/clitoral nerve stimulation used in previous animal studies^{19–22,24} include: (i) The pelvic nerve in human consists of many small branches that are not easily accessible by surgery for implanting stimulation electrode, while pudendal nerve can be accessed for electrical stimulation via a minimally invasive surgical approach²⁶; (ii) Clitoral nerve stimulation requires implantation of a stimulation electrode at an inconvenient location for sexual activities, while the main trunk of the pudendal nerve that is stimulated in this study is far away from the sexual organ; (iii) Restoring female sexual function by PNS could be integrated into the pudendal neuromodulation device that is currently in development in our lab for restoring bladder function after SCI.^{7,8}

Only continuous and intermittent PNS were tested in this study (Figure 4), but other vaginal contraction patterns that more closely mimic those during orgasm could also be induced

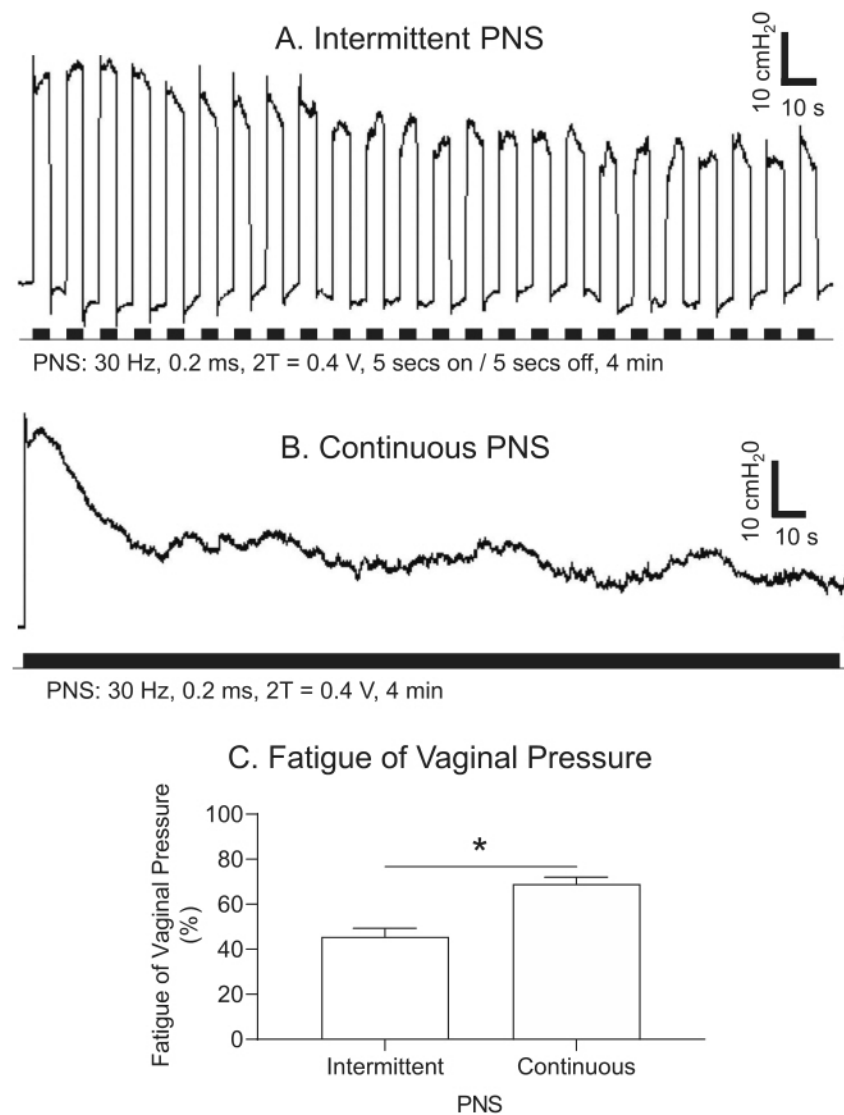


Figure 4. Fatigue of vaginal pressure increases induced by prolonged (4 minutes) pudendal nerve stimulation (PNS). A. Vaginal pressure responses induced by intermittent PNS. B. Vaginal pressure responses induced by continuous PNS. Black bars under the pressure trace indicate the PNS duration. C. Fatigue of vaginal pressure responses. The reduction in vaginal pressure responses at the end of 4-minutes PNS was normalized to the maximal response to indicate the muscle fatigue. PNS: 30 Hz, 0.2 ms, 2T = 0.32-1 V, 4 minutes. T – threshold intensity for inducing a small observable vaginal pressure increase. * indicates significantly different (paired *t*-test, *P* = .0354). The data are shown as mean + SE, N = 4 cats.

by programming the PNS intensity and duration. This study investigated the full range of vaginal pressure responses to different stimulation frequency and intensity. The maximal pressure generated by PNS in cats is about 40 cmH₂O that could be very different from pressures in humans due to the different anatomy. A maximal vaginal pressure may not be necessary to achieve the functions of vaginal contractions for improving vaginal lubrication, female organism, and male sexual sensation/organism. In clinical application, a periodic or pulsed stimulation can be used to generate different patterns of vaginal contractions and the stimulation intensity can be modulated to generate different contraction pressures. To develop a therapeutic device, a significant amount of information from female users' needs to be acquired

to determine the stimulation patterns that can be pre-programmed into the device. The current animal study provides the preclinical evidence supporting the feasibility to develop such a therapeutic device.

Since the urethra was ligated by a suture, it was unlikely that urine leaked into the vagina to change the vaginal cotton wetness. Therefore, the increase in the vaginal cotton weight must be due to fluid accumulation in the vagina during the 10-minute period. Vaginal lubrication during sexual arousal is mainly due to the increased blood flow in the vagina producing transudate on the surface of the vaginal wall.²⁷ In addition, vaginal fluid could also contain secretions from female prostate or Bartholin's glands.²⁷ Therefore, it is possible that the fluid absorbed by the

cotton in this study contains both the transudate from vaginal blood vessels and secretions from female sexual glands. However, the vaginal fluid collected by the cotton in the vagina was not analyzed for the chemical composition in this study.

In summary, this study in anesthetized spinal intact cats shows that PNS can improve vaginal lubrication and induce vaginal pressure increase/change. If these results can be obtained in a future study using spinal cord injured cats, it will support the idea of developing a pudendal neuromodulation device to restore or improve female sexual function after SCI. Since our recent studies in cats^{7,8} have shown that PNS/block can successfully restore bladder function after SCI, this current study indicates a possibility to develop a novel implantable pudendal neuromodulation device to restore both bladder and sexual function for women with SCI.

CONFLICT OF INTEREST AND FUNDING

Conflict of Interest: The authors have no conflicts of interest.

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Corresponding Author: Changfeng Tai, Urology, University of Pittsburgh, 700 Kaufmann Building, Pittsburgh, PA 15213, USA, Tel: (412) 6924142; Fax: (412) 6924380.; E-mail: cftai@pitt.edu

STATEMENT OF AUTHORSHIP

C.T. conceived the experiments. All authors performed experiments and wrote the manuscript. C.T. secured funding.

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University of Pittsburgh DARPA Bridging the GAP Plus (BG+) Preliminary Design Review Report

Period Covered by the Report: Oct. 1, 2020 through Sept. 30, 2021

(b)(4)

Date of Report: September 30, 2021

Project Title: An Implantable System to Stabilize Acute SCI and Restore Bladder, Bowel, and Sexual Functions after Chronic SCI

Contract or Agreement Number: N66001-20-C-4050

Total Dollar Value: (b)(4)

Subcontractors: Med-Ally LLC and Thermaquil, Inc.

Program Manager: (b)(4); (b)(6)

Submitted by:

(b)(4); (b)(6)

700 Kaufmann Building

University of Pittsburgh

Telephone: (b)(4); (b)(6)

Email: (b)(4); (b)(6)

1 High-Level Project Progress, including Big Wins

During the (b)(4) we have made major progresses in the following areas:

1. Scientific progress:

- Big Win – We have successfully demonstrated in anesthetized cats that pudendal nerve stimulation and block by lead electrodes can achieve a low-pressure (≤ 50 cmH₂O) voiding. This success indicates a minimally invasive surgical approach by inserting lead electrodes close to the pudendal nerves can be developed to restore bladder function after spinal cord injury. The data/results from this animal study have been processed, analyzed, peer-reviewed, and now published on Experimental Neurology, 2021; 346:113860. An abstract was accepted for presentation at American Urological Association (AUA) Annual Meeting, September 10-13, 2021, Las Vegas, Nevada. Second abstract was also accepted for presentation at International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.
- We have also successfully demonstrated in anesthetized cats that stimulation of sacral S2 spinal root at (b)(4) is most effective to induce colon contractions, while stimulation of S1/S3 root or pudendal nerve is not effective. In addition, defecation is successfully

induced by stimulation of S2 spinal root using wire electrodes. (b)(4)

(b)(4)

(b)(4)

The data/results from this animal study have been processed, analyzed, and summarized. A manuscript has been submitted for review and publication (see attachment for the submitted manuscript).

- Animal study to restore sexual function after spinal cord injury has been initiated. Results from the first 4 cats show that stimulation of sacral S1 spinal root is effective in inducing strong sustained penile erection in male cats, while stimulation of S2/S3 spinal root or pudendal nerve is ineffective. This study will continue in the (b)(4)

(b)(4)

(b)(4)

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2. Engineering progress:

(b)(4)

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- Completed the full electrical redesign schematics of a (b)(4) (b)(4) built upon the specific requirements for BG+ therapy application.

(b)(4)

-

- All safeties and electrical conditions suitable for human implantation have been reviewed, analyzed, and implemented in the new platform, thus making it suitable (b)(4) (b)(4) for both animal and human trials.

- Preliminary firmware has been written and completed to meet with the new schematics guided by BG+ schematics requirements.

- The new schematics design uses (b)(4) requiring software development. This software development has been completed.
- (b)(4)
- The PCB fabrication was successfully completed. A sample population of the PCB's were populated with acquired parts and tested, then brought up, loaded with firmware and tested for function and pulse generation. The functional PCB card was successfully tested at University of Pittsburgh to block and stimulate cat pudendal nerves.
- The enclosure design for (b)(4) was completed, with specialty hardware located and acquired. (b)(4)
- (b)(4)
- (b)(4) enclosures were successfully fabricated as proof of concept, to be integrated with the PCBA, then demonstrated to University of Pittsburgh.
- (b)(4) will be programed and controlled by (b)(4) Graphical User Interface (GUI). (b)(4) will communicate with (b)(4)
- Multiple sessions with Dr. Tai and Med-Ally GUI developer concluded in a complete GUI that meets with the BG+ therapy approach and requirements. The completed GUI is currently being interfaced with (b)(4) for full functionality.
- Progress on a (b)(4) achieved. The newly acquired (b)(4) was successfully used to fabricate (b)(4) BG+ required materials were specified, identified, located then ordered, while (b)(4)
- Tooling was designed for (b)(4) completed then sent out for fabrication. Additionally, peripheral equipment was identified and purchased for (b)(4) (b)(4) currently in house awaiting final tooling. Lastly, needed raw materials and consumables were specified, located then purchased, currently in house to be enacted on shortly.
- The mechanical design of the BG+ implanted device (b)(4) progressed significantly, (b)(4)
- The design also includes (b)(4)
- We have compared and contrasted various communication schemes and resulting mechanical antennae designs for the IPG, deciding on optimal scenarios and resultant updates to the IPG, with two main considerations to test and simulate.

- (b)(4)
-

3. Regulatory progress:

- Met with the FDA regarding (b)(4)
 - Received feedback on (b)(4)
 - Given FDA's positive and very helpful feedback, we (b)(4) and made other more minor changes.
- Drafted early feasibility human clinical protocol for the EPG system.
- Finalized logistics (b)(4), (b)(6)
- Continued to identify user needs and voice of customer to inform device requirements (b)(4)
- FDA (b)(4) was submitted, which focuses on the (b)(4)
- Met with the FDA regarding (b)(4) on the (b)(4)
 - (b)(4)
 -

4. Administrative progress:

- Two weekly meetings were hold between University of Pittsburgh, Med-Ally, and Thermoquil to coordinate the project activity.
- Monthly meeting was hold with DARPA to report our progress.
- (b)(4)

2 Tasks: Progress, Accomplishments, and Plans

Task 1.1 Institutional IACUC approval and DOD ACURO review (b)(4)

The third-year renewal of our IACUC protocol was approved by University of Pittsburgh, DOD ACURO, and NAVY IACUC.

Task 1.2 Animal studies to develop the external system and the implantable system

Sub-Task 1.2.1 Restore bladder function (b)(4)

We have successfully demonstrated in anesthetized cats that pudendal nerve can be blocked by (b)(4) stimulation using a lead electrode and a low-pressure voiding can be produced by a (b)(4) pudendal nerve stimulation combined with the pudendal nerve block. (b)(4)

(b)(4)

(b)(4) This study has now been peer-reviewed and published on Experimental Neurology (see attachment for the publication).

Nerve Block by a Lead Electrode

Our study in anesthetized cats has discovered that the pudendal nerve can be completely blocked by a lead electrode immediately following the termination of a (b)(4) stimulation, i.e., a post-stimulation block (Fig.1). Blocking nerve conduction by a (b)(4) is novel because all previous studies require a major invasive surgery to implant a cuff electrode to block a peripheral nerve conduction. Our study demonstrates in the first time (b)(4)

(b)(4) is not needed, and a nerve block can be induced (b)(4)

(b)(4)

As shown in Fig.1, Stim.B (b)(4) successfully blocked the periodic EUS contractions induced by short bursts of Stim.C (b)(4) off) during a period after terminating the (b)(4) stimulation, i.e., a post-stimulation block. Since the EUS contractions induced by (b)(4) was not changed while the contractions induced by (b)(4) was blocked, it indicated that (b)(4) blocked the pudendal nerve conduction instead of causing a EUS fatigue that should affect the responses induced by both (b)(4) and (b)(4). For long-term bladder management after SCI, repeatedly fatiguing the EUS by pudendal nerve stimulation will cause the EUS to become more and more fatigue resistant and not relax sufficiently for a low-pressure voiding.

The duration of (b)(4) post-stimulation block was dependent on the intensity and duration of the stimulation. As shown in Fig.2, the block duration was increased by increasing the intensity of (b)(4) from (b)(4). At the intensity of (b)(4) the duration for a complete post-stimulation block reached 2 mins with a recovery duration of 6 mins (see the third trace in Fig.2). When the intensity of (b)(4) increased to (b)(4) the complete block duration increased to (b)(4) mins, but the recovery duration increased dramatically to (b)(4) mins (see the fourth trace in Fig.2). In the other 2 cats, a prolonged recovery duration (58 mins or 60 mins) was induced by (b)(4) stimulation at the intensity/duration combination of (b)(4) respectively.



Fig.1: A. Experimental setup for pudendal nerve block by a hook electrode. B. Complete nerve block occurred after the end of the (b)(4) continuous stimulation. The arrows indicate the EUS contractions induced by the (b)(4) stimulation at the distal site (b)(4). The thick bars under the EUS pressure trace indicate the stimulation durations for (b)(4) and (b)(4). After ending the (b)(4) (b)(4), EUS contraction can still be induced by the distal (b)(4) but the contractions induced by the central (b)(4) are completely blocked initially and then gradually recover, indicating that nerve conduction block occurs locally at (b)(4) and the EUS is not fatigued. EUS –External Urethral Sphincter.

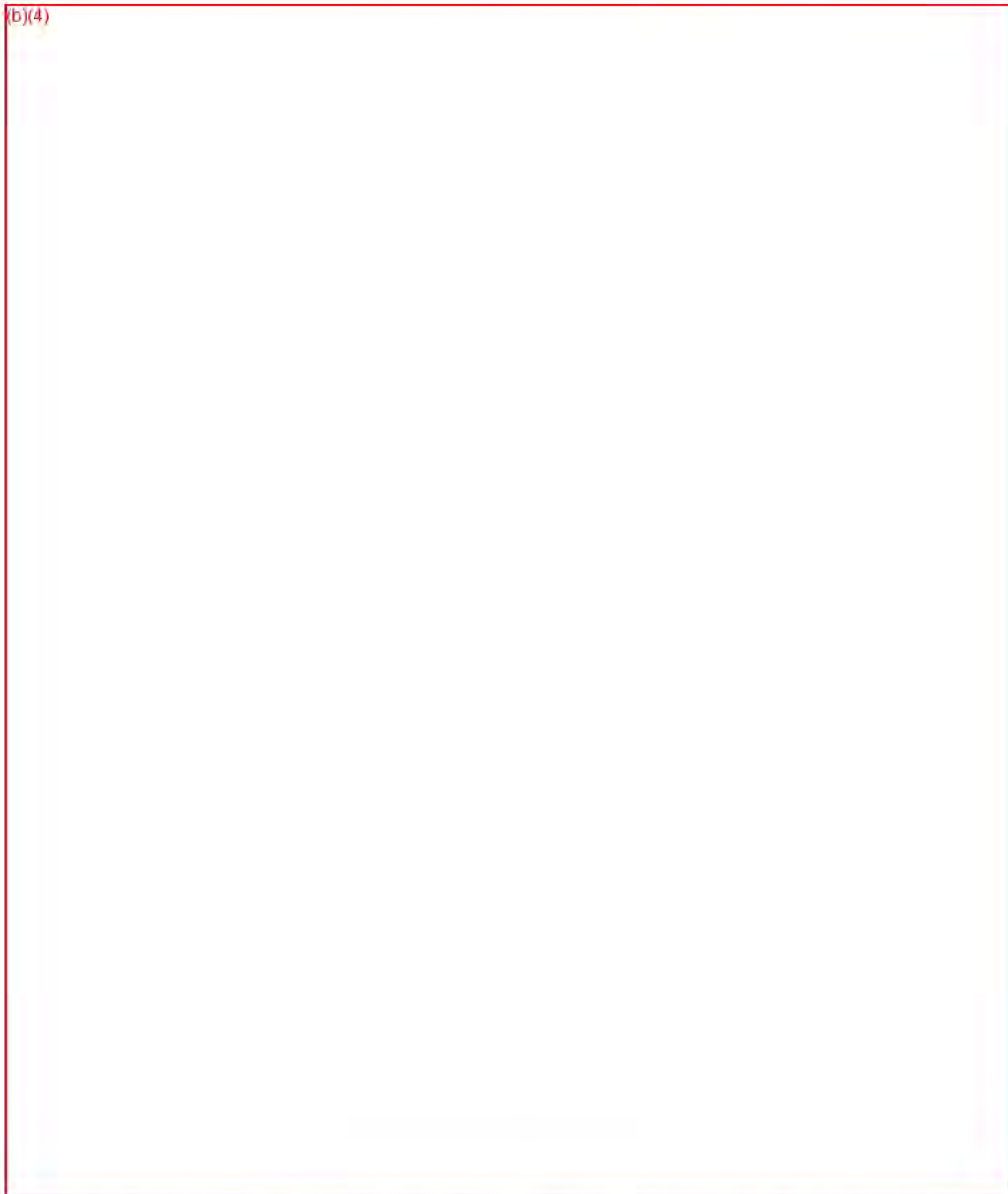


Fig. 2: Block stimulation (b)(4) produced a complete post-stimulation block of duration ≥ 2 minutes as the stimulation intensity gradually increased to (b)(4) intensity. (b)(4) produced a prolonged post-stimulation block with a recovery period of 110 minutes. The short black bar under each pressure trace indicates the stimulation duration. The duration of (b)(4) shown at the bottom applies to all pressure traces. The intensity for (b)(4) is at 3 times of the threshold (T) intensity for inducing visible anal twitch to induce maximal contractions of the external urethral sphincter.

Fig.3 summarized the intensity and duration required for (b)(4) stimulation to induce a complete post-stimulation block of (b)(4) and the corresponding duration for the block and recovery (N=6 nerves from 3 cats). In one cat, (b)(4) duration failed to induce a complete post-stimulation of duration ≥ 2 mins before its intensity increased to the level causing hindlimb movement. In this cat after increasing the stimulation duration to (b)(4) required a much lower intensity (b)(4) see the black dots in Fig.3) than 30 secs duration to induce the complete block. These results indicate that there is a combination of intensity and duration for (b)(4) to induce a complete post-stimulation block of duration (b)(4) that can recover relatively quick (b)(4) (Fig.3). However, if the intensity and/or the duration increased further, a prolonged recovery of 1-2 hours may occur (see the fourth trace in Fig.2). The complete block was defined as the EUS contraction pressure induced by (b)(4) was reduced to (b)(4) that would allow a low-pressure voiding to occur. The complete post-stimulation block of duration ≥ 2 mins would allow a duration long enough for voiding to complete.

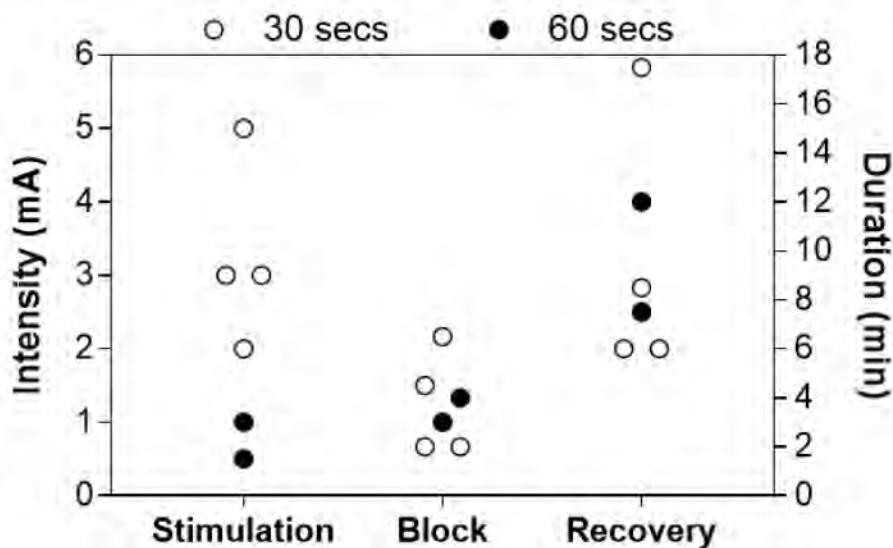


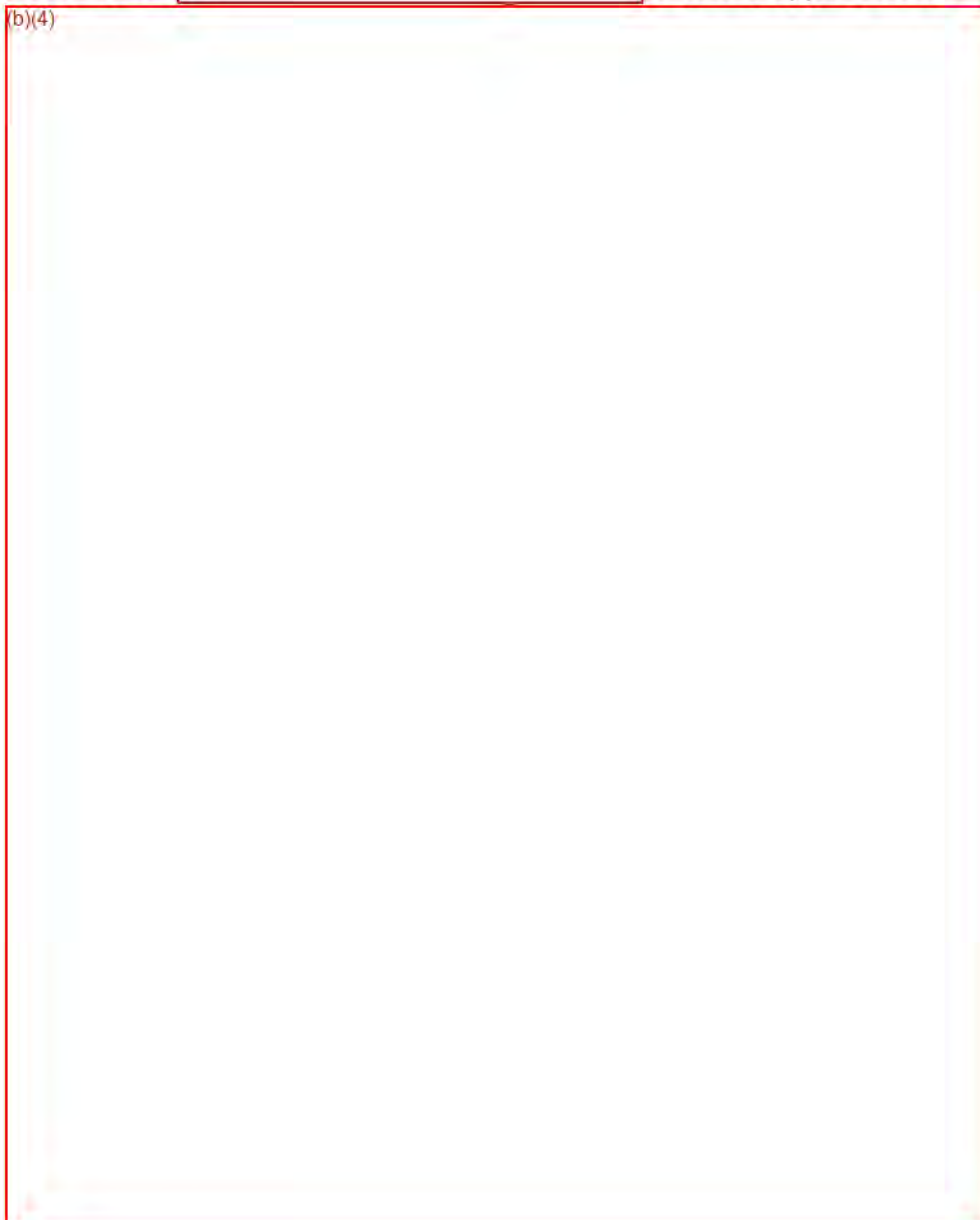
Fig.3: The intensity and duration (b)(4) required for (b)(4) stimulation to produce a complete post-stimulation block of duration ≥ 2 mins and the corresponding duration for the block and recovery. Stimulation intensity is shown on the left Y-axis. Duration for the block and recovery is shown on the right Y-axis. N = 6 nerves from 3 cats.

Low-Pressure Voiding by Lead Electrodes

More importantly, we further demonstrated that a low-pressure (b)(4) voiding can be successfully induced by a (b)(4) pudendal nerve stimulation during the post-stimulation block of bilateral pudendal nerves induced by lead electrodes (Fig.4). Low-pressure voiding induced by pudendal nerve stimulation and block is critical for long-term management of bladder dysfunction after SCI, because high-pressure voiding can cause renal failure in a long-term clinical application. Therefore, the (b)(4) pudendal nerve block as shown in our study is very important because it can prevent detrusor sphincter dyssynergia (DSD) after SCI and reduce

urethral outlet resistance for a low-pressure voiding to occur. The clinical significance of using (b)(4) electrodes to induce a low-pressure voiding is that a minimally invasive surgical approach by inserting the (b)(4) close to the pudendal nerves, instead of a major invasive surgery to implant the (b)(4) can be used to block and stimulate the pudendal nerves to restore bladder function after SCI.

Fig.4 shows that using monopolar hook electrodes the (b)(4) pudendal nerve stimulation combined with 1 kHz post-stimulation block successfully induced a low-pressure (b)(4) voiding. During the control CMG (Fig.4B), slow infusion of the bladder triggered a micturition reflex producing a low-pressure (b)(4) voiding. With the bladder partially full (Fig.4C), Stim #1 (b)(4) alone induced a voiding of a high pressure (≈ 60 cmH₂O). However, the same Stim #1 successfully induced a low-pressure (≈ 40 cmH₂O) voiding when applied immediately after termination of the (b)(4) that was applied to both Stim #2



and #3 (see Fig.4D). The high-pressure (b)(4) voiding was returned in about minutes after termination of the (b)(4) stimulation (Fig.4E).

Fig.5 shows that a low-pressure voiding can also be induced by combination of (b)(4) block and (b)(4) stimulation using bipolar hook electrodes. However, the recovery from a low-pressure voiding to a high-pressure voiding was prolonged since the (b)(4) block stimulation was too strong. As shown in Fig.5A-B, micturition reflex induced a low-pressure (b)(4)

(b)(4)

voiding while (b)(4) alone induced a high-pressure (≈ 100 cmH₂O) voiding. After applying (b)(4) stimulation (b)(4) to both (b)(4) the same Stim #1 lost the ability to induce a bladder contraction in about 10 mins, and then it successfully induced a low-pressure (b)(4) voiding (see Fig.5C). The low-pressure voiding induced by Stim

#1 alone lasted for at least an hour (Fig.5 D-E). In 3 hours, the voiding pressure returned to about (b)(4). Please note that there was no leakage of fluid from the bladder during the period immediately following termination of the (b)(4) although many non-voiding contractions occurred during this period (Fig.5C).

It is worth noting that the (b)(4) post-stimulation block is reversible within minutes if an appropriate combination of stimulation intensity and duration is used (Figs.3-4). This reversibility is needed because the (b)(4) needs to be functional after each voiding to maintain continence during urine storage. There is an optimal combination of stimulation intensity and duration for achieving a short duration (b)(4) post-stimulation block to allow a low-pressure voiding to occur while also allowing the block to recovery within 20 mins (Fig.3). In future clinical application, this optimal combination of stimulation intensity and duration will have to be determined for each individual subject after implanting the pudendal nerve stimulation and block device. If the (b)(4) stimulation is too strong or lasts too long, the pudendal nerve block will need a long time to recovery (see forth trace in Fig.3), which will result in a prolonged period of low-pressure voiding (Fig.5).

Fig.6 summarizes the results from 5 cats. On average, in the control condition the micturition reflex induced by filling the bladder produced low-pressure (b)(4) voiding (Fig.6A) of a 50% voiding efficiency (Fig.6B) with a post-void residual volume of 50% (Fig.6C) of the control bladder capacity. A slightly higher voiding efficiency, but not significantly different from control, and a lower post-void residual volume were produced by (b)(4) stimulation alone (Fig.6 B-C) at a significantly ($p<0.05$) higher voiding pressure (b)(4). However, (b)(4) stimulation combined with (b)(4) post-stimulation block significantly ($p<0.05$) increased voiding efficiency (80%, Fig.6B) and reduced post-void residual volume (20%, Fig.6C) but at a significantly ($p<0.05$) lower pressure (≈ 40 cmH₂O) that was same as in the control condition (Fig.6A). After a recovery period ranging from 15 mins to 3 hours (see Fig.4 and Fig.5), the voiding pressure induced by (b)(4) stimulation alone increased to about (b)(4) while voiding efficiency declined (50%, Fig.6B) and post-void residual volume significantly ($p<0.05$) increased (50%, Fig.6C) above that measured immediately after the (b)(4) stimulation. On average a similar amount of saline was infused into the bladder under the different conditions (Fig.6D).

(b)(4)

Fig.6. Summary of the urodynamic parameters for control condition, (b)(4) stimulation alone (b)(4) stimulation after (b)(4) stimulation (b)(4)), and recovery from the (b)(4) blocking effect (Post-kHz recovery). A. Voiding pressure. B. Voiding efficiency. C. Post-void residual. D. Bladder volume infused. * indicates a significant difference ($p < 0.05$, ANOVA). N = 5 cats.

Sub-Task 1.2.2 Restore bowel function (b)(4)

A total of 9 cats (4 females and 5 male, 4.0 ± 0.3 kg; (b)(4)) were anesthetized with isoflurane (2-5% in oxygen) during surgery and then switched to α -chloralose anesthesia (initial 65 mg/kg i.v. and supplemented as needed) during data collection. Left cephalic vein was catheterized for fluid administration. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic blood pressure. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; (b)(4)) attached to the tongue. Through an abdominal incision, one balloon catheter (G15766, (b)(4) Spencer, IN) 1.4 cm in diameter and 5 cm in length was inserted into the proximal colon via a small incision at the proximal end of the colon (Fig.7). The second balloon catheter of the same size was inserted into the distal colon via the anus. The two balloons were filled with water (5-7 ml) to a resting pressure of 10-15 cmH₂O to measure proximal and distal colon contractions in the first group of 7 cats. In the second group of 2 cats, a large balloon catheter made of a condom (3.5 cm in diameter and 15 cm in length) was inserted via the small incision

at the proximal end of the colon to occupy the entire colon so that both proximal and distal colon contractions were recorded. The condom was filled with water (b)(4) to a resting pressure of (b)(4). In these 2 cats, 1-4 glass marbles (diameter 1.5 cm) were also inserted into the rectum via the anus so that evacuation of these marbles could be videotaped simultaneously with the colon pressure recording.

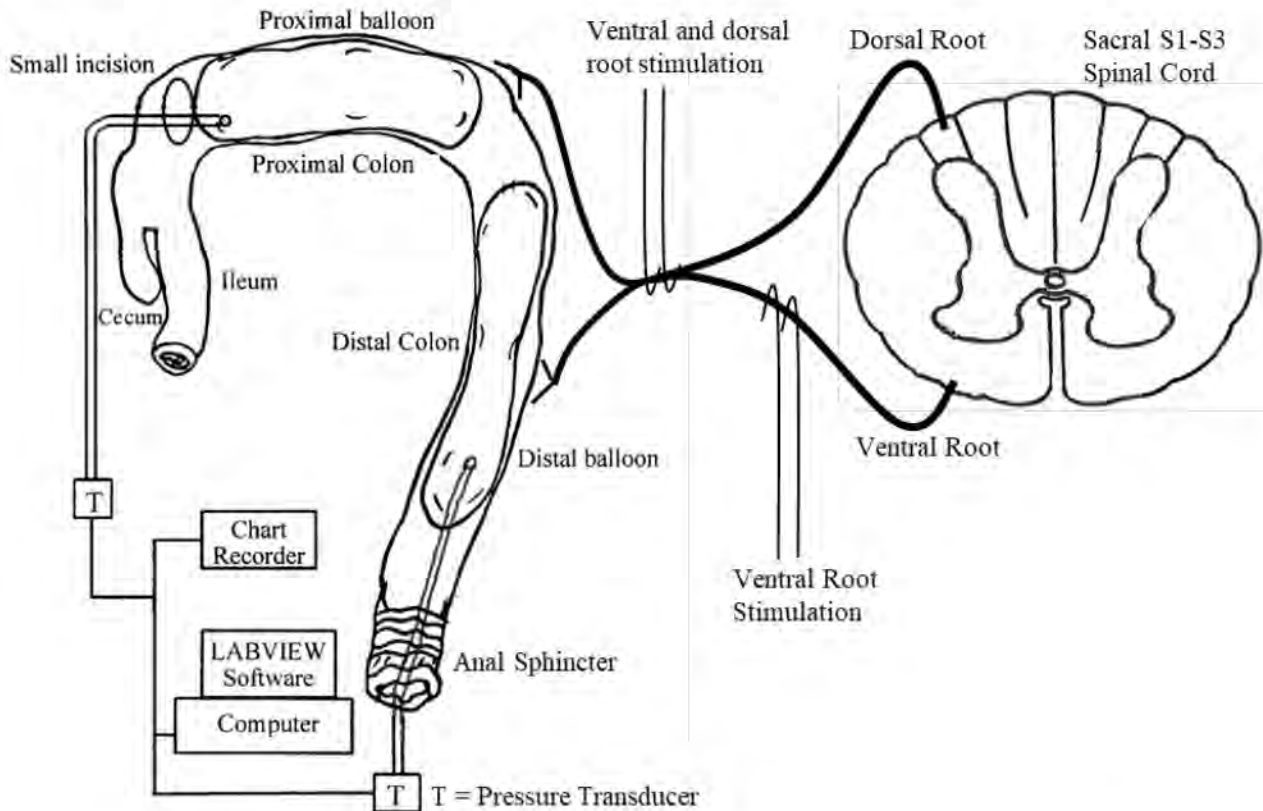


Fig.7. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce colon contractions.

The spinal cord was exposed from lumbar (b)(4) segments by a dorsal laminectomy. The dura mater was opened and each of the (b)(4) roots was identified. The dorsal and ventral roots were separated to either stimulate the ventral root individually or to stimulate both (b)(4) together (Fig.7). The animals were in a prone position during the experiment with a surgical retractor maintaining the spinal incision open to form a pool that was filled with warm (35-37 °C) mineral oil. A heating pad was used to maintain the animal body temperature between 35 and 37 °C. Bipolar stainless steel hook electrodes (b)(4) distance between the electrodes) were used to deliver the stimulation to each (b)(4) root by slightly lifting the root above the spinal cord.

Colon contraction induced by sacral ventral root stimulation is dependent on stimulation frequency and the spinal segment stimulated.

Stimulation of (b)(4) induced larger colon contractions than stimulation of (b)(4) ventral roots, and the most effective stimulation frequency was (b)(4). Fig.8 shows the distal and proximal colon pressure traces recorded from the same cat. Stimulation of (b)(4)

induced colon contractions (b)(4)

(b)(4) at frequencies (b)(4)

(b)(4) with a similar contraction

amplitude in the distal and proximal colon (Fig.8B).

Stimulation of the (b)(4) ventral root did not induce colon contractions larger than the small (b)(4)

rhythmic baseline colon contractions that were occurring asynchronously during the periods of stimulation (Fig.8 A and C). In this cat, the stimulation intensity used for (b)(4)

stimulation is 2 times the threshold intensity used during (b)(4)

stimulation to induce an observable proximal colon contraction. Due to the weak colon response to (b)(4) ventral root stimulation, the maximal stimulation intensity just below the threshold for inducing lower spine movement was applied to these roots. Hindlimb (mainly (b)(4) and/or tail (b)(4) movements were induced by stimulation of each of the three roots. Fig.9 summarizes the results from 7 cats showing that (b)(4) stimulation at (b)(4) was optimal for producing significantly (b)(4) larger colon contractions than those elicited by (b)(4) ventral root stimulation. There was no significant difference between the contraction amplitudes of distal and proximal colon (Fig.9).

roots at different frequencies (b)(4). A. S1 ventral root. B. S2 ventral root. C. S3 ventral root. Data were obtained from the same animal.

(b)(4)

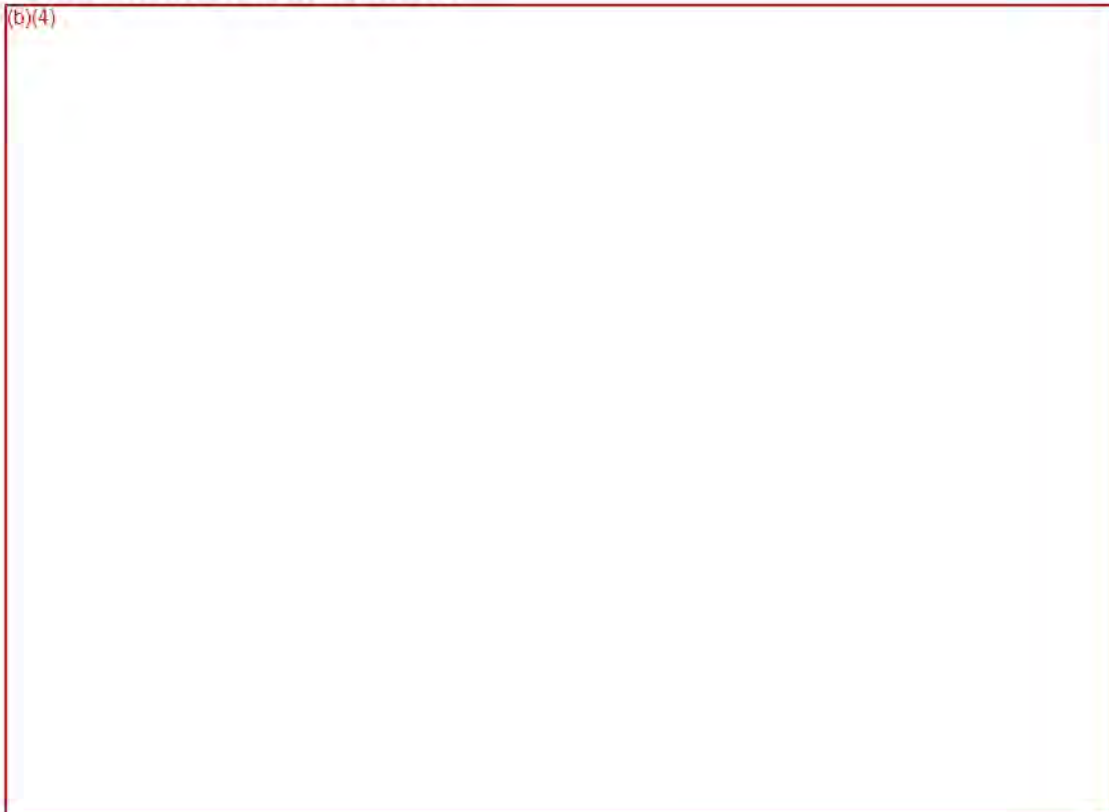
(b)(4)

Fatigue of colon contractions induced by S2 root stimulation

(b)(4) and dorsal roots together induced large amplitude (b)(4) (b)(4) contractions in both distal and proximal colon (Fig.10). Fig.10A shows that intermittent (b)(4) threshold intensity for inducing a proximal colon contraction produced a stronger contraction during the second period of stimulation and that

the large amplitude contraction was maintained throughout the next (b)(4) stimulations. However, the contraction amplitude gradually declined during a (b)(4) (b)(4), indicating a fatigue in the colon contractions (Fig.10A). On average the contraction amplitude for both the distal and proximal colon was maintained during the 5 intermittent (b)(4) Fig.10B), but was significantly ($p < 0.05$) reduced from the mean maximal amplitude of (b)(4) at the end of the 5-minute continuous stimulation (N=7 cats, Fig.10C).

A. S2 Ventral and Dorsal Roots



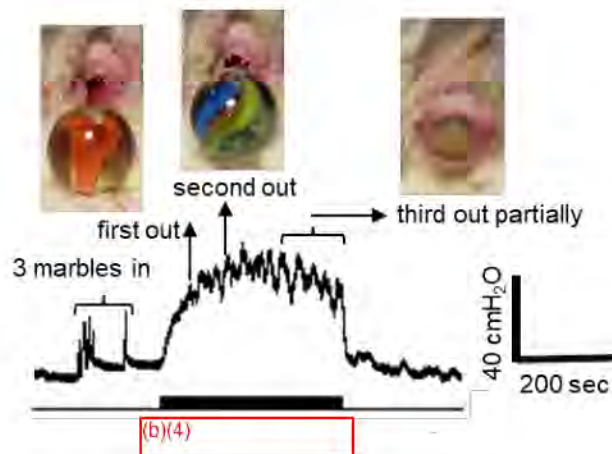
maintained during intermittent stimulation (b)(4). C. Summarized results (N = 7 cats) showing that the maximal (Max) contraction amplitude was significantly reduced from mean 30 cmH₂O to about 18 cmH₂O at the end of the continuous 5-minute stimulation (b)(4) * significantly different ($p < 0.05$, paired t-test).

Defecation induced by S2 root stimulation

Stimulation of the (b)(4) roots together induced a maximal amplitude colon contraction of (b)(4) (Fig.11A) and (b)(4) in cat #2 (Fig.11B). In cat #1 after 3 marbles were inserted into the rectum, S2 root stimulation (b)(4) induced a successful defecation that eliminated the first marble after 1 minute of stimulation and the

second marble after 2 minutes of stimulation (Fig.11A). The elimination of the third marble which started during the last 2 minutes of the 6-minute stimulation was incomplete (Fig.11A). In cat #2 after 1 marble was inserted into the rectum, the (b)(4) stimulation (b)(4) ms) eliminated the marble after 2.5 minutes of stimulation (Fig.11B). However, after 4 marbles were inserted into the rectum, the stimulation eliminated only 1 of the 4 marbles after 4.5 minutes of stimulation with 3 marbles in the rectum at the end of 11 minutes of stimulation (Fig.11B). Although stimulation of S2 ventral and dorsal roots together only eliminated 1-2 marbles in these two cats, stimulating the S2 ventral root alone without the dorsal root successfully eliminated all 4 marbles in both cats.

A. Cat #1



B. Cat #2

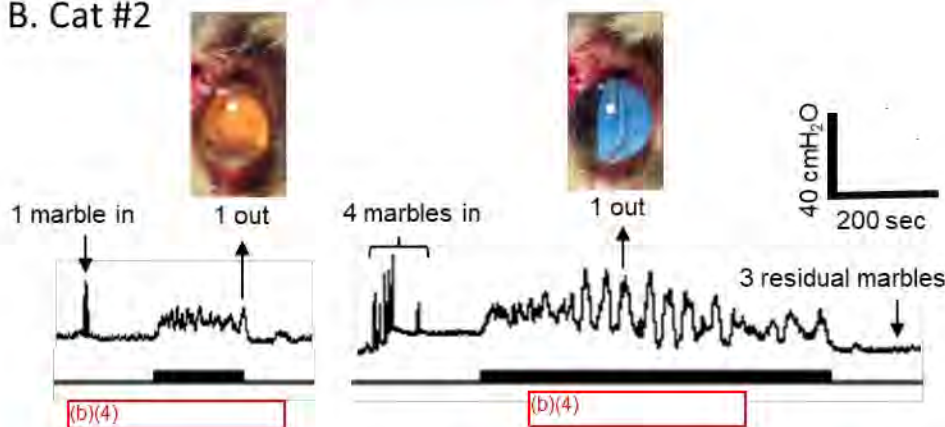
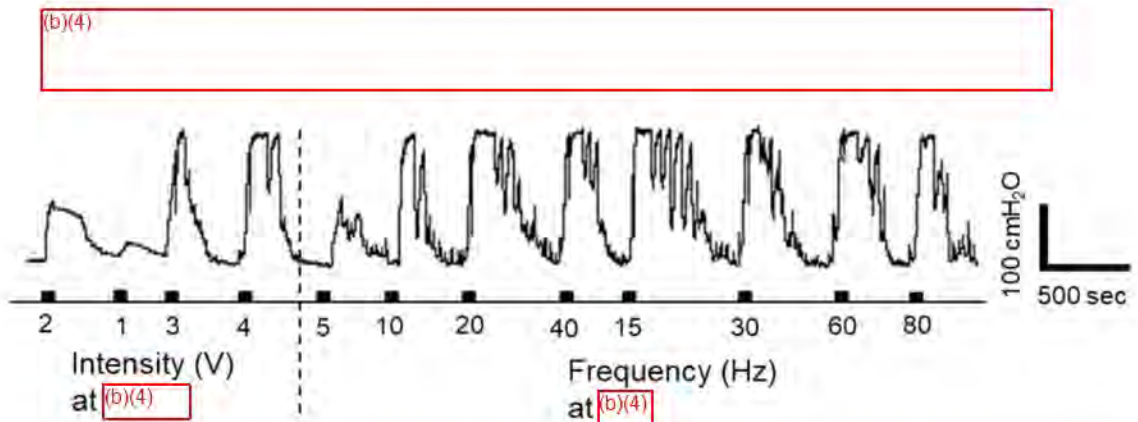
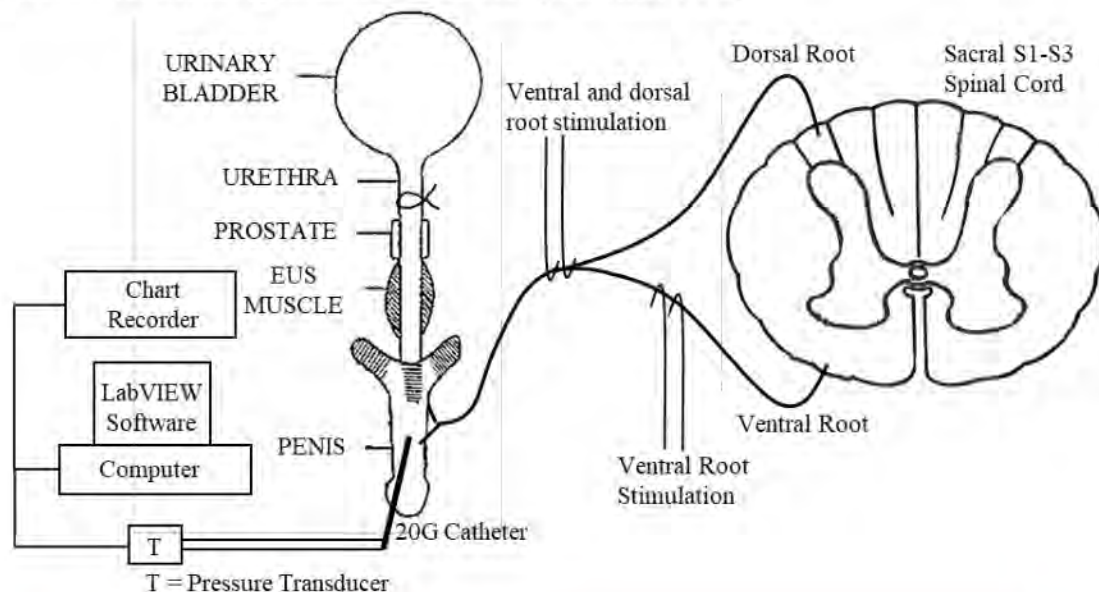


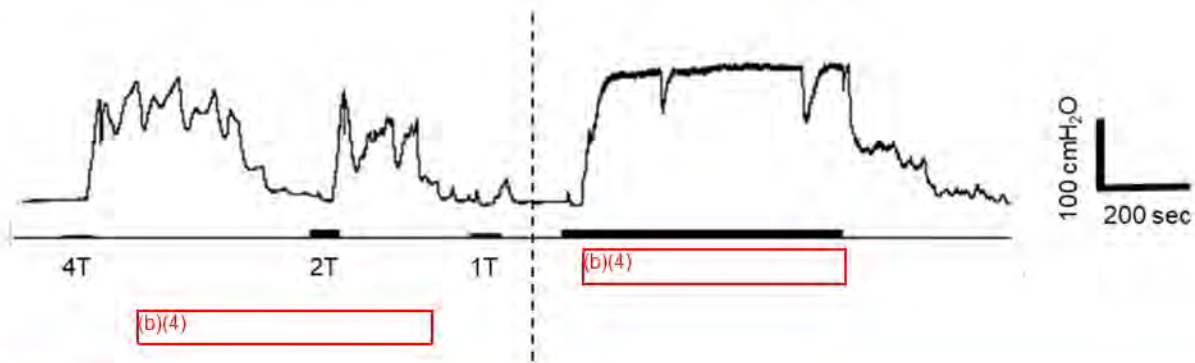
Fig.11. Defecation induced by stimulation of (b)(4) A. In cat #1 after inserting three marbles into the rectum, the defecation induced by sacral S2 root stimulation eliminated 2 marbles after 1 minute and 2 minutes of stimulation, respectively, with the last marble partially evacuated during the last 2 minutes of the 6-minute stimulation. B. In cat #2 after inserting 1 marble into the rectum, it was defecated after 2.5 minutes of stimulation. After inserting 4 marbles, stimulation of 11 minutes produced defecation of 1 marble at 4.5 minutes with 3 residual marbles in the rectum. Colon contraction pressure was measured by a large condom catheter with the condom covering the proximal and distal colon.

Sub-Task 1.2.3 Restore sexual function (b)(4)

Our study in 4 anesthetized cats so far is also very successful in restore sexual function. Fig.12 shows that experimental setup where a 20G needle catheter was inserted into the (b)(4) of the penis to record the pressure during penile erection. Laminectomy was performed to expose the sacral spinal cord and roots. (b)(4) and ventral root were isolated for electrical stimulation using a stainless-steel bipolar hook electrode. S1 ventral root stimulation successfully induced large pressure increase (b)(4) in the corpus cavernosum indicating a strong penile erection. Frequencies (b)(4) are most effective as shown in Fig.13, while (b)(4) root and pudendal nerve stimulation is not effective. Stimulation of S1 dorsal and ventral roots together can also induce large (b)(4) (b)(4), sustained, pressure increase in the penis lasting more than 10 minutes as shown in Fig.14. These results indicates that a minimally invasive approach can be used to insert a lead electrode to stimulate the S1 spinal root to restore penile erection in addition to stimulated S2 spinal root for restoring defecation function (see Task 1.2.2).



(b)(4)



(b)(4)

penile pressure lasting more than 10 minutes.

Task 1.3 Test Bed Environment (b)(4)

Sub-Task 1.3.1 Complete initial PCB schematic design with design review (b)(4)

The development of the test bed environment is based on (b)(4) The original (b)(4)

(b)(4)

Sub-Task 1.3.2 Complete initial PCB board layout, fabrication/assembly and evaluation (b)(4)

The two adapted test beds were already fabricated and assembled. These are referred to as (b)(4)

(b)(4)

Sub-Task 1.3.3 (b)(4) test application with board connectivity (b)(4)

(b)(4)

Sub-Task 1.3.4 Integration of (b)(4) in enclosure with firmware and (b)(4) connectivity

(b)(4)

The (b)(4) test bed has been delivered for evaluation to UPitt. (b)(4)

(b)(4)

(b)(4)

The waveform gets distorted the more the hardware is pushed past its limitations, which was anticipated. The (b)(4) bed only has (b)(4) The knowledge

gained from the (b)(4) provides valuable inputs for the (b)(4) prototype and ultimately the final (b)(4)

(b)(4)

(b)(4)

(b)(4)

University of Pittsburgh provided feedback on the capabilities of the (b)(4) test bed prototypes.

(b)(4)

Task 1.4 Implantable pulse generator with custom (b)(4)

(b)(4)

Sub-Task 1.4.1 Can/Header design with design review complete (b)(4)

(b)(4)

Sub-Task 1.4.2 Can samples developed and fully evaluated (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

1.4.3 Integrate pulse generator circuit into can (b)(4)

The (b)(4) PCB has been completed and received, currently under test and evaluation. Any modifications to the design will be identified and implemented for generation 2 of the (b)(4)

(b)(4)

(b)(4)

1.4.4 Final (b)(4) test application with wireless board connectivity (b)(4)

(b)(4) GUI has been completed and currently in integration with the PCBA. The GUI was created by working in conjunction with (b)(4) thus guided on the clinical application and requirements therein. (b)(4) This series of testing and communication activity tests will become the prelude of similarly configured (b)(4)

(b)(4) and other tests as part and parcel of Verification & Validation activity.

1.4.5 Full integration and test completed for initial prototype (b)(4)

(b)(4)

Task 1.5 Stimulation (b)(4) for animal studies (b)(4)

Sub-Task 1.5.1 Finalize on the (b)(4)

(b)(4)

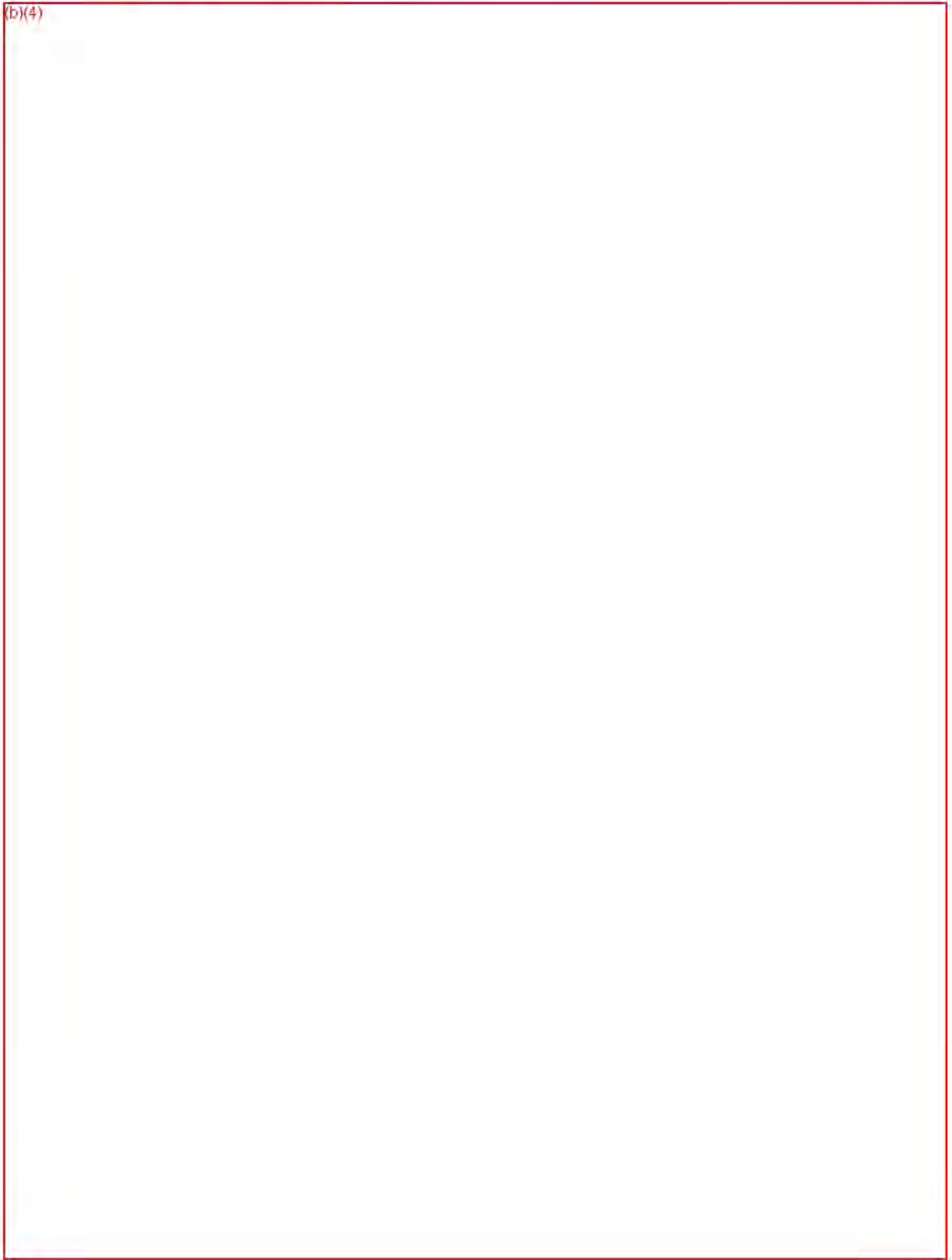
(b)(4)

Sub-Task 1.5.2 Finalize design of cat (b)(4)

The (b)(4) design has been finalized to follow those specifications as determined in our previous cat studies. An example of the desired (b)(4) sizes for cat and (b)(4)

(b)(4) are shown in Figs.21-22. Examples of desired surgical accessories used (b)(4)
(b)(4) can be seen in Fig.23.

(b)(4)



Sub-Task 1.5.3 First prototypes of leads/cuffs delivered for test (b)(4)

The first percutaneous lead prototype was delivered to (b)(4) for evaluation. The (b)(4) nerve block was achieved using this prototype lead (Fig.20). A (b)(4) prototype to be used in animal studies (cats) has been conceived and a prototype will be provided - (b)(4)

(b)(4)

Sub-Task 1.5.4 Finalize design and (b)(4) electrode production (b)(4)

Suppliers have been contacted and are motivated to produce our finalized (b)(4) designs. A (b)(4) was purchased to bring the (b)(4) production in house. Bringing this process in house can expedite prototyping and production time. The (b)(4) has passed our in-house validation process and quality standards. A protocol is now developed for operation. Finally, the (b)(4) will need to meet quality and performance standards and testing. (b)(4)

(b)(4)

(b)(4)

(b)(4) University of Pittsburg for evaluation.

(b)(4)

Task 1.6 External pulse generator for (b)(4)

(b)(4) has progressed from an initial design and concept to populated and in test PCB Boards with firmware. The physical board incorporating the electrical design, board layout, material sourcing, purchasing then populating, and lastly incorporation with custom written firmware has all been completed. This (b)(4) PCB board has now been successfully tested in animal studies at University of Pittsburgh to stimulate and block the (b)(4) nerves.

Sub-Task 1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)

Sub-Task 1.6.2 Integration of the (b)(4) with firmware and the enclosure (b)(4)

(b)(4) enclosure was updated to fit the new (b)(4) PCB (Fig.25 and Fig.26).

(b)(4)

The Boards have been received fully populated (Fig.27) (b)(4)

(b)(4)

Once received, firmware was loaded on the Boards and bring up was started immediately. To date, this task is progressing well with no major issues nor obstacles encountered. No effort is being spared, with the team working on this task continuously since PCBA receipt. The (b)(4) PCB board has now been tested successfully in animal studies at University of Pittsburgh to stimulate and block (b)(4) nerves.

(b)(4)

(b)(4)

Task 1.7 (b)(4) for human use (b)(4)

Sub-Task 1.7.1 Finalize on the (b)(4) sizes and lengths (b)(4)

(b)(4)

Sub-Task 1.7.2 Finalize design of human (b)(4)

(b)(4)

(b)(4)

Sub-Task 1.7.3 First prototypes of (b)(4) delivered for test (b)(4) (b)(4) have been requested to be delivered to (b)(4). To work towards this near-term goal, the (b)(4) lead manufacturing team have sent out multiple iterations of the (b)(4) attached to a (b)(4) representative model (Fig.29). The (b)(4) bodies contain a (b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

Task 1.8 Physician/Investigator controller for (b)(4)

The first incarnation is the current GUI already in integration, with learnings and adjustments needed, if any, transposed to future controllers, example in Fig.36, below. This is a (b)(4) GUI (b)(4)

(b)(4)

Sub-Task 1.8.1 Schematic design complete (b)(4)

Control of the (b)(4) to be accomplished by using a (b)(4) GUI, (b)(4)

(b)(4) The shown GUI (Fig.36) is written (b)(4)

Sub-Task 1.8.2 Evaluation board fabricated/assembled and initial evaluation complete

(b)(4)

The GUI is running (b)(4)

Sub-Task 1.8.3 Integration with a simple enclosure with display and firmware (b)(4)

(b)(4)

Task 1.9 Patient controller for (b)(4)

The current GUI will be used as a trial towards full system integration and future amelioration with learnings and real clinical use.

Sub-Task 1.9.1 Firmware features complete to restrict use as a patient controller (b)(4)

The software is structured to (b)(4)

(b)(4)

Task 1.10 Software Development Kit (SDK) (b)(4)

Sub-Task 1.10.1 Requirements understood for SDK (b)(4)

The SDK will be (b)(4) development for the EPG and IPG. The requirements for the SDK are in the process of being established.

Task 1.11 GLP animal study for (b)(4)

The purpose of the GLP safety studies is to provide support for the safe initiation of an Early Feasibility IDE Study of (b)(4) in humans. (b)(4)

(b)(4)

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

(b)(4)



Task 1.13 Early feasibility human clinical protocol for (b)(4)

Thermaquil team has drafted an early feasibility clinical study for the (b)(4) with input from clinicians during (b)(4). This clinical protocol is needed for the FDA IDE submission. We submitted the clinical protocol for FDA feedback in (b)(4) (Fig. 41).

(b)(4)



Withheld pursuant to exemption

(b)(4)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4); (b)(6)

of the Freedom of Information Act.

Withheld pursuant to exemption

(b)(4)

of the Freedom of Information Act.

(b)(4), (b)(6)

3 Publications and Presentations

Jicheng Wang, Zhijun Shen, Bing Shen, Jianan Jian, Travis Hannan, Khari Goosby, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai.
"Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats".
American Journal of Physiology - Gastrointestinal and Liver Physiology, submitted.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai
"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"
Experimental Neurology, 2021; 346:113860.

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai
"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"
American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Spinal Intact Cats"

International Continence Society (ICS) Annual Meeting, October 14-17, 2021, Online.

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [9/30/2020] – [10/31/2020]

10/30/2020





Overview – Technical Update

- IACUC protocol is approved by University of Pittsburgh and DOD ACURO. It is also submitted to NIWC IACUC and is now waiting for NIWC IACUC approval.
- (b)(4) electrode size and length are determined.
- Development of the Test Bed Environment is in preparation.
- FDA IDE (b)(4) is in preparation.
- (b)(4)
- Reviewed previous clinical protocols and FDA feedback for applicability and developing new clinical protocol for (b)(4)



Overview – Financial Update

- University of Pittsburgh is processing to create the financial account for this project.
- University of Pittsburgh is authorized by DARPA for the first installation of funding of total \$1,380,172.00



Overview – Administrative Update

- Draft subcontracts were sent to Med-Ally and Thermaquil for review
- Med-Ally and Thermaquil are now negotiating with University of Pittsburgh on the terms of the subcontracts
- It is expected that the subcontracts will be signed shortly.



4 Week work plan from previous Report

- This is the first monthly status report
- There is not 4-week work plan from previous report yet



COVID related updates

- Current MSR period – (b)(4) (9/30/2020-10/31/2020)
 - (b)(4)



Big Wins

- This is the first MSR. There is no Big Wins to report yet



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date:10/30/2020

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.1 Institutional IACUC approval and DOD ACURO review	(b)(4)	(b)(4)					(b)(4)
1.1.1 IACUC approval	(b)(4)					Milestone 1.1.1 – Deliverable 1.1.1	
1.1.2 DOD ACURO approval						Milestone 1.1.2 – Deliverable 1.1.2	
1.3 Test Bed Environment	(b)(4)						
1.3.1 Initial PCB design	(b)(4)					Milestone 1.3.1	
1.5 Stimulation Leads and cuff electrodes for animal studies	(b)(4)						
1.5.1 Finalized on lead/cuff size and length	(b)(4)					Milestone 1.5.1	
1.5.2 Finalize design of cat lead/cuff						Milestone 1.5.2	
1.14 FDA IDE	(b)(4)						
1.14.1 FDA IDE for	(b)(4)					Milestone 1.14.1 – Deliverable 1.14.1	
1.12	(b)(4)						
1.12	(b)(4)					Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for	(b)(4)						
1.13 Early feasibility human clinical protocol for	(b)(4)					Milestone 1.13 – Deliverable 1.13	



Technical Progress Summary

- The (b)(4) sizes for cat study are finalized. We will determine if the (b)(4) sized for human application is suitable for cat study.
- Human (b)(4) size is determined (b)(4)
(b)(4)
- Development of the (b)(4) Environment is in preparation.
- FDA IDE (b)(4) is in preparation.



Task 1.5: Stimulation leads and cuff electrodes for animal studies

(b)(4)

This figure shows the geometry of the (b)(4) for human subject study. (b)(4)

(b)(4)



Work plan for next 4 weeks (b)(4)

- Task 1.2.1: Restore bladder function (b)(4)

Obtain NIWC PACIFIC IACUC approval. Submission is completed and is now waiting for approval

To achieve pudendal nerve block by lead electrode in 2 cats (b)(4)

- Task 1.3: Test Bed Environment (b)(4)

Complete initial PCB schematic design with design review (b)(4)

- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)

Finalize on the lead/cuff size and length (b)(4)

- Task 1.14: FDA IDE (b)(4)

Prepare and draft FDA IDE (b)(4)

- (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol for (b)(4)

Consultations with clinicians and regulatory experts to finalize clinical protocol



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



Miscellaneous

- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [11/1/2020] – [11/30/2020]

11/30/2020





Overview – Technical Update

- IACUC protocol is approved by NIWC IACUC and animal study is initiated.
- Pudendal nerve block by a lead electrode is conducted in 2 cat experiments.
- Development of the Test Bed Environment is started at (b)(4)
- FDA IDE (b)(4) is outlined with the FDA regulatory consultant and Thermoquil is drafting (b)(4)
- (b)(4)
- Drafted potential GLP swine study protocol to meet FDA IDE safety requirements
- Started drafting the early feasibility human clinical protocol to meet FDA IDE clinical requirements



Overview – Financial Update

- University of Pittsburgh created the financial account for this project.
- University of Pittsburgh is now setting up the payment process for the subcontractors.



Overview – Administrative Update

- Subcontracts with (b)(4) and Thermaquil are signed.
- The project team for engineering and manufacturing was formed at (b)(4):
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)
 - (b)(6) - (b)(4); (b)(6)



4 Week work plan from previous Report

- Task 1.2.1: Restore bladder function (b)(4)
Obtain NIWC PACIFIC IACUC approval - (b)(4)
To achieve pudendal nerve block by lead electrode in 2 cats (b)(4)

- Task 1.3: Test Bed Environment (b)(4)

Complete initial PCB schematic design with design review (b)(4)

- Task 1.5: Stimulation (b)(4) for animal studies (b)(4)

Finalize on the lead/cuff size and length (b)(4)

- Task 1.14: FDA IDE (b)(4)

Prepare and draft FDA IDE (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol for (b)(4)

(b)(4)



COVID related updates

- Current MSR period – (b)(4) (11/1/2020-11/30/2020)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date:11/30/2020

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.1 Restore bladder function (b)(4)	(b)(4)					Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.3 Test Bed Environment (b)(4)							
1.3.1 Complete initial PCB schematic design with design review	(b)(4)					Milestone 1.3.1	
1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)							
1.5.1 Finalized on lead/cuff size and length	(b)(4)					Milestone 1.5.1	
1.5.2 Finalize design of cat lead/cuff						Milestone 1.5.2	
1.14 FDA IDE (b)(4)							
1.14.1 FDA IDE (b)(4)	(b)(4)					Milestone 1.14.1 – Deliverable 1.14.1	
1.12 (b)(4)							
1.12 (b)(4)	(b)(4)					Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)					Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study	(b)(4)					Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- (b)(4) nerve block by a lead electrode is successfully achieved in 2 cat experiments.
- Development of the Test Bed Environment is started at (b)(4)
- FDA IDE (b)(4) is outlined with the FDA regulatory consultant and Thermaquil starts drafting this (b)(4)
- Start to draft the (b)(4) GLP swine study protocol, and early feasibility human clinical protocol.



(b)(4)

(b)(4)



(b)(4)

(b)(4)



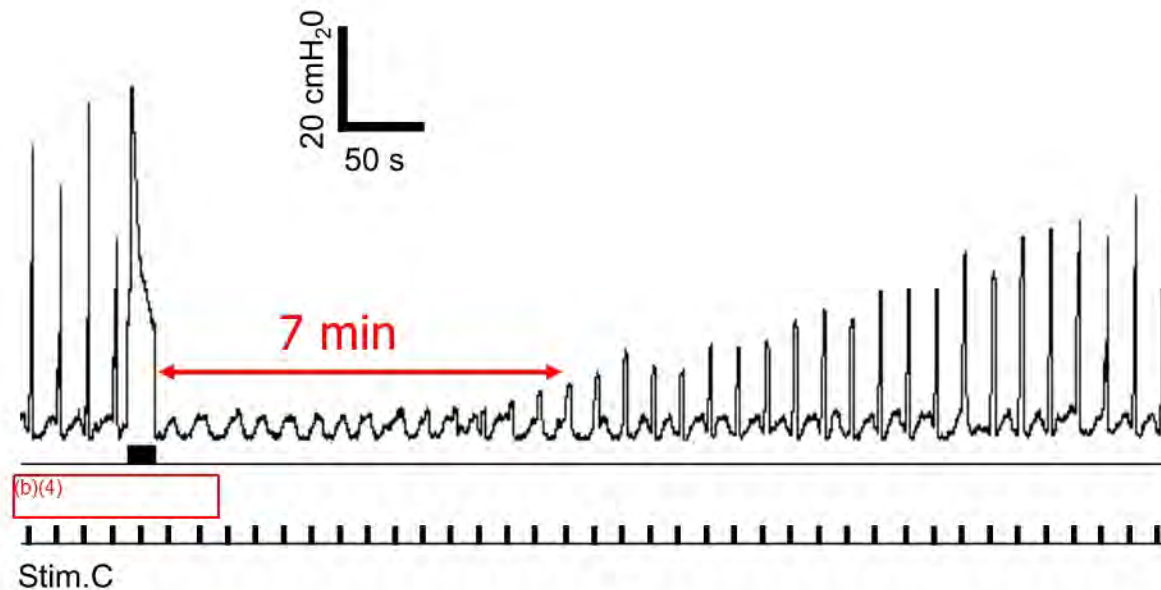
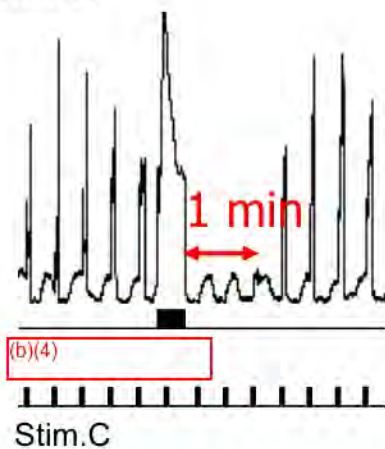
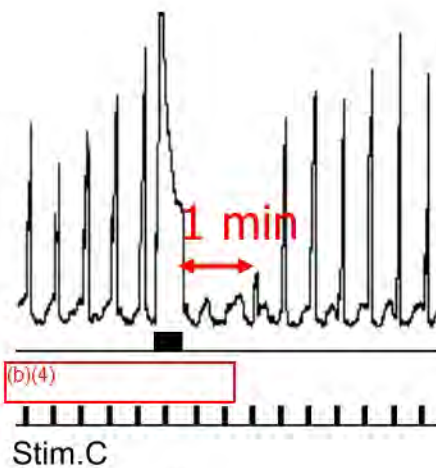
(b)(4)

(b)(4)

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Task 1.2.1: Block (b)(4) Nerve by a (b)(4)



(b)(4)



Work plan for next 4 weeks (b)(4)

- Task 1.2.1: Restore bladder function (b)(4)
 - To achieve (b)(4) nerve block by a lead electrode in 1 more cats (b)(4)
 - To achieve low pressure voiding in 1 cats (b)(4)
- Task 1.3: (b)(4) Environment (b)(4)
 - Complete initial PCB board layout, fabrication/assembly and evaluation (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
 - Finalize design of cat lead/cuff (b)(4)
- Task 1.14: FDA IDE (b)(4)
 - Complete FDA IDE (b)(4)
- (b)(4)
 - (b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
 - Drafting human clinical protocol
- Task 1.11: GLP animal study (b)(4)
 - Finalize GLP animal study protocol



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



Miscellaneous

- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4), (b)(6)

Reporting Period: [12/1/2020] – [12/31/2020]

12/31/2020





Overview – Technical Update

- (b)(4) nerve block by a lead electrode is confirmed in another experiment.
- (b)(4) nerve block by lead electrodes significantly reduced voiding pressure.
- Two independent (b)(4) Environments under development at Med-Ally. The first simpler one with high current output made by (b)(4) is received by University of Pittsburgh.
- (b)(4) provided the first sample of lead electrode for testing in cat experiment.
- Updated initial specifications for animal IPG
- Finalized animal IPG User/Customer Requirements
- (Beta) (b)(4) - full device functionality under firmware test
- (Beta) (b)(4) for GUI is re-programmed to interface with test bed system and under test
- FDA IDE (b)(4) is submitted to the FDA by Thermaquil on (b)(4).
- (b)(4)
- Final GLP swine study protocol was included in (b)(4)
- Continued drafting early feasibility human clinical protocol for (b)(4)
- Started drafting the bench testing plan for (b)(4)



Overview – Financial Update

- University of Pittsburgh started the payments for the subcontractors.



Overview – Administrative Update

- Administrative/progress meeting is hold weekly to coordinate the works among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week work plan from previous Report

- Task 1.2.1: Restore bladder function (b)(4)
To achieve (b)(4) nerve block by a lead electrode in 1 more cats (b)(4)
To achieve low pressure voiding in 1 cats (b)(4)
- Task 1.3: (b)(4) Environment (b)(4)
Complete initial PCB board layout, fabrication/assembly and evaluation (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
Finalize design of cat lead/cuff (b)(4)
- Task 1.14: FDA IDE (b)(4)
Complete FDA IDE (b)(4)
- (b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Drafting human clinical protocol – (b)(4)
- Task 1.11: GLP animal study (b)(4)
Finalize GLP animal study protocol – (b)(4)



COVID related updates

- Current MSR period – (b)(4) (12/1/2020-12/31/2020)

- (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 12/31/3030

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.1 Restore bladder function (b)(4)		(b)(4)				Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.3 Test Bed Environment (b)(4)							
1.3.1 Complete initial PCB schematic design with design review		(b)(4)				Milestone 1.3.1	
1.3.2 Complete initial PCB board layout, fabrication/assembly and evaluation						Milestone 1.3.2	
1.4 Implantable pulse generator with custom (b)(4)							
1.4.1 Can/Header design with design review complete		(b)(4)				Milestone 1.4.1	
1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)							
1.5.2 Finalize design of cat lead/cuff		(b)(4)				Milestone 1.5.2	
1.5.3 First prototypes of leads/cuffs delivered for test						Milestone 1.5.3	
1.14 FDA IDE (b)(4)							
1.14.1 FDA IDE (b)(4) for 1 the (b)(4)		(b)(4)				Milestone 1.14.1 – Deliverable 1.14.1	
1.14.2 FDA IDE (b)(4) 2 meeting						Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)							
1.15.1 FDA IDE (b)(4) for 1 the (b)(4)		(b)(4)				Milestone 1.15.1 – Deliverable 1.15.1	
1.12 (b)(4)						Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)		(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study		(b)(4)				Milestone 1.11 – Deliverable 1.11	

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Technical Progress Summary

- Bilateral (b)(4) nerve block by lead electrodes successfully reduced voiding pressure.
- The first (b)(4) Environment is (b)(4)
- Sample lead electrode for cat study is (b)(4)
- FDA IDE (b)(4) is submitted.
- (b)(4)
- Final GLP swine study protocol was included in (b)(4) for FDA feedback

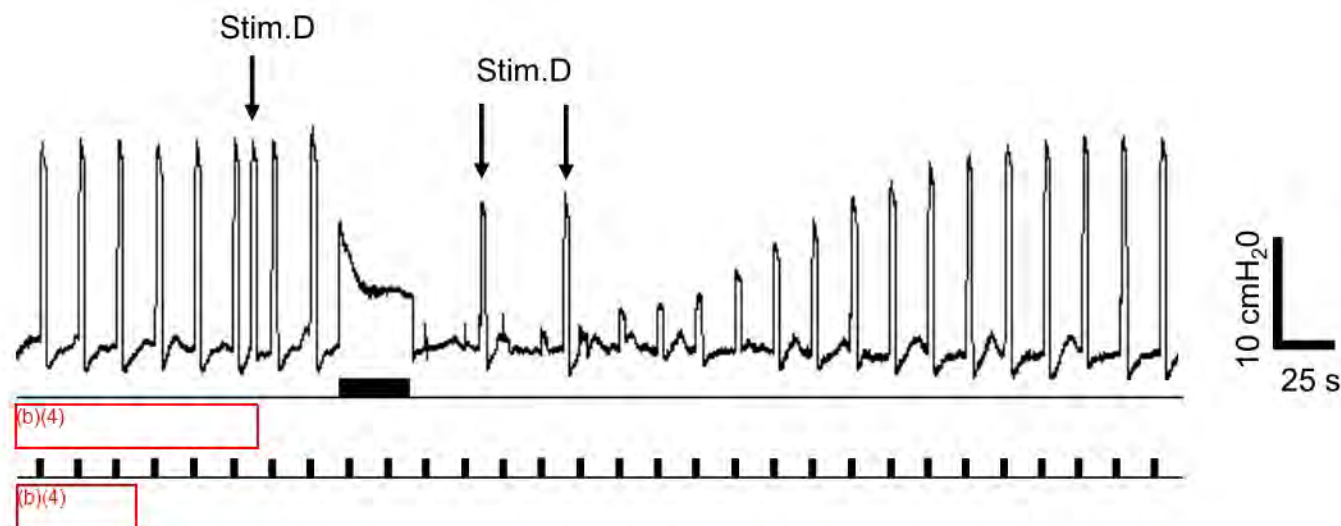


(b)(4)



(b)(4)

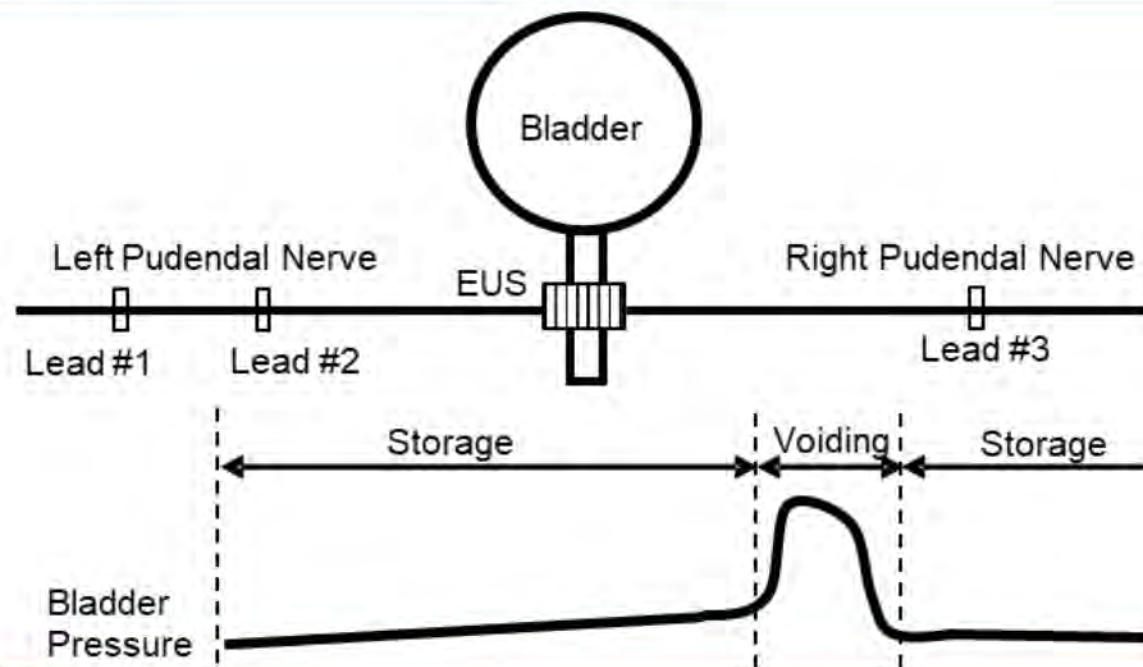




(b)(4)

During the (b)(4) nerve block period, the response induced by (b)(4) is partially reduced in this cat, indicating that the blocking (b)(4) might have partially fatigued the EUS muscle or it might have partially influenced the excitability of the pudendal nerve at the site of the (b)(4)

Note: The distance between (b)(4)



(b)(4)

(b)(4)



Task 1.2.1: Low Pressure Voiding Induced by Lead Electrodes

(b)(4)

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Task 1.5: Stimulation lead electrode for animal studies

(b)(4)

A large rectangular area of the slide is completely redacted, indicated by a thin red border. The text "(b)(4)" is written in the top left corner of this area.

Figure 5: Sample

(b)(4)

A rectangular area at the bottom of the slide is redacted, indicated by a thin red border. The text "(b)(4)" is written in the top left corner of this area.



Task 1.5: Stimulation lead electrode for animal studies

(b)(4)

Figure 6: This figure shows the geometry of the sample lead electrode provided by (b)(4)

(b)(4)



Task 1.5: Stimulation lead electrode for animal studies

(b)(4)



Task 1.14: FDA IDE

(b)(4)

(b)(4), (b)(6)



Work plan for next 4 weeks

(b)(4)

- Task 1.2.1: Restore bladder function

(b)(4)

To achieve low pressure voiding in another 2 cats

(b)(4)

- Task 1.2.5: Test electrode and device prototypes

(b)(4)

To test the first prototype of animal lead

(b)(4)

To test the first prototype of the

(b)(4)

Environment

(b)(4)

- Task 1.3: Test Bed Environment

(b)(4)

(b)(4) test application with board connectivity

(b)(4)

(b)(4)

Integration of

(b)(4)

(b)(4)

connectivity

(b)(4)

A prototype unit to University of Pittsburgh researchers to use for early animal research. Two set of the prototypes will be provided at

(b)(4)

for the tasks 1.2 at University of Pittsburgh

(b)(4)

- Task 1.4: Implantable pulse generator with

(b)(4)

Can/Header design with design review complete

(b)(4)

- Task 1.5: Stimulation Leads and cuff electrodes for animal studies

(b)(4)

Finalize design and lead/cuff electrode production

(b)(4)



Work plan for next 4 weeks (b)(4)

- Task 1.6: External pulse generator for (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the (b)(4) size and length (b)(4)
- Task 1.11: GLP animal study (b)(4)
Summarize protocol for (b)(4) meeting with FDA
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Complete draft of early feasibility human clinical protocol for review by clinicians
- Task 1.14: FDA IDE (b)(4)
(b)(4) for FDA IDE (b)(4) meeting (b)(4)
- Task 1.15: FDA IDE (b)(4)
(b)(4) FDA IDE (b)(4)



Monthly Financial Overview

(b)(4)

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Administrative issues

- No administrative issues to report



Miscellaneous

- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4), (b)(6)

Reporting Period: [1/1/2021] – [1/31/2021]

1/31/2021





Overview – Technical Update

- Pudendal nerve block was achieved by Med-Ally (b)(4)
- Prolonged recovery from low-pressure to high-pressure voiding was confirmed.
- Med-Ally delivered the first prototype of the Test Bed Environments for testing at University of Pittsburgh. It generated (b)(4) with an intensity range of (b)(4). Based on the feedback from University of Pittsburgh, Med-Ally is now working on a (b)(4) Test Bed to deliver (b)(4).
- Med-Ally authored, reviewed and released draft of system bench testing protocols for (b)(4).
- Thermaquil drafted (b)(4).
- Thermaquil prepared for (b)(4) meetings with the FDA.



Overview – Financial Update

- University of Pittsburgh received the second (b)(4)
- DARPA also informed us that we will receive the rest of the phase 1 fund by the end of (b)(4)
- (b)(4) the clean room monitoring equipment for builds has been purchased currently under calibration and installation when readied. Laser marking equipment has been purchased with an estimated delivery (b)(4) with validations and testing to follow.



Overview – Administrative Update

- Administrative/progress meeting is hold weekly to coordinate the activity among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week work plan from previous Report

- Task 1.2.1: Restore bladder function (b)(4)
To achieve low pressure voiding in another 2 cats (b)(4)
- Task 1.2.5: Test electrode and device prototypes (b)(4)
To test the first prototype of animal lead (b)(4)
To test the first (b)(4)
- Task 1.3: (b)(4)
PC test application with board connectivity (b)(4)
(b)(4)
(b)(4) connectivity (b)(4)
A prototype unit to University of Pittsburgh researchers to use for early animal research. Two set of the prototypes will be provided at (b)(4) for the tasks 1.2 at University of Pittsburgh (b)(4)
(b)(4)
- Task 1.4: Implantable pulse generator with (b)(4)
Can/Header design with design review complete (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
Finalize design and lead/cuff electrode production (b)(4)



4 Week work plan from previous Report

- Task 1.6: External pulse generator (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the lead size and length (b)(4)
- Task 1.11: GLP animal study (b)(4)
Summarize protocol for (b)(4) meeting with FDA – (b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Complete draft of early feasibility human clinical protocol for review by clinicians – (b)(4)
- Task 1.14: FDA IDE (b)(4)
Preparing for FDA (b)(4) meeting (b)(4)
- Task 1.15: FDA IDE (b)(4)
Preparing FDA IDE (b)(4)



COVID related updates

- Current MSR period – (b)(4) (1/1/2021-1/31/2021)

- (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 1/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		(b)(4)
1.2.1 Restore bladder function	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.2.5.To test the first prototype of 1 animal lead	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.2.5.1	
1.2.5.To test the first prototype of the 2 Test Bed Environment	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.2.5.2	
1.3 Test Bed Environment	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
1.3.3 application with board connectivity	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.3.3	
Integration of (b)(4) in enclosure	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.3.4	
1.3.4 with firmware and connectivity	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
1.4 Implantable pulse generator with custom	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
1.4.1 Can/Header design with design review complete	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.4.1	
1.5 Stimulation Leads and cuff electrodes for animal studies	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
1.5.4 Finalize design and lead/cuff electrode production	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.5.4	
1.6 External pulse generator for human study	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
1.6.1 Industrial design and CAD drawings for the custom enclosure	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.6.1	
Integration of the (b)(4) components with firmware and the enclosure	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.6.2	
1.7 (b)(4) electrodes for human use	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)		
1.7.1 Finalize on the (b)(4) size and length	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.7.1	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 1/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller for (b)(4)							(b)(4)
1.8.1 Schematic design complete (b)(4)		(b)(4)				Milestone 1.8.1	
Evaluation board							
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)						Milestone 1.8.1	
Integration with a simple							
1.8.3 enclosure with display and firmware (b)(4)						Milestone 1.8.1	
1.10 Software Development Kit (SDK) (b)(4)							
1.10. Requirements understood for 1 (b)(4)						Milestone 1.10.1	
1.14 FDA IDE (b)(4)							
1.14. FDA IDE (b)(4) 2 meeting		(b)(4)				Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)							
1.15. FDA IDE (b)(4) for 1 (b)(4)		(b)(4)				Milestone 1.15.1 – Deliverable 1.15.1	
(b)(4)							
						Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)		(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- (b)(4) was achieved by (b)(4)
- Prolonged (b)(4) indicating acute safety of (b)(4)
- The first prototype of Test Bed Environment delivered by (b)(4) and tested at University of Pittsburgh
- (b)(4) drafted the system bench testing protocols for (b)(4)
- (b)(4) drafted (b)(4) that focuses on the (b)(4)
- (b)(4) prepared for (b)(4) meetings with the FDA. (b)(4)
- Informational Meeting for (b)(4) is scheduled for (b)(4)



(b)(4)

(b)(4)



(b)(4)

[Redacted content]

(b)(4)

[Redacted content]

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(b)(4)

[Redacted content]

(b)(4)

[Redacted content]

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1264



(b)(4)

(b)(4)



Task 1.3: Test Bed Environment

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1266



Task 1.15: FDA IDE

(b)(4)

(b)(4)



Work plan for next 4 weeks

(b)(4)

- Task 1.2.1: Restore bladder function

(b)(4)

(b)(4)

- Task 1.3: Test Bed Environment

(b)(4)

Integration of (b)(4) in enclosure with firmware and (b)(4) connectivity (b)(4)

A prototype unit to University of Pittsburgh researchers to use for early animal research. Two set of the prototypes will be provided at (b)(4) for the tasks 1.2 at University of Pittsburgh (b)(4)

- Task 1.4: Implantable pulse generator with

(b)(4)

Can/Header design with design review complete (b)(4)

- Task 1.5: Stimulation Leads and cuff electrodes for animal studies

(b)(4)

Finalize design and lead/cuff electrode production (b)(4)

- Task 1.6: External pulse generator

(b)(4)

Industrial design and CAD drawings for the custom enclosure (b)(4)

Integration of the (b)(4) components with updated firmware and hardware (b)(4)

- Task 1.7: (b)(4) electrodes for human use (b)(4)

Finalize on the (b)(4)



Work plan for next 4 weeks (b)(4)

- Task 1.8: Physician/Investigator controller for (b)(4)
The GUI will continue to be updated. (b)(4)

- Task 1.10: (b)(4) (b)(4)
Continuing to define SDK requirements

- Task 1.11: GLP animal study (b)(4)
Finalize protocol summary for Meeting (b)(4) with FDA

- Task 1.12: (b)(4)

- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Acquire clinician feedback on early feasibility human clinical protocol

- Task 1.14: FDA IDE (b)(4)
Finalize presentation for Meeting (b)(4) with FDA

- Task 1.15: FDA IDE (b)(4)
Submit (b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4), (b)(6)

Reporting Period: [2/1/2021] – [2/28/2021]

2/28/2021





Overview – Technical Update

- Pudendal nerve block was achieved by (b)(4)
- Low-pressure voiding was achieved in 4 animals.
- After delivering the first prototype of the Test Bed, (b)(4) is now working on developing the (b)(4) based on the feedback from University of Pittsburgh. The first version (b)(4) will be used as the deliverable of the final Test Bed.
- Cuff lead for cat was delivered to University of Pittsburgh for evaluation. University of Pittsburgh has provided feedback as inputs for the final cuff lead design.
- (b)(4) schematic is almost ready to be implemented as hardware. (b)(4)
(b)(4)
- Variations of (b)(4)
(b)(4)
- All (b)(4) configurations have Solidworks models.
- Had Informational Meeting for (b)(4) with the FDA on (b)(4)
- Continued preparing (b)(4)
- Continued drafting early feasibility human clinical protocol for (b)(4)



Overview – Financial Update

- DARPA informed us that we will receive the rest of the phase 1 fund by the end of (b)(4). We are now still waiting for DARPA to give us any update on this.
- We will be able to order new equipment if we can get full funding as budgeted.
- Clean room monitoring equipment has arrived, validations and testing in progress at (b)(4).
- Laser marking equipment has arrived, validations and testing in progress at (b)(4).



Overview – Administrative Update

- Administrative/progress meeting is hold weekly to coordinate the activity among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week work plan from previous Report

- Task 1.2.1: Restore bladder function (b)(4)
(b)(4) voiding for statistically significant results (b)(4)
(b)(4) - Done
- Task 1.3: Test Bed Environment (b)(4)
After delivering the first prototype of the Test Bed, (b)(4) is now working on developing (b)(4) based on the feedback from University of Pittsburgh. The first version (b)(4) will be used as the deliverable of the final Test Bed. — (b)(4)
- Task 1.4: Implantable pulse generator with (b)(4)
Can/Header design with design review complete (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
Finalize design and lead/cuff electrode production (b)(4)
- Task 1.6: External pulse generator for human study (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
Integration of the EPG components with updated firmware and hardware (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the (b)(4) size and length (b)(4)



4 Week work plan from previous Report

- Task 1.8: Physician/Investigator controller for (b)(4)
The GUI will continue to be updated. As the hardware changes on the (b)(4) test bed, the user interface may require minor modifications. – (b)(4)
- Task 1.10: Software Development Kit (SDK) (b)(4)
Continuing to define SDK requirements – (b)(4)
- Task 1.11: GLP animal study (b)(4)
Finalize protocol summary for Meeting (b)(4) with FDA – (b)(4)
Schedule study – (b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Acquire clinician feedback on early feasibility human clinical protocol – (b)(4)
- Task 1.14: FDA IDE (b)(4)
Finalize presentation for Meeting (b)(4) with FDA – (b)(4)
- Task 1.15: FDA IDE (b)(4)
Submit (b)(4)



COVID related updates

- Current MSR period – (b)(4) (2/1/2021-2/28/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 1/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.2.1 Restore bladder function	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.3 Test Bed Environment	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	
1.3.4 Integration of (b)(4) in enclosure with firmware and (b)(4) connectivity	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.3.4	
1.4 Implantable pulse generator with	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	
1.4.1 Can/Header design with design review complete	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.4.1	
1.5 Stimulation Leads and cuff electrodes for animal studies	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	
1.5.4 Finalize design and lead/cuff electrode production	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.5.4	
1.6 External pulse generator	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	
1.6.1 Industrial design and CAD drawings for the custom enclosure	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.6.1	
1.6.2 Integration of the (b)(4) components with firmware and the enclosure	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	
1.7.1 Finalize on the (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.7.1	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 1/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller for (b)(4)	(b)(4)	(b)(4)					(b)(4)
1.8.1 Schematic design complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
Evaluation board							
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
Integration with a simple							
1.8.3 enclosure with display and firmware (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
1.10 Software Development Kit (SDK) (b)(4)	(b)(4)	(b)(4)					
1.10. Requirements understood for	(b)(4)	(b)(4)					
1 SDK (b)(4)	(b)(4)	(b)(4)				Milestone 1.10.1	
1.14 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.14. FDA IDE (b)(4)	(b)(4)	(b)(4)					
2 meeting						Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.15. FDA IDE (b)(4)	(b)(4)	(b)(4)					
1 the (b)(4)	(b)(4)	(b)(4)				Milestone 1.15.1 – Deliverable 1.15.1	
1.12 Human cadaver study (b)(4)	(b)(4)	(b)(4)					
1.12 (b)(4)	(b)(4)	(b)(4)				Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)	(b)(4)					
1.13 Early feasibility human clinical protocol for (b)(4)	(b)(4)	(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)	(b)(4)	(b)(4)					
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- (b)(4) was achieved by (b)(4)
- Low-pressure voiding was achieved in 4 animals.
- After delivering the first prototype of the Test Bed, (b)(4) is now working on developing the (b)(4) based on the feedback from University of Pittsburgh. The first version of the (b)(4) will be used as the delivery of the final Test Bed.
- (b)(4) schematic is almost ready to be implemented as hardware. (b)(4)
(b)(4)
- Variations (b)(4)
(b)(4)
- Had Informational Meeting for (b)(4) with the FDA on (b)(4)
- Continued preparing (b)(4) that focuses (b)(4)
- Continued drafting early feasibility human clinical protocol for (b)(4)



Task 1.2.1: Block Pudendal Nerve by 100 Hz Stimulation

(b)(4)



Task 1.2.1: Block Pudendal Nerve by 100 Hz Stimulation

(b)(4)

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Task 1.2.1: Low Pressure Voiding Induced by Bipolar Hook Electrodes

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1286



Task 1.2.1: Low Pressure Voiding Induced by Bipolar Hook Electrodes

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1287



Task 1.6: External Pulse Generator for

(b)(4)

(b)(4)

Figure 5: The schematic

(b)(4)

(b)(4)

(b)(4)

by

(b)(4)

(provided



Task 1.7: (b)(4) Electrodes for Human Use

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1289

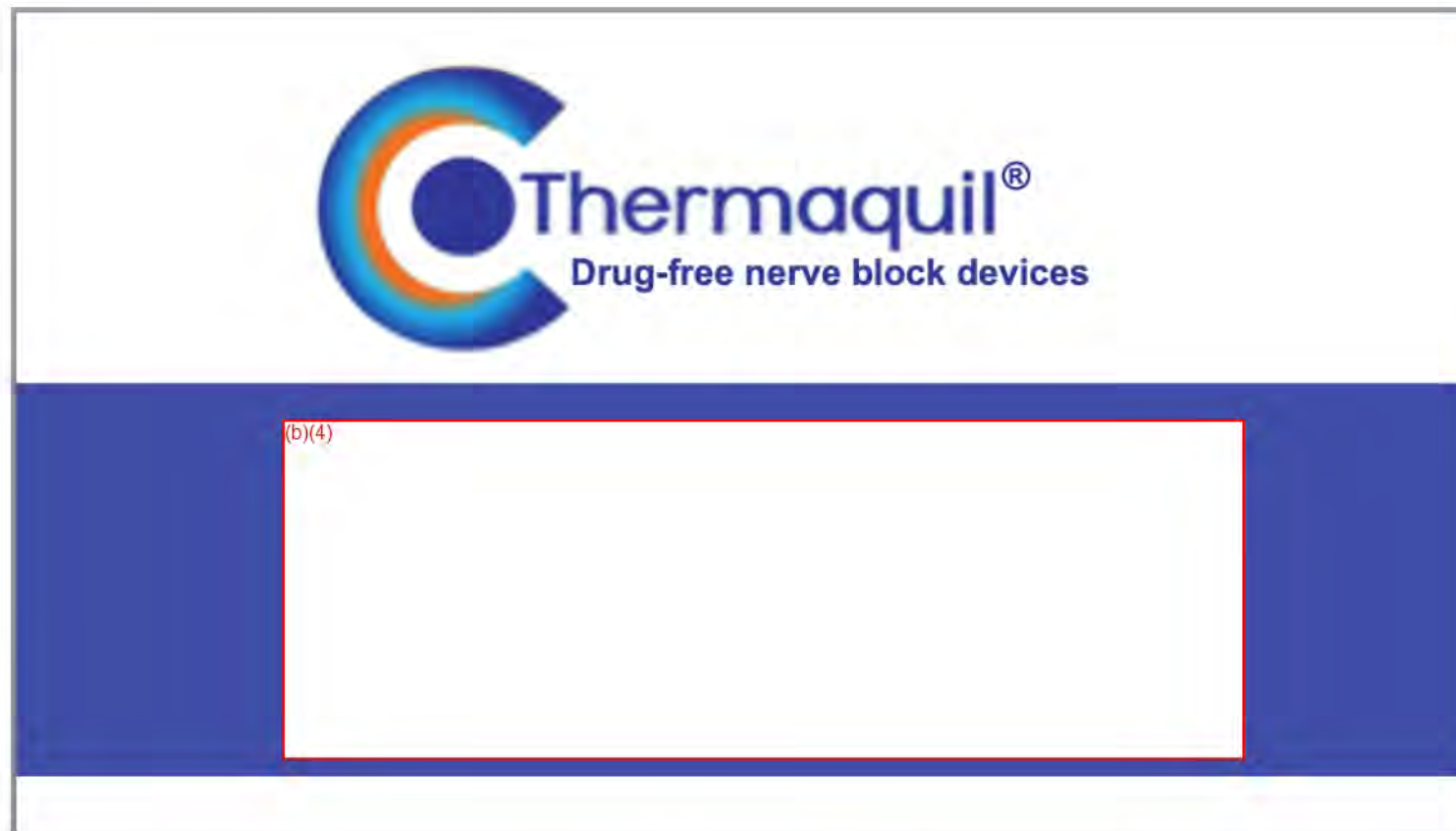


Figure 7: (b)(4) FDA IDE (b)(4) was hold on (b)(4) with DARPA BG+ team participation (provided by Thermaquil).



Work plan for next 4 weeks (b)(4)

- Task 1.2.1: Restore bladder function (b)(4)
 - Increase number of animals to achieve statistically significant results (b)(4)
 - Analyze the results from Task 1.2.1 and prepare a manuscript for publication
- Task 1.4: Implantable pulse generator with (b)(4)
 - Can/Header design with design review complete (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
 - Finalize design and lead/cuff electrode production (b)(4)
- Task 1.6: External pulse generator for (b)(4)
 - Industrial design and CAD drawings for the custom enclosure (b)(4)
 - Integration of the (b)(4) components with updated firmware and hardware (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
 - Finalize on the (b)(4) size and length (b)(4)
- Task 1.8: Physician/Investigator controller (b)(4)
 - Schematic design complete (b)(4)
 - Evaluation board fabricated/assembled and initial evaluation complete (b)(4)
 - Integration with a simple enclosure with (b)(4) and firmware (b)(4)



Work plan for next 4 weeks

(b)(4)

- Task 1.10: Software Development Kit (SDK) (b)(4)
Continuing to define SDK requirements
- Task 1.11: GLP animal study (b)(4)
Schedule study and continue to finalize study details
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol
- Task 1.14: FDA IDE (b)(4)
Schedule Meeting with the FDA
- Task 1.15: FDA IDE (b)(4)
Submit (b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [3/1/2021] – [3/31/2021]

3/31/2021





Overview – Technical Update

- Completed the animal studies of (b)(4)
(b)(4)
- Experimental data are processed, analyzed, and a manuscript is finished and ready for submission.
- The (b)(4) is now under evaluation by (b)(4)
(b)(4)
- (b)(4) layout with different combinations of (b)(4) have been updated to meet the (b)(4)
(b)(4) is ready to be implemented as hardware. (b)(4) has been updated to meet the new (b)(4).
- (b)(4)
(b)(4)
- Preparing for the FDA (b)(4) meeting.
- Continued preparing (b)(4) that focuses (b)(4)
(b)(4)
- Finalized (b)(4)



Overview – Financial Update

- We received the rest of the phase 1 fund.

(b)(4)

(b)(4)



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.1: (b)(4)
Increase number of animals to achieve statistically significant results (b)(4)
(b)(4) and prepare a manuscript for publication - (b)(4)
- Task 1.4: (b)(4)
(b)(4)
- Task 1.5: Stimulation (b)(4) for animal studies (b)(4)
Finalize design and (b)(4) production (b)(4)
- Task 1.6: (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
Integration of the (b)(4)
- Task 1.7: (b)(4) for human use (b)(4)
Finalize on the (b)(4)
- Task 1.8: Physician/Investigator controller for (b)(4)
Schematic design complete (b)(4)
Evaluation (b)(4) and initial evaluation complete (b)(4)
Integration with a simple (b)(4)



4 Week Work Plan from Previous Report

- Task 1.10: (b)(4)
Continuing to define (b)(4)
- Task 1.11: (b)(4)
Schedule study and continue to finalize study details – (b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early (b)(4)
Continue to amend the early feasibility human clinical protocol – (b)(4)
- Task 1.14: (b)(4)
Schedule Meeting with (b)(4)
- Task 1.15: (b)(4)
Submit (b)(4)



COVID related updates

-

(b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

(b)(4)



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month

(b)(4)

Date: 3/31/2021

(b)(4)



Technical Progress Summary

- Animal studies of (b)(4)
- Data analysis is completed, and a manuscript is ready for submission.
- The (b)(4)
- (b)(4)
- (b)(4)
- Variations of (b)(4)
- (b)(4)
- Prepared for meeting on (b)(4) with FDA regarding (b)(4)
- (b)(4)
- (b)(4)



(b)(4)



(b)(4)



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(b)(4)

(b)(4)

(b)(4)

(b)(4)



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1309



Task 1.2.1: Low Pressure Voiding by Stimulation and Block Using Lead Electrodes

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1310



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1311



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1312



(b)(4)

(b)(4), (b)(6)

(b)(4), (b)(6)

(b)(4)

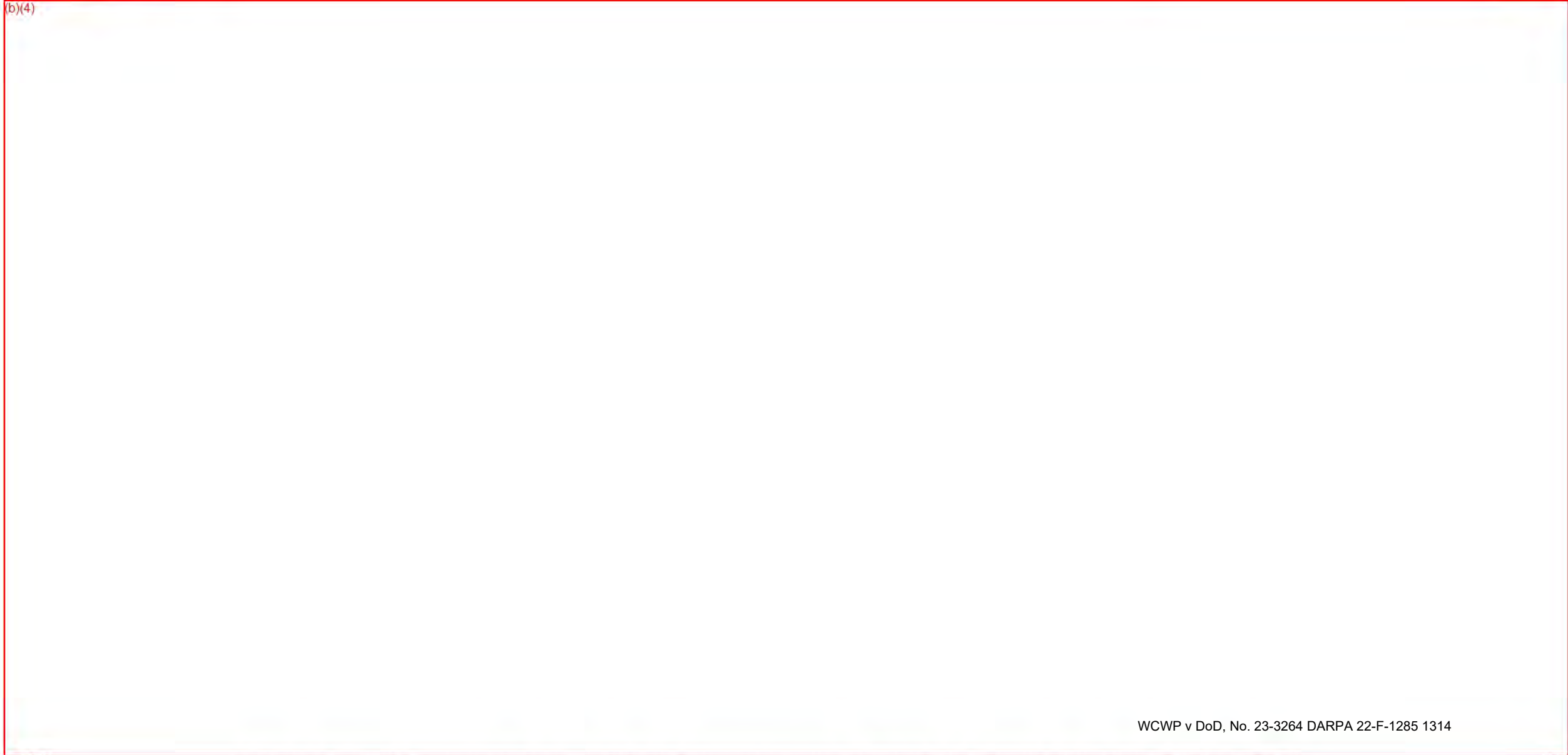
to be submitted.



(b)(4)



(b)(4)



WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1314



(b)(4)



(b)(4)



WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1315



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1316



(b)(4)



(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1317



(b)(4)

(b)(4); (b)(6)



(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

(b)(4)

-
-
-
-
-



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



Miscellaneous

-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4), (b)(6)

Reporting Period: [3/1/2021] – [3/31/2021]

3/31/2021





Overview – Technical Update

- Completed the animal studies of pudendal nerve block using lead electrodes and successfully induced low-pressure voiding by pudendal nerve block and stimulation.
- Experimental data are processed, analyzed, and a manuscript is finished and ready for submission.
- The (b)(4) machine is now under evaluation by (b)(4) electrodes are also ordered from other manufacturers to support (b)(4)
- (b)(4)
(b)(4) EPG schematic is ready to be implemented as hardware. (b)(4)
(b)(4)
- Variations of the surgical tools (b)(4)
(b)(4)
- Preparing for the FDA (b)(4) meeting.
- Continued preparing (b)(4)
(b)(4)
- Finalized logistics and scheduled (b)(4)



Overview – Financial Update

- We received the rest of the (b)(4) University of Pittsburgh has extended the subcontracts with Med-Ally and Thermaquil.



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week Work Plan from Previous Report

- Task 1.2.1: Restore bladder function (b)(4)
Increase number of animals to achieve statistically significant results (b)(4)
Analyze the results from Task 1.2.1 and prepare a manuscript for publication - (b)(4)
- Task 1.4: Implantable pulse generator with (b)(4)
Can/Header design with design review complete (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
Finalize design and lead/cuff electrode production (b)(4)
- Task 1.6: External pulse generator for (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
Integration of the (b)(4) components with updated firmware and hardware (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the (b)(4) size and length (b)(4)
- Task 1.8: Physician/Investigator controller (b)(4)
Schematic design complete (b)(4)
Evaluation board fabricated/assembled and initial evaluation complete (b)(4)
Integration with a simple enclosure with (b)(4) and firmware (b)(4)



4 Week Work Plan from Previous Report

- Task 1.10: Software Development Kit (SDK) (b)(4)
Continuing to define SDK requirements - (b)(4)
- Task 1.11: GLP animal study (b)(4)
Schedule study and continue to finalize study details - (b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Continue to amend the early feasibility human clinical protocol - (b)(4)
- Task 1.14: FDA IDE (b)(4)
Schedule Meeting with the FDA - (b)(4)
- Task 1.15: FDA IDE (b)(4)
Submit (b)(4)



COVID related updates

- Current MSR period – (b)(4) (3/1/2021-3/31/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.1 Restore bladder function (b)(4)	(b)(4)	(b)(4)				Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.3 Test Bed Environment (b)(4)							
1.3.4 Integration of EPG in enclosure with firmware and PC application connectivity (b)(4)	(b)(4)	(b)(4)				Milestone 1.3.4	
1.4 Implantable pulse generator with custom spiral lead for animal study (version 0) (b)(4)							
1.4.1 Can/Header design with design review complete (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.1	
1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)							
1.5.4 Finalize design and lead/cuff electrode production (b)(4)	(b)(4)	(b)(4)				Milestone 1.5.4	
1.6 External pulse generator (b)(4)	(b)(4)						
1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)	(b)(4)	(b)(4)				Milestone 1.6.1	
1.6.2 Integration of the (b)(4) components with firmware and the enclosure (b)(4)	(b)(4)	(b)(4)				Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)							
1.7.1 Finalize on the (b)(4) size and length (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.1	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller for (b)(4)							(b)(4)
1.8.1 Schematic design complete (b)(4)		(b)(4)				Milestone 1.8.1	
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)						Milestone 1.8.1	
1.8.3 enclosure with display and firmware (b)(4)						Milestone 1.8.1	
1.10 Software Development Kit (SDK) (b)(4)							
1.10. Requirements understood for 1 SDK (b)(4)						Milestone 1.10.1	
1.14 FDA IDE (b)(4)							
1.14. FDA IDE 2 meeting (b)(4)		(b)(4)				Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)							
1.15. FDA IDE 1 (b)(4)		(b)(4)				Milestone 1.15.1 – Deliverable 1.15.1	
1.12 (b)(4)							
1.12 (b)(4)		(b)(4)				Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)		(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies of pudendal nerve block and low-pressure voiding are completed.
- Data analysis is completed, and a manuscript is ready for submission.
- The wire coiling machine is under evaluation by (b)(4)
- (b)(4) schematic is ready to be implemented as hardware. (b)(4) enclosure has been updated to meet (b)(4)
- Variations of the surgical tools needed for (b)(4)
- Prepared for meeting on (b)(4) with FDA regarding (b)(4)
- Continued preparing (b)(4) that focuses on the (b)(4)



Task 1.2.1: Block Pudendal Nerve Using a (b)(4) Electrode

(b)(4)



Task 1.2.1: Block Duration Increases with Increasing Stimulation Intensity

(b)(4)

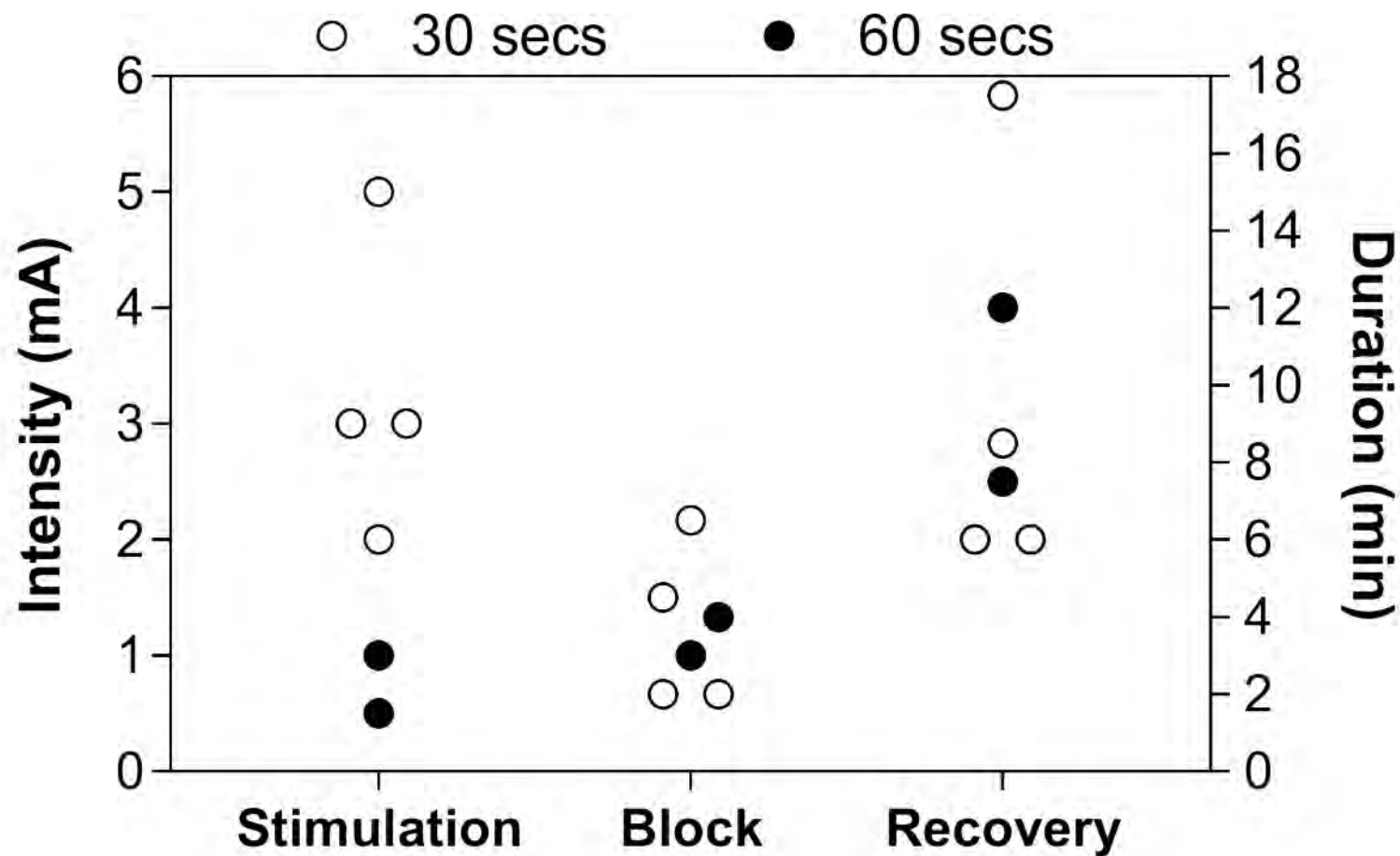


Figure 3: Summary for pudendal nerve block.



(b)(4)



(b)(4)



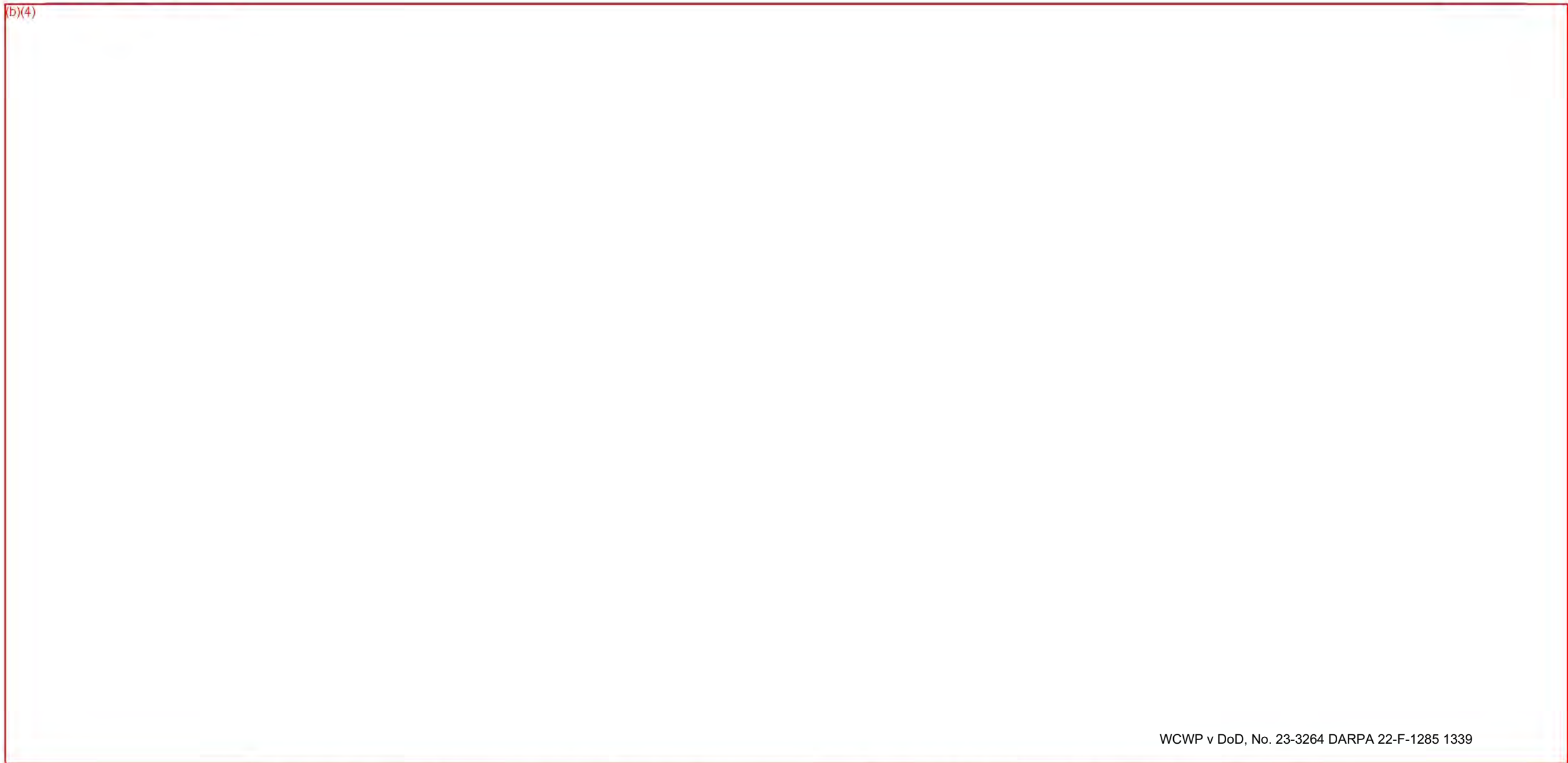
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(b)(4)



(b)(4)



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Task 1.2.1: Summary of the Urodynamic Parameters

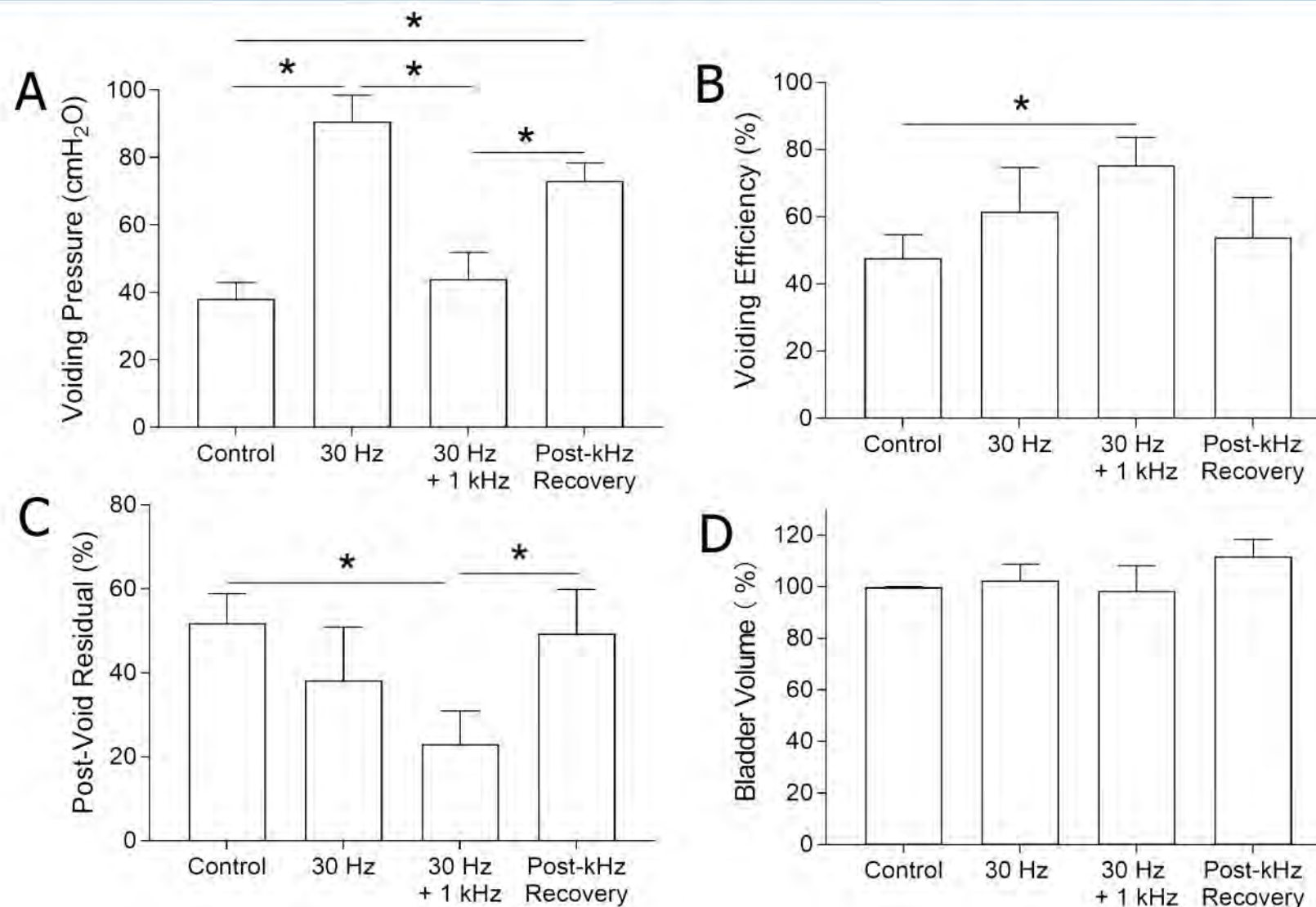


Figure 6: Summary for low pressure voiding.



Task 1.2.1: Meeting Abstracts and Publications

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Pudendal Nerve Stimulation and Block Using Lead Electrodes in Cats"
American Urological Association (AUA) Annual Meeting, September 10 – 13, 2021, Las Vegas, Nevada

Jialiang Chen, Jianan Jian, Jicheng Wang, Zhijun Shen, Bing Shen, William Wang, Jonathan Beckel, William C. de Groat, Christopher Chermansky, Changfeng Tai

"Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats"
Experimental Neurology, 2021, to be submitted.



Task 1.7: (b)(4) Electrodes for Human Use

(b)(4)



Task 1.6: External Pulse Generator

(b)(4)

(b)(4)

Figure 8: Current layout of the EPG system

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Task 1.4: Implantable Pulse Generator for Animal Study

(b)(4)



Task 1.4: Implantable Pulse Generator for Animal Study

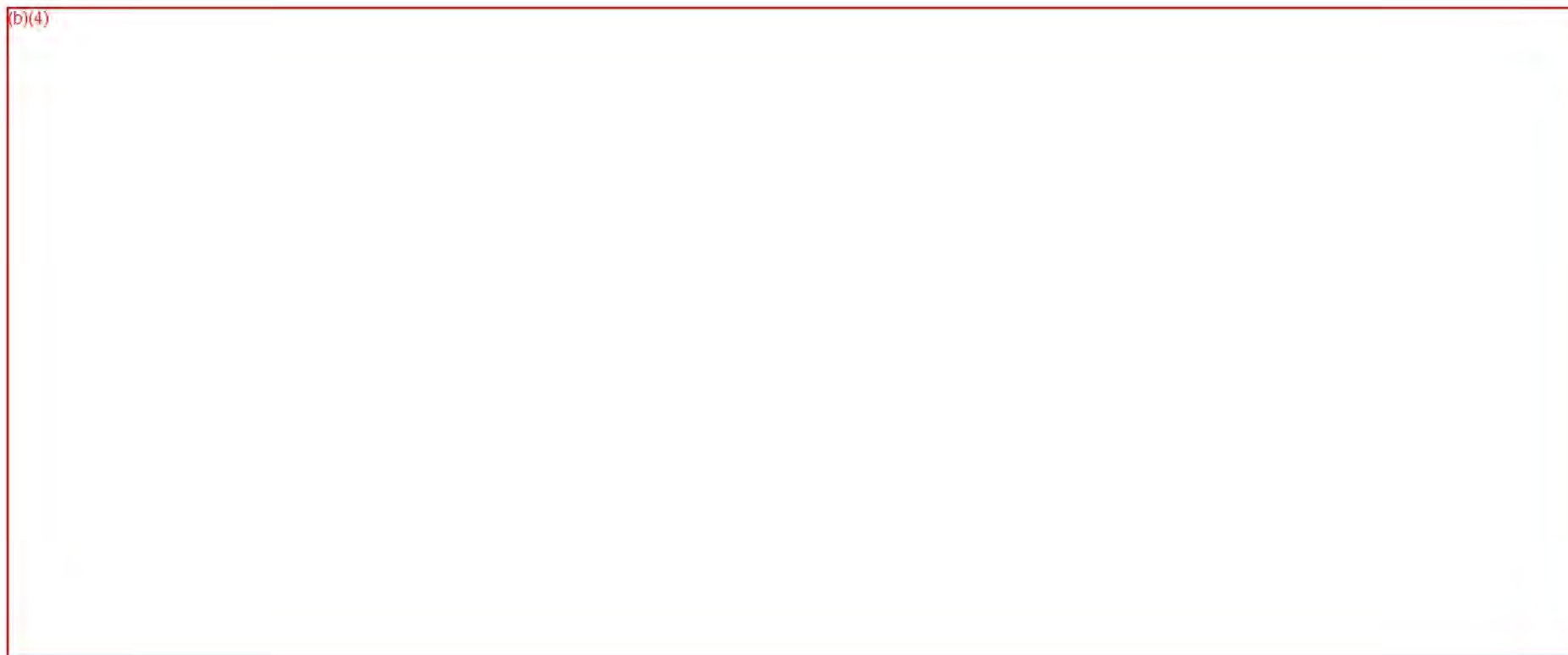


Figure 10:



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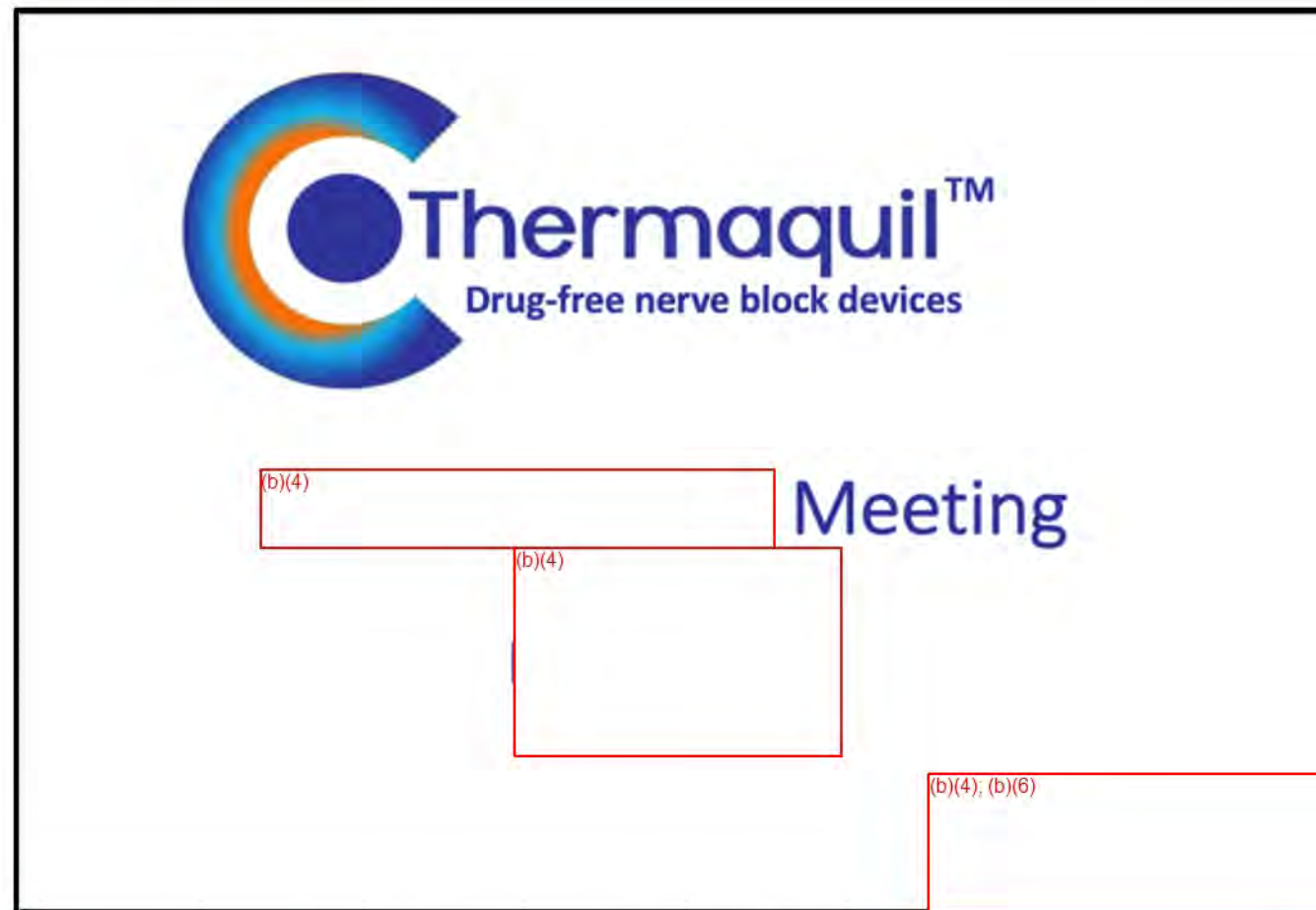


Figure 11: FDA IDE (b)(4) meeting is scheduled on (b)(4) with DARPA BG+ team participation (provided by Thermaquil).



Work Plan for Next

(b)(4)

- Task 1.2.2: Restore bowel function

(b)(4)

Induce colon contractions in 2 cats by pudendal nerve or

(b)(4)

- Task 1.4: Implantable pulse generator with

(b)(4)

Can samples will be developed and fully evaluated

(b)(4)

- Task 1.5: Stimulation Leads and cuff electrodes for animal studies

(b)(4)

Finalize design and lead/cuff electrode production

(b)(4)

- Task 1.6: External pulse generator for human study

(b)(4)

Industrial design and CAD drawings for the custom enclosure

(b)(4)

Integration of the EPG components with updated firmware and hardware

(b)(4)

- Task 1.7: electrodes for human use

(b)(4)

(b)(4)

Finalize on the

(b)(4)

size and length

(b)(4)

- Task 1.8: Physician/Investigator controller for

(b)(4)

Schematic design complete

(b)(4)

Evaluation board fabricated/assembled and initial evaluation complete

(b)(4)

Integration with a simple enclosure with

(b)(4)

and firmware

(b)(4)



Work Plan for Next

(b)(4)

- Task 1.11: GLP animal study (b)(4)

Schedule study and continue to finalize study details

Amend study based on FDA feedback from our meeting (b)(4)

- Task 1.12: (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol for (b)(4)

Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol

Amend study based on FDA feedback from our meeting (b)(4)

- Task 1.14: FDA IDE (b)(4)

Meeting with the FDA (b)(4)

- Task 1.15: FDA IDE (b)(4)

Submit (b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



Miscellaneous

-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [3/1/2021] – [3/31/2021]

3/31/2021





Overview – Technical Update

- Completed the animal studies of (b)(4)
(b)(4)
- Experimental data are processed, analyzed, and a manuscript is finished and ready for submission.
- The (b)(4) is now under evaluation by (b)(4)
(b)(4)
- (b)(4) system layout with different combinations of (b)(4) have been updated to meet the (b)(4)
(b)(4) schematic is ready to be implemented as hardware. (b)(4) has been updated to meet the (b)(4)
- (b)(4)
(b)(4)
- Preparing for the FDA (b)(4) meeting.
- Continued preparing (b)(4) that focuses on (b)(4)
(b)(4)
- (b)(4)



Overview – Financial Update

- We received the rest of the phase 1 fund. (b)(4)

(b)(4)



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.1: (b)(4)
Increase number of animals to achieve statistically significant results (b)(4)
(b)(4) and prepare a manuscript for publication - (b)(4)
- Task 1.4: (b)(4) with (b)(4)
(b)(4)
- Task 1.5: (b)(4) for animal studies (b)(4)
Finalize design and (b)(4) production (b)(4)
- Task 1.6: (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
Integration of the (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the (b)(4) size and length (b)(4)
- Task 1.8: Physician/Investigator controller (b)(4)
Schematic design complete (b)(4)
Evaluation (b)(4) and initial evaluation complete (b)(4)
Integration with a simple (b)(4)



4 Week Work Plan from Previous Report

- Task 1.10: (b)(4)
Continuing to define (b)(4)
- Task 1.11: (b)(4)
Schedule study and continue to finalize study details – (b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: (b)(4)
Continue to amend the early (b)(4)
- Task 1.14: (b)(4)
Schedule Meeting with the (b)(4)
- Task 1.15: (b)(4)
Submit (b)(4)



COVID related updates

- (b)(4) (3/1/2021-3/31/2021)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	(b)(4)
(b)(4)					



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	(b)(4)
(b)(4)					



Technical Progress Summary

- Animal studies of (b)(4)
- Data analysis is completed, and a manuscript is ready for submission.
- The (b)(4)
- (b)(4)
- Variations of (b)(4)
- (b)(4)
- Prepared for meeting (b)(4) with FDA (b)(4)
- (b)(4)
- (b)(4)



(b)(4)



(b)(4)



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(b)(4)

(b)(4)

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Task 1.2.1:

(b)(4)

(b)(4)

Figure 3:

(b)(4)



(b)(4)

(b)(4)

(b)(4)

(b)(4)



(b)(4)

(b)(4)

(b)(4)



Task 1.2.1:

(b)(4)

(b)(4)

Figure 6:

(b)(4)

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Task 1.2.1:

(b)(4)

(b)(4), (b)(6)

(b)(4)

to be submitted.



(b)(4)

(b)(4)

(b)(4)

(b)(4)



(b)(4)

(b)(4)

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Task 1.4:

(b)(4)

(b)(4)



Task 1.4: Implantable Pulse Generator for Animal Study

(b)(4)

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(b)(4)

(b)(4); (b)(6)



(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

(b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



Miscellaneous

-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [3/1/2021] – [3/31/2021]

3/31/2021





Overview – Technical Update

- Completed the animal studies of pudendal nerve block using lead electrodes and successfully induced low-pressure voiding by pudendal nerve block and stimulation.
- Experimental data are processed, analyzed, and a manuscript is finished and ready for submission.
- The (b)(4) is now under evaluation by (b)(4) electrodes are also ordered (b)(4) (b)(4)
- (b)(4) layout with different combinations (b)(4) have been updated to meet (b)(4) (b)(4) schematic is ready to be implemented as hardware. (b)(4) enclosure has been updated to meet (b)(4)
- Variations of the surgical tools needed (b)(4) (b)(4)
- Preparing for the FDA (b)(4) meeting.
- Continued preparing (b)(4) that focuses on (b)(4) (b)(4)
- (b)(4)



Overview – Financial Update

- We received the rest of the

(b)(4)

(b)(4)



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week Work Plan from Previous Report

- Task 1.2.1: Restore bladder function (b)(4)
Increase number of animals to achieve statistically significant results (b)(4)
Analyze the results from Task 1.2.1 and prepare a manuscript for publication - (b)(4)
- Task 1.4: Implantable pulse generator (b)(4)
Can/Header design with design review complete (b)(4)
- Task 1.5: Stimulation Leads and cuff electrodes for animal studies (b)(4)
Finalize design and lead/cuff electrode production (b)(4)
- Task 1.6: External pulse generator (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
Integration of the (b)(4) components with updated firmware and hardware (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the (b)(4) size and length (b)(4)
- Task 1.8: Physician/Investigator controller (b)(4)
Schematic design complete (b)(4)
Evaluation board fabricated/assembled and initial evaluation complete (b)(4)
Integration with a simple enclosure with display and firmware (b)(4)



4 Week Work Plan from Previous Report

- Task 1.10: Software Development Kit (SDK) (b)(4)
Continuing to define SDK requirements - (b)(4)
- Task 1.11: GLP animal study (b)(4)
Schedule study and continue to finalize study details – (b)(4)
- Task 1.12: (b)(4)
Schedule study and continue to finalize study details – (b)(4)
- Task 1.13: Early feasibility human clinical protocol (b)(4)
Continue to amend the early feasibility human clinical protocol – (b)(4)
- Task 1.14: FDA IDE (b)(4)
Schedule Meeting with the FDA - (b)(4)
- Task 1.15: FDA IDE (b)(4)
Submit (b)(4)



COVID related updates

- Current MSR period – (b)(4) (3/1/2021-3/31/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.1 Restore bladder function (b)(4)	(b)(4)					Milestone 1.2.1(1-4) – Deliverable 1.2.1	
1.3 Test Bed Environment (b)(4)							
1.3.4 Integration of (b)(4) in enclosure with firmware and application connectivity (b)(4)	(b)(4)					Milestone 1.3.4	
1.4 Implantable pulse generator with (b)(4)							
1.4.1 Can/Header design with design review complete (b)(4)	(b)(4)					Milestone 1.4.1	
1.5 Stimulation Leads and cuff electrodes for animal studies (b)(4)							
1.5.4 Finalize design and lead/cuff electrode production (b)(4)	(b)(4)					Milestone 1.5.4	
1.6 External pulse generator for (b)(4)							
1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)	(b)(4)					Milestone 1.6.1	
1.6.2 Integration of the (b)(4) components with firmware and the enclosure (b)(4)	(b)(4)					Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)							
1.7.1 Finalize on the (b)(4) size and length (b)(4)	(b)(4)					Milestone 1.7.1	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 3/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller for (b)(4)							(b)(4)
1.8.1 Schematic design complete (b)(4)						Milestone 1.8.1	
Evaluation board							
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)						Milestone 1.8.1	
Integration with a simple							
1.8.3 enclosure with display and firmware (b)(4)						Milestone 1.8.1	
1.10 Software Development Kit (SDK) (b)(4)							
1.10. Requirements understood for							
1 SDK (b)(4)						Milestone 1.10.1	
1.14 FDA IDE pre-submission #1 (b)(4)							
1.14. FDA IDE (b)(4)							
2 meeting						Milestone 1.14.2 – Deliverable 1.14.2	
1.15 FDA IDE (b)(4)							
1.15. FDA IDE (b)(4)							
1 (b)(4)						Milestone 1.15.1 – Deliverable 1.15.1	
1.12 (b)(4)							
1.12 (b)(4)						Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for EPG system (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)						Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies of pudendal nerve block and low-pressure voiding are completed.
- Data analysis is completed, and a manuscript is ready for submission.

- The (b)(4)

- (b)(4)

- (b)(4)

- Prepared for meeting (b)(4) with FDA regarding (b)(4)

- (b)(4) that focuses on (b)(4)

- (b)(4)



Task 1.2.1:

(b)(4)

(b)(4)



Task 1.2.1:

(b)(4)

[Redacted content]

(b)(4)

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Task 1.2.1:

(b)(4)

(b)(4)

Figure 3:

(b)(4)



Task 1.2.1:

(b)(4)

(b)(4)

(b)(4)



Task 1.2.1:

(b)(4)

(b)(4)



Task 1.2.1:

(b)(4)

(b)(4)



Task 1.2.1:

(b)(4)

(b)(4)

(b)(4)

to be submitted.



Task 1.7:

(b)(4)

(b)(4)



(b)(4)

(b)(4)

(b)(4)

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Task 1.4: Implantable Pulse Generator for Animal Study

(b)(4)

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(b)(4)

(b)(4), (b)(6)

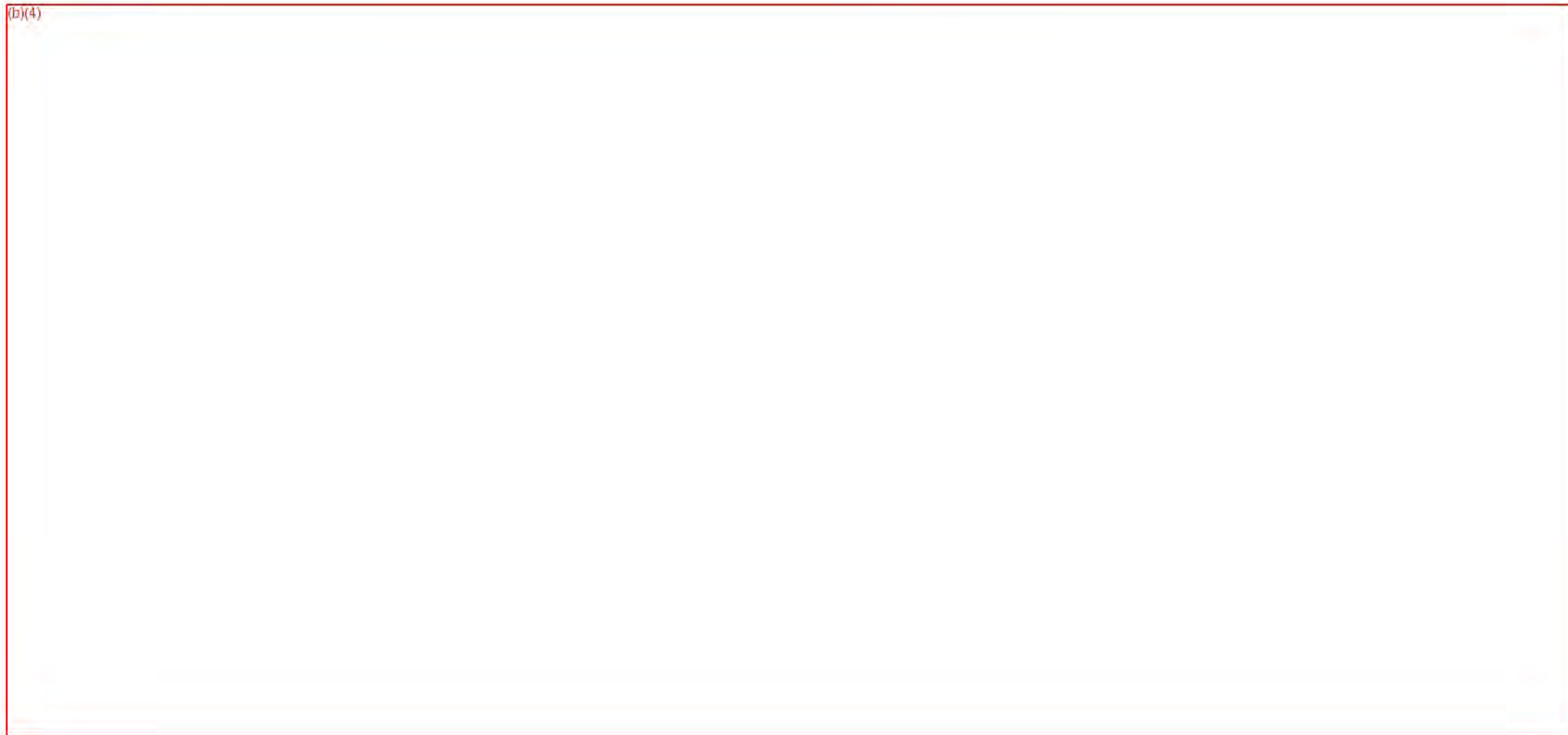
(b)(4)



(b)(4)



(b)(4)





Work Plan for Next 4 Weeks

(b)(4)

(b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- No administrative issues to report



Miscellaneous

-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [4/1/2021] – [4/30/2021]

4/30/2021





Overview – Technical Update

- Begin animal studies to induce colon contractions for restoring defecation function.
- The (b)(4) have been produced and have been (b)(4)
- The (b)(4) has been updated.
- The surgical approach has been further adapted and defined. (b)(4)
- (b)(4) have been acquired.
- The (b)(4) has made great progress and is under ongoing review with clinicians and the internal team.
- (b)(4)
- Continued preparing (b)(4)
- (b)(4)
- Continued drafting early feasibility human clinical protocol for (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements



Overview – Financial Update

- Nothing to report



Overview – Administrative Update

-

(b)(4)



4 Week Work Plan from Previous Report

(b)(4)

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4 Week Work Plan from Previous Report

(b)(4)

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COVID related updates

- Current MSR period – (b)(4)
- (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] (b)(4)

Date: 4/30/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
(b)(4)							



Milestones and Deliverables Summary

[University of Pittsburgh] (b)(4)

Date: 4/30/2021

(b)(4)



Technical Progress Summary

- Animal studies to (b)(4) were performed in (b)(4)
- (b)(4)
- The (b)(4) has been updated.
- The surgical approach has been further adapted and defined. (b)(4)
- (b)(4)
- The (b)(4) has made great progress and is under ongoing review with clinicians and the internal team
- Met with FDA regarding (b)(4) Given FDA's positive feedback, we amended our safety and efficacy studies accordingly
- Continued drafting (b)(4)
- (b)(4)
- Continued drafting the early (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements



Task 1.2.2: Restore Bowel Function

(b)(4)



(b)(4)

(b)(4)



Task 1.2.2: Restore Bowel Function

(b)(4)



Task 1.3: Early Feasibility Human Clinical Protocol

(b)(4)

(b)(4)



(b)(4)

(b)(4)

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(b)(4)

Electrode Manufacturing

(b)(4)

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(b)(4)

Electrode Design

(b)(4)

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(b)(4)



(b)(4)





(b)(4)

(b)(4)

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(b)(4)

Figure 15

(b)(4)

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(b)(4)

(b)(4)

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(b)(4)

(b)(4)



IPG Design for

(b)(4)

(b)(4)

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Task 1.15: FDA IDE

(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

(b)(4)

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Monthly Financial Overview

(b)(4)



Administrative issues

- Nothing to report



-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [4/1/2021] – [4/30/2021]

4/30/2021





Overview – Technical Update

- Begin animal studies to (b)(4)
- The (b)(4) have been produced and have been (b)(4) Currently (b)(4)
- (b)(4)
- (b)(4)
- (b)(4)
- (b)(4)
- (b)(4)
- (b)(4)
- Continued preparing (b)(4)
- (b)(4)
- Continued drafting (b)(4)
- Continued identifying user needs and voice of (b)(4)



Overview – Financial Update

- Nothing to report



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week Work Plan from Previous Report

(b)(4)

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4 Week Work Plan from Previous Report

(b)(4)

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COVID related updates

- Current MSR period – (b)(4) (4/1/2021-4/30/2021)
 - (b)(4)



Big Wins

- Nothing to report.



(b)(4)

[University of Pittsburgh] (b)(4)

Date: 4/30/2021

(b)(4)



(b)(4)

[University of Pittsburgh] (b)(4)

Date: 4/30/2021

(b)(4)



Technical Progress Summary

- (b)(4)
-
-
-
-
-
-
- Met with FDA regarding (b)(4) Given FDA's positive feedback, we amended our safety and efficacy studies accordingly
- Continued drafting (b)(4)
- (b)(4)
- (b)(4)



Task 1.2.2: Restore Bowel

(b)(4)

(b)(4)



(b)(4)

(b)(4)



(b)(4)

(b)(4)



Task 1.3:

(b)(4)

(b)(4)



(b)(4)

(b)(4)



(b)(4)

(b)(4)

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(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1455



(b)(4)

(b)(4)



EPG Stimulation Output Stage - Electrical Design

(b)(4)



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1458



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1459



Figure 18

(b)(4)





(b)(4)

(b)(4)



Task 1.15: FDA IDE

(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

(b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- Nothing to report



Miscellaneous

-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4), (b)(6)

Reporting Period: [5/1/2021] – [5/31/2021]

5/31/2021





Overview – Technical Update

- Conducted additional (b)(4)
- The (b)(4) is currently being fabricated on to the (b)(4)
- The (b)(4) has been finalized.
- (b)(4)
- New (b)(4) were conceived.
- Multiple (b)(4) were evaluated after they were (b)(4) It was determined that the (b)(4)
- The graphical user interface (GUI) has made great progress and is under ongoing review with clinicians and the internal team.
- (b)(4)
- Continued preparing (b)(4)
- (b)(4)
- Continued drafting early (b)(4)
- Continued identifying user needs and voice of customer to (b)(4)



Overview – Financial Update

- Nothing to report



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among University of Pittsburgh, Med-Ally, and Thermaquil.



4 Week Work Plan from Previous Report

- Task 1.2.2: Restore bowel function (b)(4)
(b)(4)
- Task 1.4: (b)(4)
Can samples will be developed and fully evaluated (b)(4)
- Task 1.6: External pulse generator for human study (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
Integration of the (b)(4) with updated firmware and hardware (b)(4)
- Task 1.7: Leads and cuff electrodes for human use (b)(4)
Finalize on the (b)(4)
Finalize design of (b)(4)
- Task 1.8: Physician/Investigator controller for (b)(4)
(b)(4)
Evaluation board (b)(4)
Integration with a simple (b)(4)
- Task 1.9: Patient controller for (b)(4)
Firmware features complete to restrict use as a patient controller (b)(4)



4 Week Work Plan from Previous Report

- Task 1.12: (b)(4)
(b)(4)
- Task 1.11: (b)(4)
Include revised study plan and confirm need for (b)(4)
(b)(4)
Schedule study and continue to finalize study details (b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol –
(b)(4)
Include protocol in (b)(4)
- Task 1.15: FDA (b)(4)
Continue to prepare (b)(4)



COVID related updates

- Current (b)(4) (5/1/2021-5/31/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] (b)(4)

Date: 5/31/2021

(b)(4)



Milestones and Deliverables Summary

[University of Pittsburgh] (b)(4)

Date: 5/31/2021

(b)(4)



Technical Progress Summary

- Animal studies to induce colon contractions were performed in 4 cats.

- (b)(4) is most effective in (b)(4)

- (b)(4)

- The (b)(4) is currently being fabricated on to the boards. The (b)(4)

- Multiple (b)(4) designs were evaluated after they were laser sintered.
- The (b)(4) has made great progress.

- Continued drafting (b)(4)

- (b)(4)



Task 1.2.2: Restore Bowel Function

(b)(4)



Task 1.2.2: Restore Bowel Function

(b)(4)



Task 1.2.2: Restore Bowel Function

(b)(4)

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Task 1.2.2: Restore Bowel Function

(b)(4)



(b)(4)

(b)(4)

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(b)(4)

(b)(4)

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Task 1.6.2 Integration of the

(b)(4)

(b)(4)

(b)(4)



(b)(4)

(b)(4)

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Task 1.4 Possible

(b)(4)

Figure 9:

(b)(4)

(b)(4)

(b)(4)



Manufacture of (b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

- Task 1.2.2: Restore bowel function

(b)(4)

Induce colon contraction and defecation in 2 more cats by sacral root stimulation

(b)(4)

- Task 1.4:

(b)(4)

Can samples will be developed and fully evaluated

(b)(4)

Final PC test application with wireless board connectivity

(b)(4)

-

(b)(4)

(b)(4)

(b)(4)

- Task 1.8:

(b)(4)

Schematic design complete

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

- Task 1.9:

(b)(4)

(b)(4)

(b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

- Task 1.11: GLP animal study (b)(4)

Schedule study and continue to finalize study details (b)(4)

- Task 1.12: (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol for (b)(4)

Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol

Include protocol in (b)(4)

- Task 1.15: FDA IDE (b)(4)

Continue to prepare (b)(4)



Monthly Financial Overview

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1491



Administrative issues

- Nothing to report



Miscellaneous

-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [5/1/2021] – [5/31/2021]

5/31/2021





Overview – Technical Update

- Conducted additional 2 animal studies to induce colon contractions by sacral root stimulation.
- (b)(4)
-
-
-
-
-
- The graphical user interface (GUI) has made great progress and is under ongoing review with clinicians and the internal team.
- Carried out (b)(4)
- Continued preparing (b)(4)
- (b)(4)
- Continued drafting early feasibility human clinical protocol for (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements



Overview – Financial Update

- Nothing to report



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.2: Restore bowel function (b)(4)
Induce colon contractions in 2 more cats by sacral root stimulation (b)(4)
- Task 1.4: (b)(4)
Can samples will be developed and fully evaluated (b)(4)
- Task 1.6: (b)(4)
Industrial design and CAD drawings for the custom enclosure (b)(4)
(b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
Finalize on the (b)(4) size and length (b)(4)
Finalize design of human lead (b)(4)
- Task 1.8: Physician/Investigator controller for (b)(4)
Schematic design complete (b)(4)
Evaluation board fabricated/assembled and initial evaluation complete (b)(4)
(b)(4)
- Task 1.9: (b)(4)
(b)(4)



4 Week Work Plan from Previous Report

- Task 1.12: (b)(4)
(b)(4)
- Task 1.11: GLP animal study (b)(4)
Include revised study plan (b)(4)
(b)(4)
Schedule study and continue to finalize study details (b)(4)
- Task 1.13: Early feasibility human clinical protocol (b)(4)
Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol –
(b)(4)
Include protocol in (b)(4)
- Task 1.15: FDA IDE (b)(4)
Continue to prepare (b)(4)



COVID related updates

- (b)(4) (5/1/2021-5/31/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 5/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.2 Restore bowel function (b)(4)	(b)(4)	(b)(4)				Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment (b)(4)							
1.3.4 (b)(4)	(b)(4)					Milestone 1.3.4	
1.4 (b)(4) with custom (b)(4)							
1.4.2 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.2	
1.4.4 (b)(4)						Milestone 1.4.4 – Deliverable 1.4	
1.6 External pulse generator for (b)(4)							
1.6.1 Industrial design and CAD drawings for the custom enclosure (b)(4)	(b)(4)	(b)(4)				Milestone 1.6.1	
1.6.2 (b)(4)						Milestone 1.6.2	
1.7 (b)(4) for human use (b)(4)							
1.7.1 Finalize on the (b)(4) size and length (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.1	
1.7.2 Finalize design of human (b)(4)	(b)(4)					Milestone 1.7.2	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 5/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller for (b)(4)							(b)(4)
1.8.1 Schematic design complete (b)(4)						Milestone 1.8.1	
1.8.2 Evaluation board (b)(4)						Milestone 1.8.1	
1.8.3						Milestone 1.8.1	
1.9 Patient controller for (b)(4)							
1.9.1 (b)(4)						Milestone 1.9.1 – Deliverable 1.9	
1.15 FDA IDE (b)(4)							
1.15. FDA IDE (b)(4)						Milestone 1.15.1 – Deliverable 1.15.1	
1 (b)(4)							
1.12 (b)(4)							
1.12 (b)(4)						Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)						Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies to induce colon contractions were performed in 4 cats.

- (b)(4)

- New (b)(4) designs were conceived. (b)(4)

- (b)(4)
-
-

- Multiple (b)(4) designs were evaluated after they were (b)(4)

- The graphical user interface (GUI) has made great progress.

- Continued drafting (b)(4)

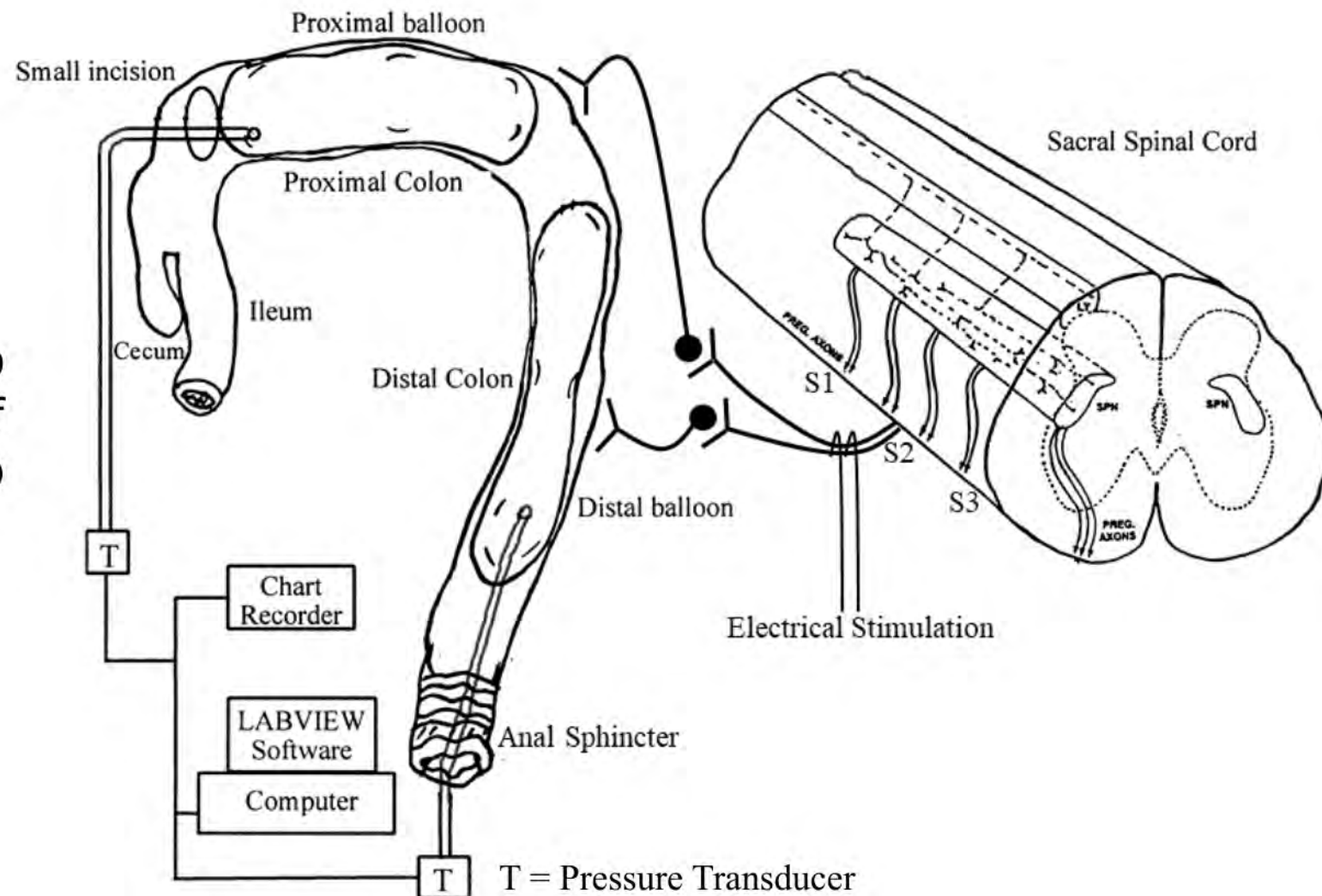
- (b)(4)

- Continued drafting the early feasibility human clinical protocol for (b)(4)

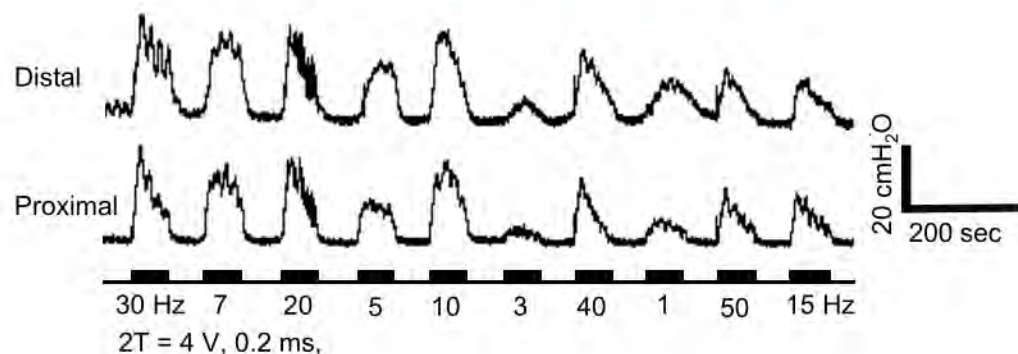
- Continued identifying user needs and voice of customer to inform device requirements

- (b)(4)

Figure 1: Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce colon contractions.



S2 Ventral Root (Frequency)



S2 Ventral Root (Duration)

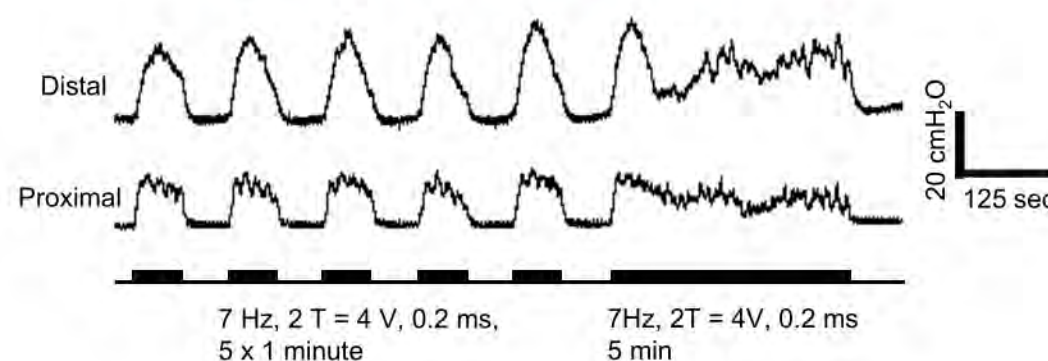


Figure 2: Proximal and distal colon responses to stimulation of S2 ventral root.

S2 Spinal Root (Ventral + Dorsal)

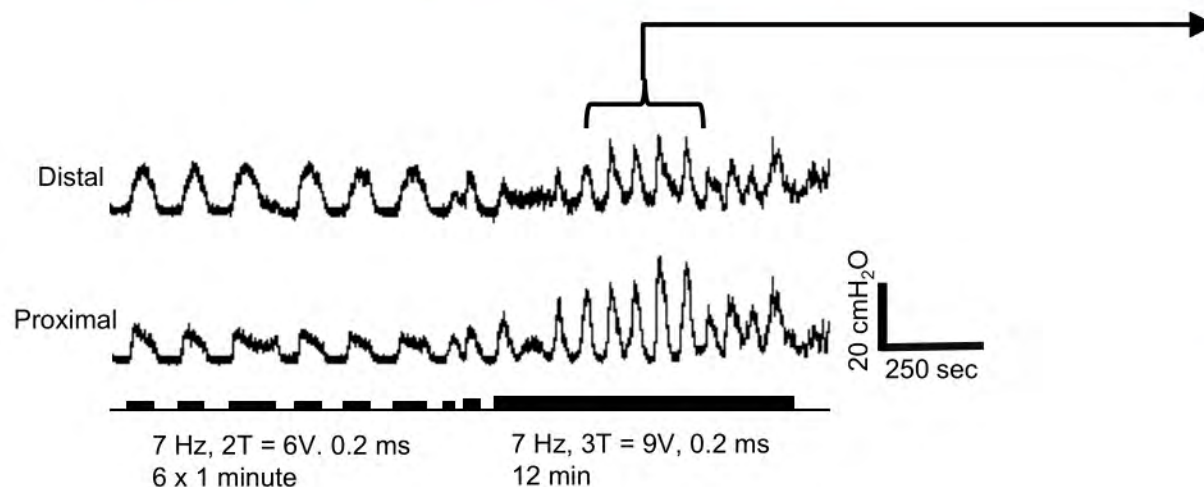


Figure 3: Defecation induced by stimulation of S2 spinal root (ventral and dorsal).



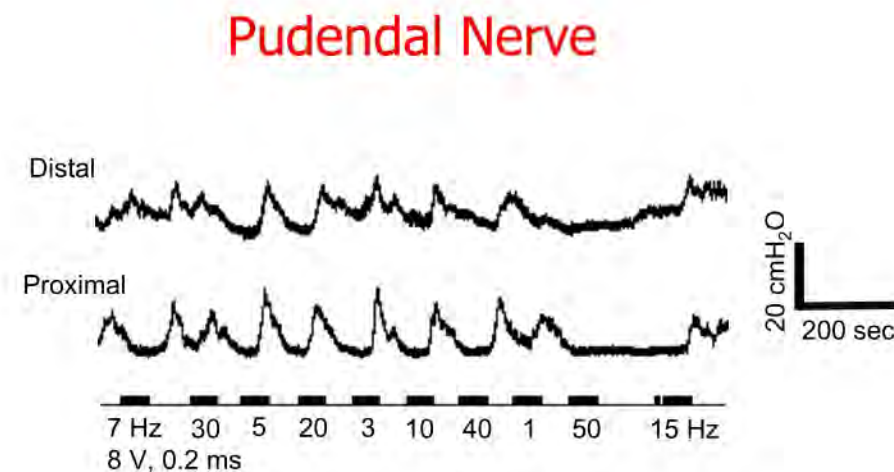
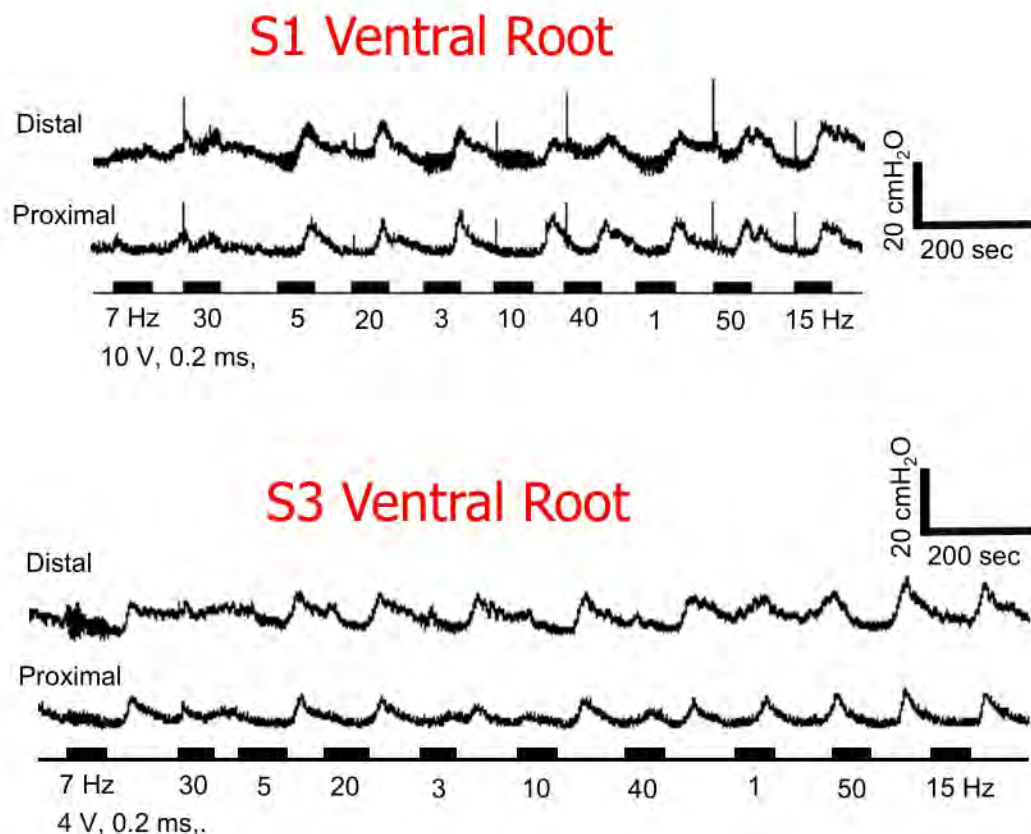


Figure 4: Proximal and distal colon responses to stimulation of S1 or S3 ventral root or the pudendal nerve.



(b)(4)



(b)(4)



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(b)(4)

(b)(4)

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Task 1.6.2 Integration of the external pulse generator components with firmware and the enclosure

(b)(4)

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(b)(4)

(b)(4)



Task 1.4 Possible IPG design

(b)(4)

(b)(4)

(b)(4)



Manufacture of (b)(4)

(b)(4)



Work Plan for Next 4 Weeks (b)(4)

- Task 1.2.2: Restore bowel function (b)(4)

Induce colon contraction and defecation in 2 more cats by sacral root stimulation (b)(4)

- Task 1.4: (b)(4)

Can samples will be developed and fully evaluated (b)(4)

(b)(4)

- Task 1.6: External pulse generator for (b)(4)

(b)(4)

- Task 1.8: (b)(4)

Schematic design complete (b)(4)

(b)(4)

(b)(4)

- Task 1.9: Patient controller for (b)(4)

(b)(4)



Work Plan for Next 4 Weeks (b)(4)

- Task 1.11: GLP animal study (b)(4)
(b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol
Include protocol in (b)(4)
- Task 1.15: FDA IDE (b)(4)
Continue to prepare (b)(4)



Monthly Financial Overview

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1517



Administrative issues

- Nothing to report



-

(b)(4)

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [6/1/2021] – [6/30/2021]

6/30/2021





Overview – Technical Update

- Conducted additional 2 animal studies to induce colon contraction and defecation by sacral root stimulation.
- (b)(4)
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-
-
- (b)(4) samples have begun to be created. Materials such as (b)(4) were purchased.
- A new biocompatible resin was purchased to print (b)(4)
- (b)(4)
- Submitted (b)(4)
- Completed drafting early feasibility human clinical protocol (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements



Overview – Financial Update

- Nothing to report



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.2: Restore bowel function (b)(4)
Induce colon contraction and defecation in 2 more cats by sacral root stimulation (b)(4)
- Task 1.4: (b)(4)
(b)(4)
- Task 1.6: (b)(4)
(b)(4)
- Task 1.8: (b)(4)
Schematic design complete (b)(4)
(b)(4) and initial evaluation complete (b)(4)
(b)(4)
- Task 1.9: (b)(4)
(b)(4)



4 Week Work Plan from Previous Report

- Task 1.11: GLP animal study (b)(4)
Schedule study and continue to finalize study details (b)(4)
- Task 1.12: (b)(4)
(b)(4)
- Task 1.13: Early feasibility human clinical protocol for (b)(4)
Continue to acquire clinician feedback on and amend the early feasibility human clinical protocol – (b)(4)
Include protocol in (b)(4)
- Task 1.15: FDA IDE (b)(4)
Continue to prepare (b)(4)



COVID related updates

- Current MSR period – (b)(4) (6/1/2021-6/30/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 6/30/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.2 Restore bowel function (b)(4)	(b)(4)	(b)(4)				Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment (b)(4)							
1.3.4 (b)(4)	(b)(4)	(b)(4)				Milestone 1.3.4	
1.4 Implantable pulse generator with (b)(4)							
1.4.2 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.2	
1.4.3						Milestone 1.4.3 – Deliverable 1.4	
1.4.4						Milestone 1.4.4 – Deliverable 1.4	
1.6 (b)(4)							
1.6.1 Industrial design and CAD drawings (b)(4)	(b)(4)	(b)(4)				Milestone 1.6.1	
1.6.2						Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)							(b)(4)
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.3 – Deliverable 1.7	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 6/30/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller for human EPG and animal IPG	(b)(4)	(b)(4)					(b)(4)
1.8.1 Schematic design complete	(b)(4)	(b)(4)				Milestone 1.8.1	
1.8.2	(b)(4)	(b)(4)				Milestone 1.8.1	
1.8.3	(b)(4)	(b)(4)				Milestone 1.8.1	
1.9 Patient controller	(b)(4)	(b)(4)					
1.9.1	(b)(4)	(b)(4)				Milestone 1.9.1 – Deliverable 1.9	
1.15 FDA IDE	(b)(4)	(b)(4)					
1.15. FDA IDE	(b)(4)	(b)(4)				Milestone 1.15.1 – Deliverable 1.15.1	
1	(b)(4)	(b)(4)					
1.12	(b)(4)	(b)(4)				Milestone 1.12 – Deliverable 1.12	
1.12	(b)(4)	(b)(4)					
1.13 Early feasibility human clinical protocol	(b)(4)	(b)(4)					
1.13	(b)(4)	(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study	(b)(4)	(b)(4)					
1.11 GLP animal study	(b)(4)	(b)(4)				Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies to induce colon contractions and/or defecation were performed in 6 cats.

(b)(4)

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- New IPG designs were sent out for quote to multiple component suppliers.

(b)(4)

-
-

- The graphical user interface (GUI) has made additional progress.

(b)(4)

-

submitted on (b)(4) accepted by FDA on (b)(4), and FDA meeting was scheduled for (b)(4)

- Drafted the early feasibility human clinical protocol, included in (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements

(b)(4)

-

Task 1.2.2: Restore Bowel Function

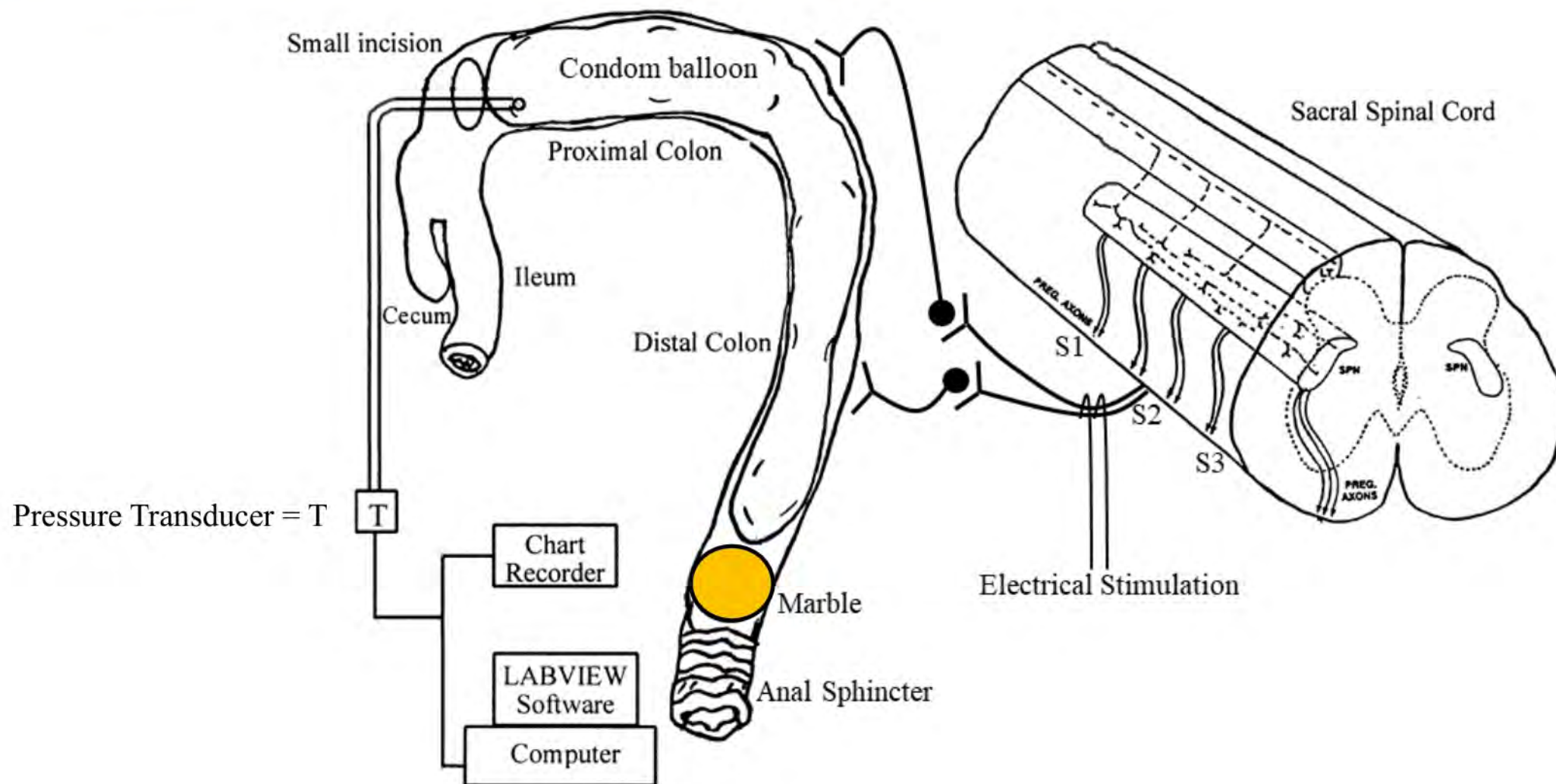
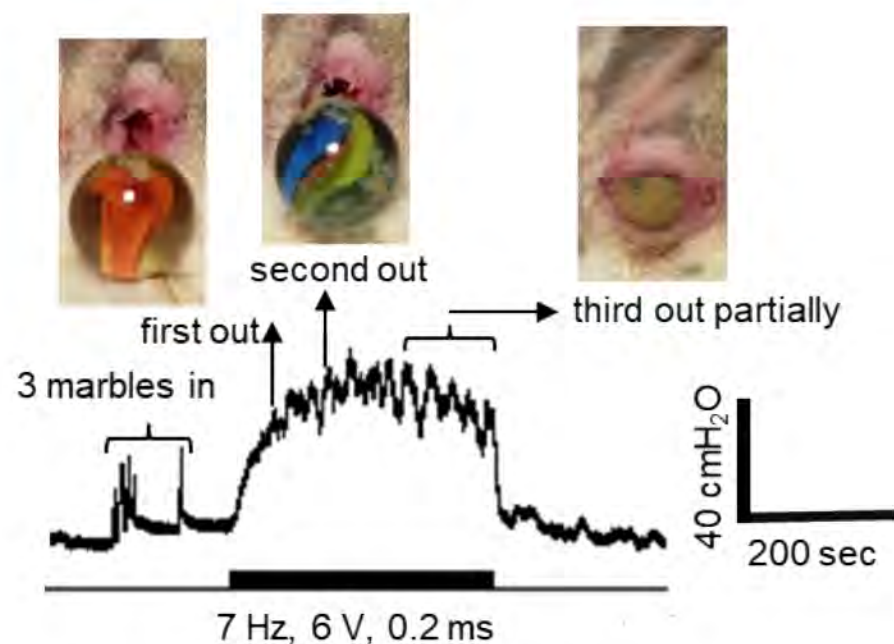


Figure 1: Experimental setup for electrical stimulation of sacral S2 root to induce defecation

WCWFF v. 5.0b, No. 23-3264 DARPA A2-F-12851631

A. Cat #1



B. Cat #2

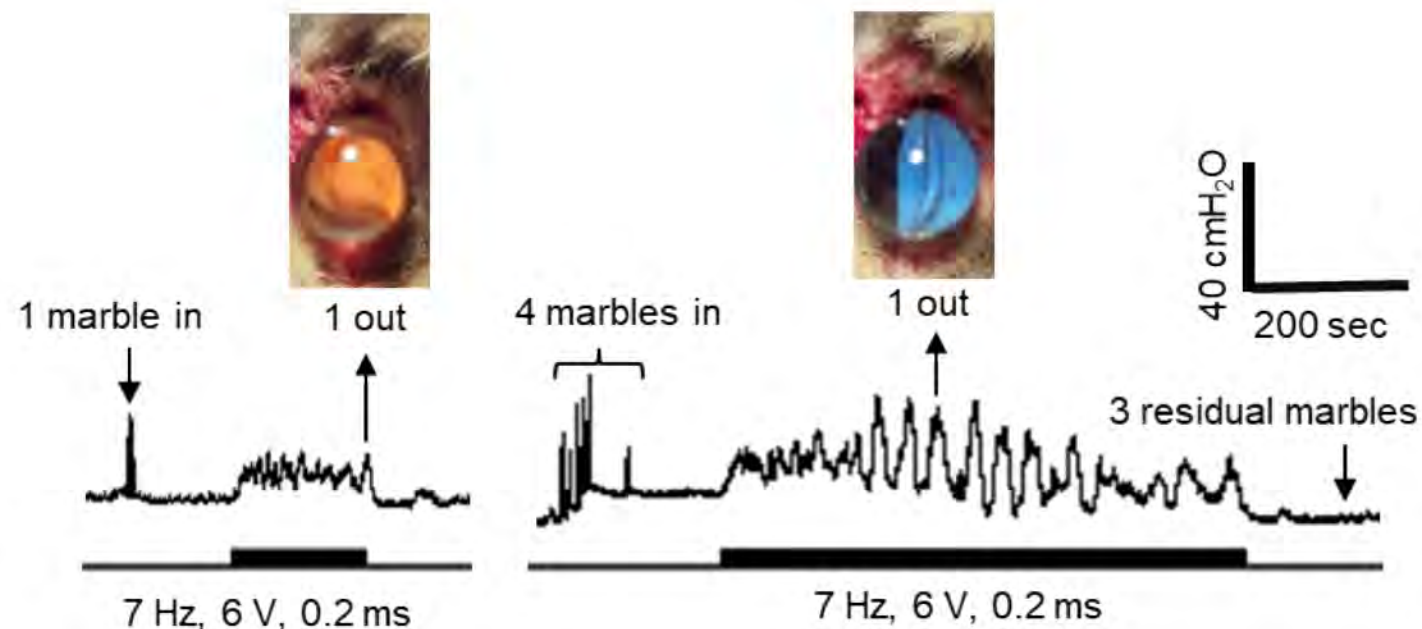


Figure.2. Defecation induced by stimulation of sacral S2 root (ventral and dorsal).



Task 1.2.2: Restore Bowel Function

Video needs to be sent separately due to the large file size.

Figure 3: Defecation induced by stimulation of S2 ventral root in Cat #1.

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1533



(b)(4)

(b)(4)



Task 1.7.3: First prototypes of leads delivered for test

(b)(4)

(b)(4)



Task 1.8: Physician/Investigator controller

(b)(4)

(b)(4)

(b)(4)



Task 1.15: FDA IDE

(b)(4)

Figure 7: Snippet of Table of Contents
(b)(4) as submitted.

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

- Task 1.2.2: Restore bowel function (b)(4)

Induce colon contractions in 2 more cats by sacral root stimulation (b)(4)

- Task 1.4: (b)(4)

(b)(4)

(b)(4)

- Task 1.6: (b)(4)

(b)(4)

- Task 1.7: (b)(4)

First prototypes of leads delivered for test (b)(4)

- Task 1.8: (b)(4)

(b)(4)

(b)(4)

- Task 1.9: (b)(4)

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

- Task 1.12:

(b)(4)

(b)(4)

- Task 1.11: GLP animal study

(b)(4)

Schedule study and continue to finalize study details

(b)(4)

- Task 1.13: Early feasibility human clinical protocol

(b)(4)

Waiting for feedback from FDA

(b)(4)

- Task 1.15: FDA IDE

(b)(4)

Prepare for

(b)(4)



Monthly Financial Overview

(b)(4)



Administrative issues

- Nothing to report



-

(b)(4)



Figure 3: Defecation induced by stimulation of S2 ventral root in Cat #1.

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1543

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [7/1/2021] – [7/31/2021]

7/31/2021





Overview – Technical Update

- Studies to restore bowel function are completed, data are processed and analyzed, and a manuscript is written for publication.

(b)(4)

-
-
-
-

(b)(4) selected to designs. (b)(4)
made soon. (b)(4)

A material decision will be

(b)(4)

- The graphical user interface (GUI) has made additional progress and is under ongoing review with clinicians and the internal team.
- (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.



Overview – Financial Update

-

(b)(4)



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.2: Restore bowel function (b)(4)
Induce colon contractions in 2 more cats by sacral root stimulation (b)(4)
- Task 1.4: (b)(4)
(b)(4)
(b)(4)
- Task 1.6: (b)(4)
(b)(4)
- Task 1.7: (b)(4)
(b)(4)
- Task 1.8: (b)(4)
(b)(4)
(b)(4)
- Task 1.9: (b)(4)
(b)(4)



4 Week Work Plan from Previous Report

- Task 1.11: GLP animal study (b)(4)

Schedule study and continue to finalize study details (b)(4)

- Task 1.12: (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol (b)(4)

Waiting for feedback from FDA from (b)(4)

- Task 1.15: FDA IDE (b)(4)

Prepare for (b)(4)



COVID related updates

- Current MSR period – (b)(4) (7/1/2021-7/31/2021)
 - (b)(4)



Big Wins

- Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 7/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
(b)(4) 1.2.2 Restore bowel function (b)(4)	(b)(4)	(b)(4)				Milestone 1.2.2(1-4) – Deliverable 1.2.2	
1.3 Test Bed Environment (b)(4)							
Integration of (b)(4) in enclosure							
1.3.4 with firmware and (b)(4)						Milestone 1.3.4	
1.4 Implantable pulse generator with custom spiral lead for animal study (version 0) (MAC2-MAC12)							
1.4.2 (b)(4)	(b)(4)					Milestone 1.4.2	
1.4.3 (b)(4)						Milestone 1.4.3 – Deliverable 1.4	
Final (b)(4) with							
1.4.4 wireless board connectivity (b)(4)						Milestone 1.4.4 – Deliverable 1.4	
Full integration and test							
1.4.5 completed for initial prototype (MAC11)						Milestone 1.4.5 – Deliverable 1.4	
1.6 External pulse generator (b)(4)							
1.6.2 (b)(4)	(b)(4)					Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)							
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)		(b)(4)				Milestone 1.7.3 – Deliverable 1.7	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 7/31/2021

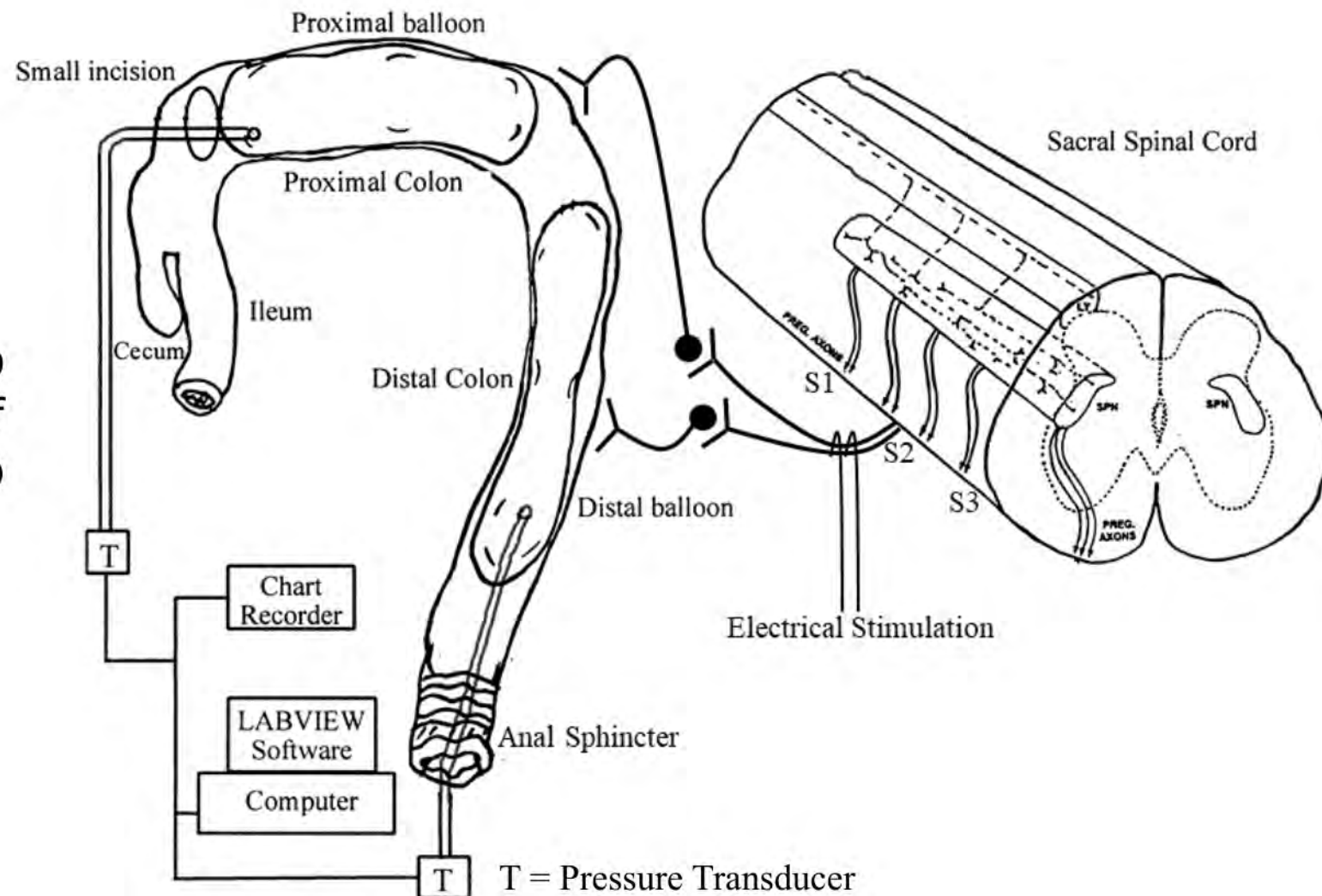
SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller (b)(4)	(b)(4)	(b)(4)					(b)(4)
1.8.2 (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
1.8.3 (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
1.9 Patient controller for (b)(4)	(b)(4)	(b)(4)					
1.9.1 (b)(4)	(b)(4)	(b)(4)				Milestone 1.9.1 – Deliverable 1.9	
1.15 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.15.FDA IDE (b)(4) 2 meeting	(b)(4)	(b)(4)				Milestone 15.2.1 – Deliverable 15.1.2	
1.12 (b)(4)	(b)(4)	(b)(4)					
1.12 (b)(4)	(b)(4)	(b)(4)				Milestone 1.12 – Deliverable 1.12	
1.13 Early feasibility human clinical protocol (b)(4)	(b)(4)	(b)(4)					
1.13 Early feasibility human clinical protocol (b)(4)	(b)(4)	(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)	(b)(4)	(b)(4)					
1.11 GLP animal study	(b)(4)	(b)(4)				Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies to restore bowel function are completed and manuscript is fully written for submission.
- (b)(4)
- (b)(4) are currently at the fabrication house being made.
- (b)(4)
-
-
- (b)(4) has made additional progress and is under ongoing review with clinicians and the internal team.
- (b)(4)
- Scheduled FDA meeting for (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.

Figure 1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce colon contractions.



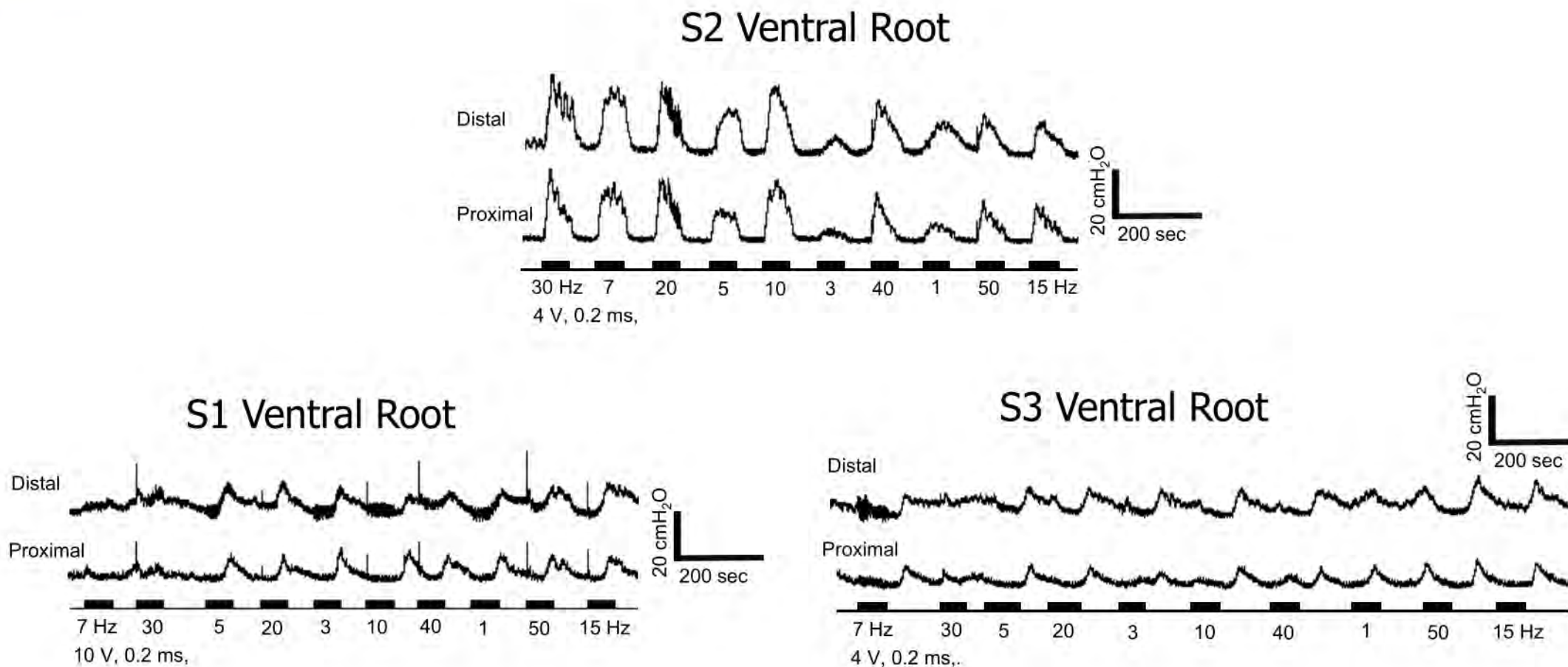


Figure 2. Colon responses to different frequency stimulation of different spinal roots.

Task 1.2.2 S2 Ventral Root at 7-10 Hz Is Most Effective

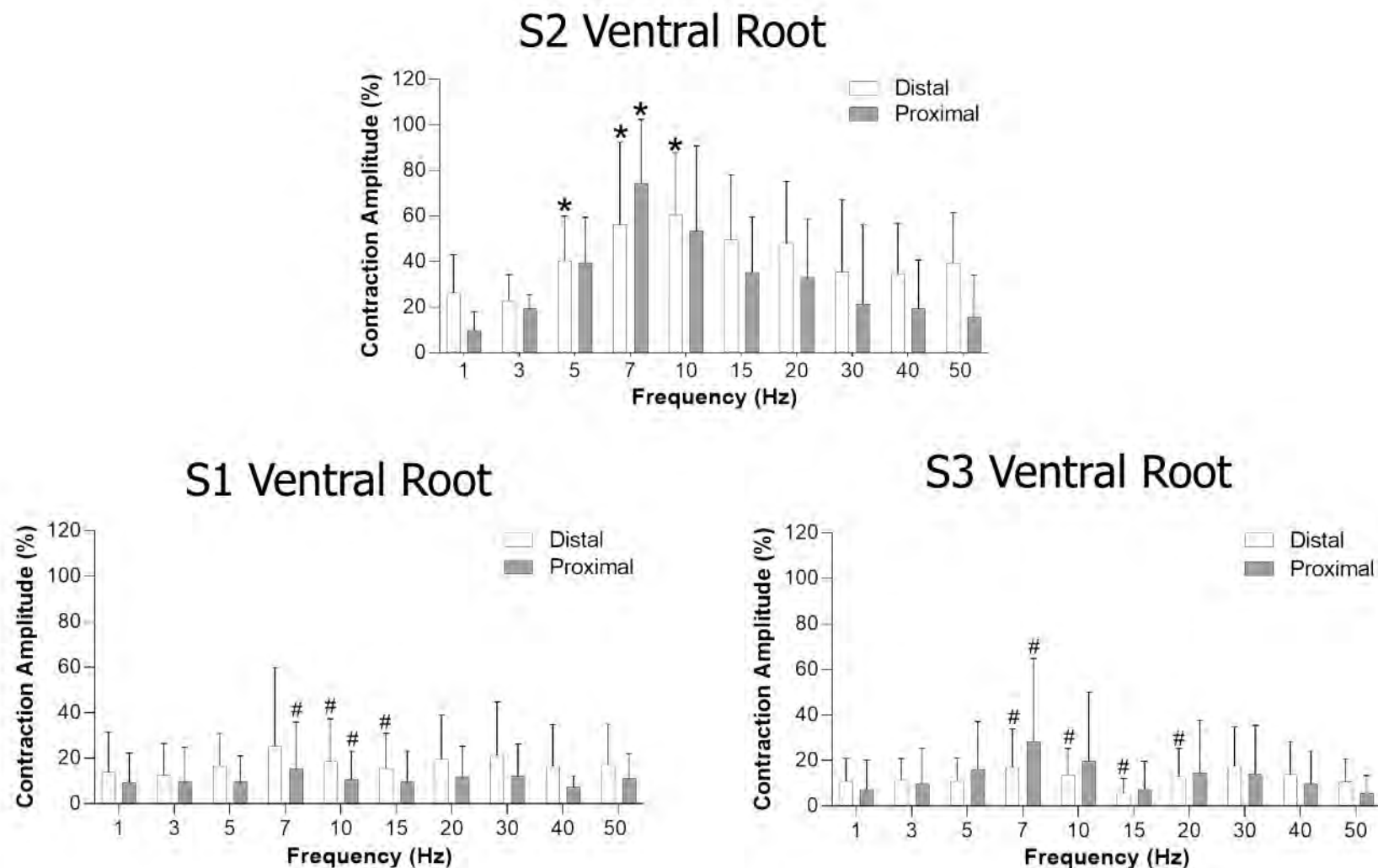
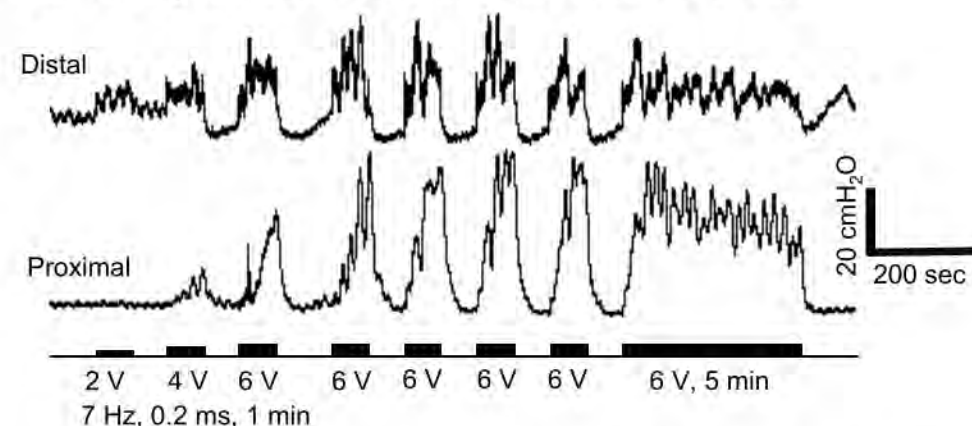


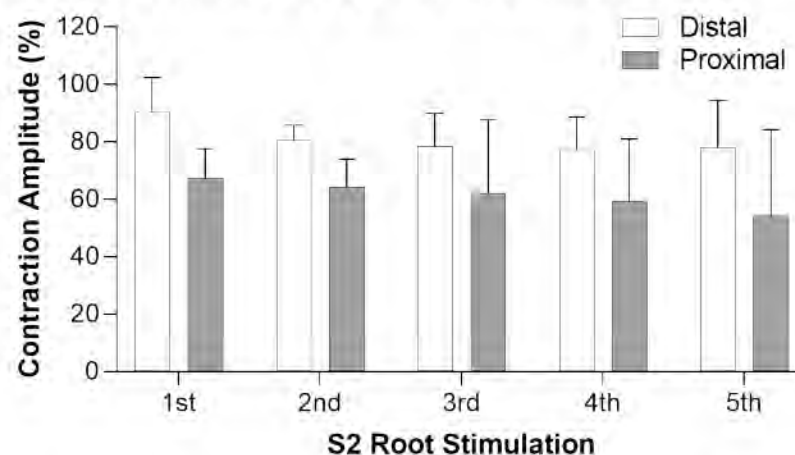
Figure 3. Summarized results showing frequency and segmental effects.

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A. S2 Spinal Root Stimulation at 7 Hz



B. Intermittent 1-min Stimulation



C. 5-min Continuous Stimulation

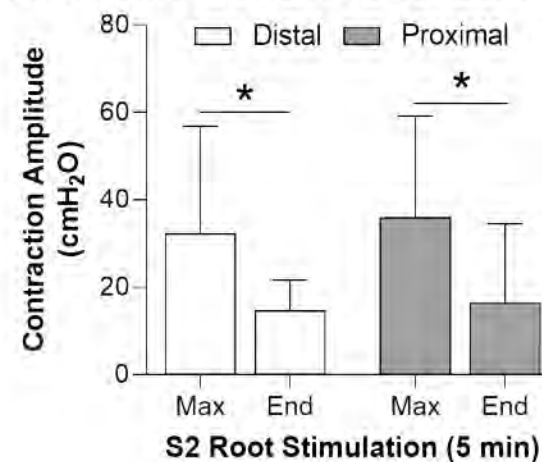


Figure 4. Effects of intermittent and continuous stimulation on colon contractions.

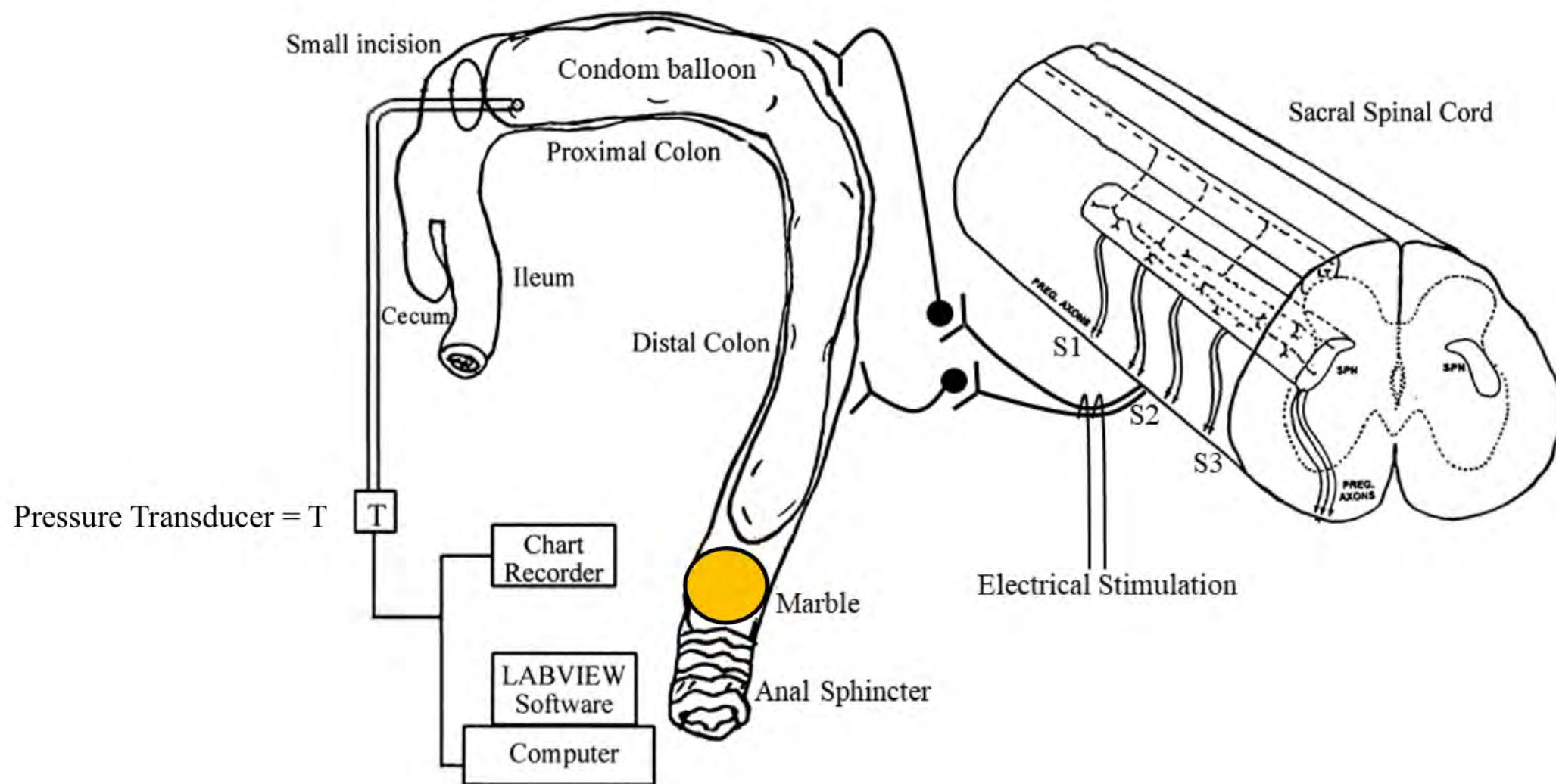
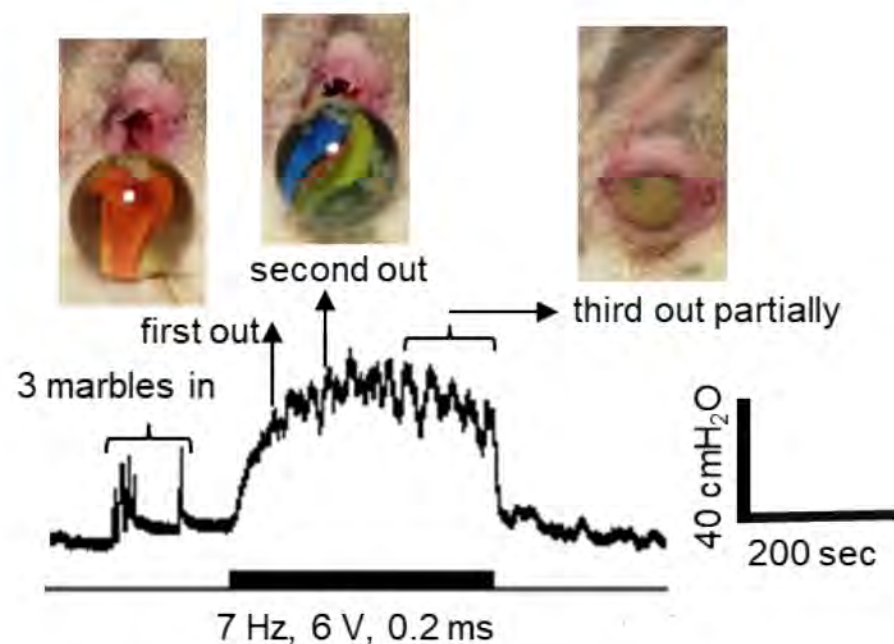


Figure 5. Experimental setup to induce defecation by sacral root stimulation.

A. Cat #1



B. Cat #2

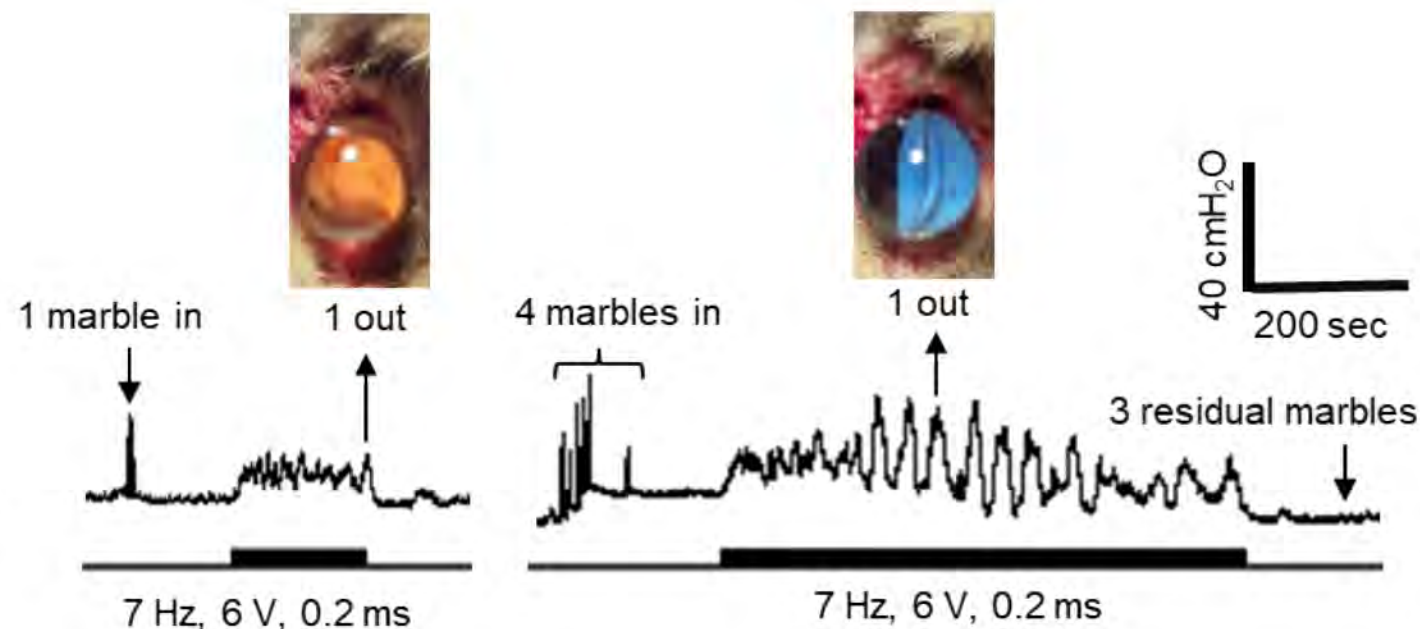


Figure 6. Defecation was successfully induced by stimulation of S2 spinal root at 7 Hz.



Defecation Induced by Stimulation of Sacral S2 Spinal Root in Cats

Jicheng Wang¹, Zhijun Shen¹, Bing Shen¹, Jianan Jian¹,
Travis Hannan¹, Khari Goosby¹, William Wang¹,
Jonathan Beckel², William C. de Groat², Christopher Chermansky¹, Changfeng Tai^{1,2,3}

¹ Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA

³ Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

⁴ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA



Task 1.12

(b)(4)

(b)(4)



(b)(4)

(b)(4)

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(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1564



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1565



Work Plan for Next 4 Weeks (b)(4)

- Task 1.2.3: Restore sexual function (b)(4)

Restoring penile erection in 2 cats by pudendal nerve stimulation (b)(4)

- Task 1.4: (b)(4)

(b)(4)

(b)(4)

- Task 1.6: (b)(4)

(b)(4)

- Task 1.7: (b)(4)

First prototypes of leads delivered for test (b)(4)

- Task 1.8: (b)(4)

Evaluation board fabricated/assembled and initial evaluation complete (b)(4)

(b)(4)

- Task 1.9: (b)(4)

(b)(4)



Work Plan for Next 4 Weeks (b)(4)

- Task 1.11: GLP animal study (b)(4)

Schedule study and continue to finalize study details (b)(4)

- Task 1.13: Early feasibility human clinical protocol (b)(4)

Waiting for feedback from FDA (b)(4)

- Task 1.15: FDA IDE (b)(4)

Conduct meeting with FDA on (b)(4)



Monthly Financial Overview

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1568



Administrative issues

- Nothing to report



- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [8/1/2021] – [8/31/2021]

8/31/2021





Overview – Technical Update

- Studies to restore sexual function are initiated. Penile erection study was performed in 2 cats
- (b)(4)
-
-
- (b)(4); (b)(8) in a cat experiment that successfully stimulated the bladder and blocked the urethra using low and high frequency stimulation paradigms
- (b)(4)
- All long lead item materials were ordered while process validation progresses in earnest. Manufacturing tools have been received and currently under evaluation and lead fabrication
- (b)(4)
- Held FDA (b)(4) meeting for IDE (b)(4)
 - (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.



Overview – Financial Update

-

(b)(4)



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.3: Restore sexual function (b)(4)
Restoring penile erection in 2 cats by pudendal nerve stimulation (b)(4)
- Task 1.4: (b)(4)
(b)(4)
(b)(4)
- Task 1.6: (b)(4)
Integration of the (b)(4) components with updated firmware and hardware (b)(4)
(b)(4)
- Task 1.7: (b)(4)
First prototypes of (b)(4) delivered for test (b)(4)
(b)(4)
- Task 1.8: Physician/Investigator controller (b)(4)
Evaluation board fabricated/assembled and initial evaluation complete (b)(4)
(b)(4)
- Task 1.9: (b)(4)
(b)(4)



4 Week Work Plan from Previous Report

- Task 1.11: GLP animal study (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol (b)(4)

Revising protocol based on feedback from FDA (b)(4)

- Task 1.15: FDA IDE (b)(4)

Conducted meeting with FDA (b)(4)



COVID related updates

- Current MSR period – (b)(4) (8/1/2021-8/31/2021)
 - (b)(4)



Big Wins

Experimental Neurology Manuscript EXNR-21-296R3: Final Decision

em.yexnr.0.75b611.01d9680e@editorialmanager.com <em.yexnr.0.75b611.01d9680e@editorialmanager.com>

on behalf of

Experimental Neurology <em@editorialmanager.com>

Wed 9/1/2021 1:31 PM

To: Tai, Changfeng <cftai@pitt.edu>

Ms. No.: EXNR-21-296R3

Title: Low Pressure Voiding Induced by Stimulation and 1 kHz Post-Stimulation Block of the Pudendal Nerves in Cats

Corresponding Author: Dr. Changfeng Tai

Authors: Jialiang Chen; Jianan Jian; Jicheng Wang; Zhijun Shen; Bing Shen; William Wang; Jonathan Beckel; William de Groat; Christopher Chermansky

Dear Dr. Tai,

We are pleased to inform you that your manuscript referenced above has been accepted for publication in Experimental Neurology.

With kind regards,

Ahmet Hoke

Editor-in-Chief

Experimental Neurology



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 8/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.3 Restore sexual function (b)(4)	(b)(4)	(b)(4)				Milestone 1.2.3(1-4) – Deliverable 1.2.3	
1.3 Test Bed Environment (b)(4)							
1.3.4 (b)(4)	(b)(4)	(b)(4)				Milestone 1.3.4	
1.4 Implantable pulse generator with (b)(4)							
1.4.3 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.3 – Deliverable 1.4	
1.4.4 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.4 – Deliverable 1.4	
1.4.5 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.5 – Deliverable 1.4	
1.6 External pulse generator for (b)(4)							
1.6.2 (b)(4)	(b)(4)	(b)(4)				Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)							
1.7.3 First prototypes of delivered for test (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.3 – Deliverable 1.7	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 8/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 (b)(4)	(b)(4)	(b)(4)					(b)(4)
1.8.2 (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
1.8.3 (b)(4)	(b)(4)	(b)(4)				Milestone 1.8.1	
1.9 Patient controller (b)(4)	(b)(4)	(b)(4)					
1.9.1 (b)(4)	(b)(4)	(b)(4)				Milestone 1.9.1 – Deliverable 1.9	
1.15 FDA IDE (b)(4)	(b)(4)	(b)(4)					
1.15. FDA IDE (b)(4)	(b)(4)	(b)(4)				Milestone 15.2.1 – Deliverable 15.1.2	
2 meeting							
1.13 Early feasibility human clinical protocol (b)(4)	(b)(4)	(b)(4)					
1.13 Early feasibility human clinical protocol (b)(4)	(b)(4)	(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)	(b)(4)	(b)(4)					
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies to restore sexual function are performed in 2 cats.

(b)(4)

[Redacted content]

(b)(4) More materials are being evaluated using these final designs (b)(4)

(b)(4)

[Redacted content]

- Held FDA (b)(4) meeting for IDE (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements

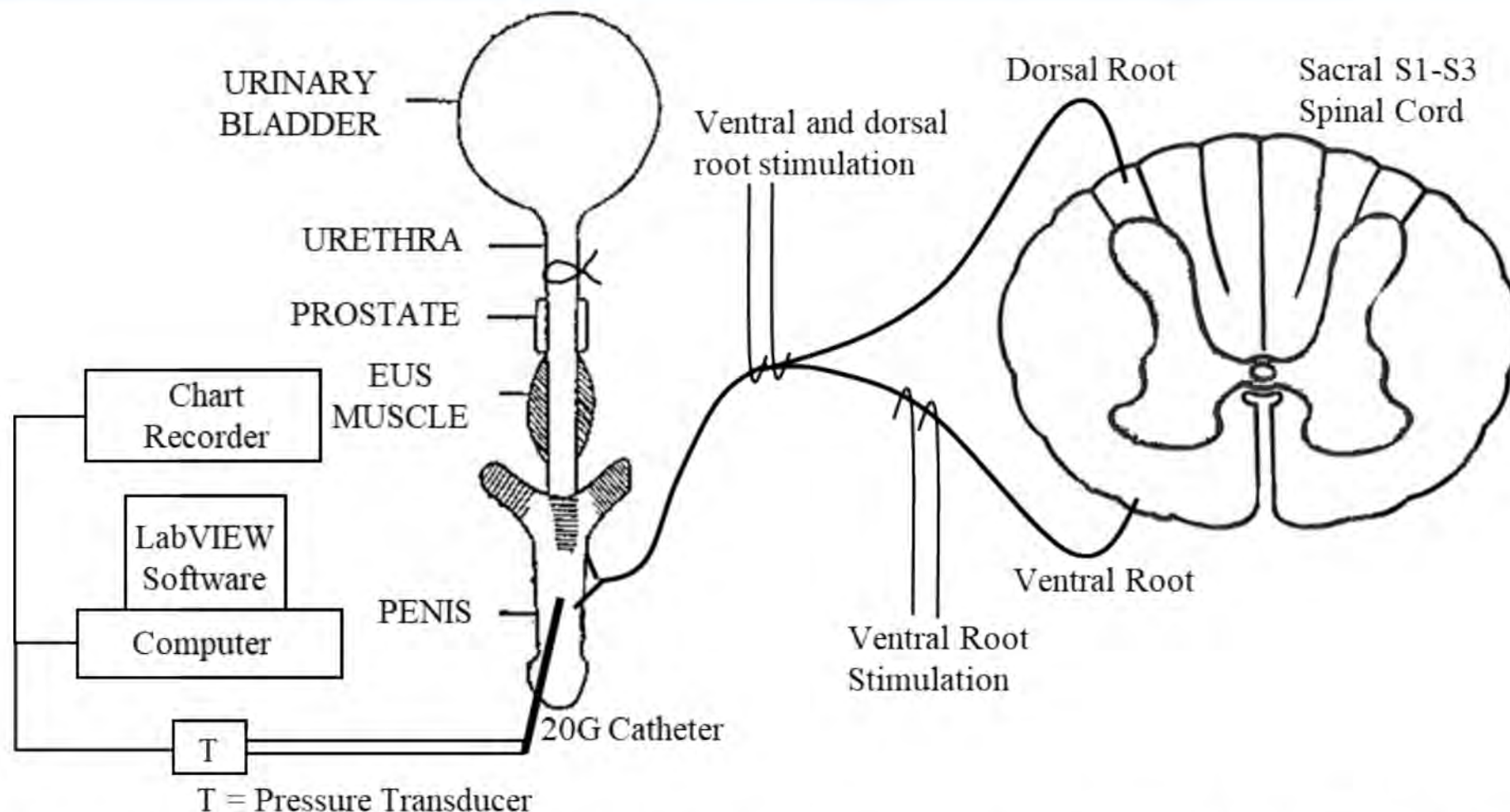


Figure 1. Experimental setup for electrical stimulation of sacral roots S1, S2 or S3 to induce penile erection.

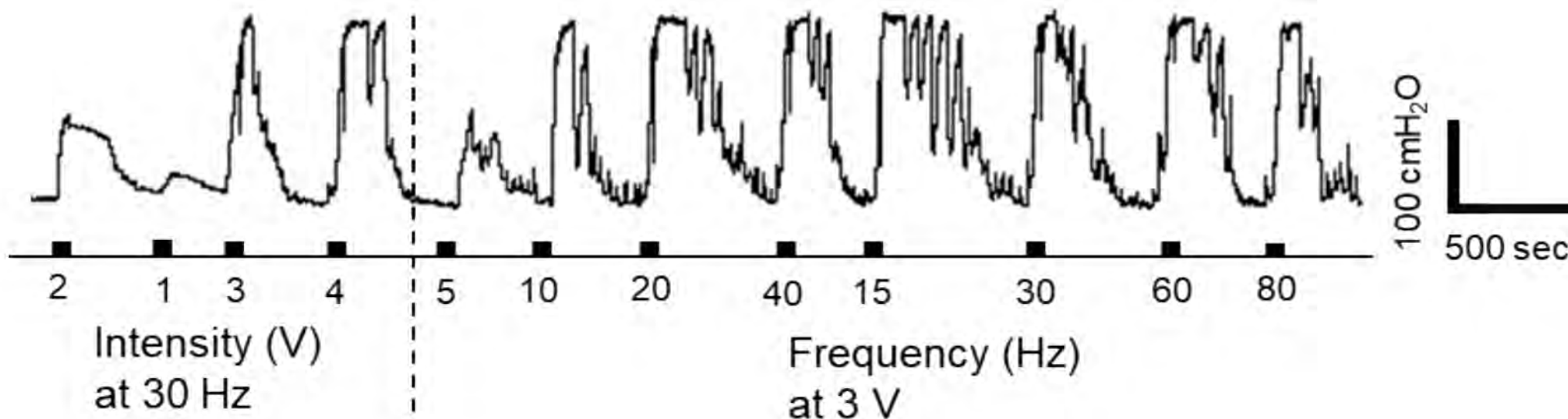


Figure 2. Penile pressure induced by S1 ventral stimulation of different frequencies.

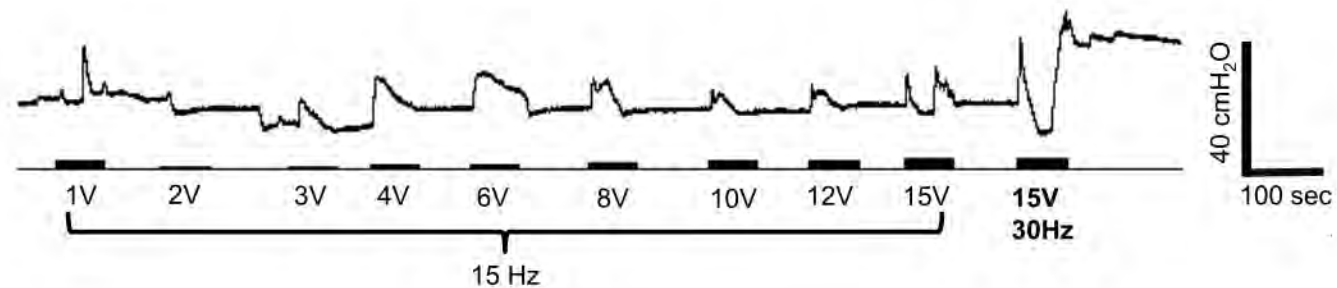


Task 1.2.3

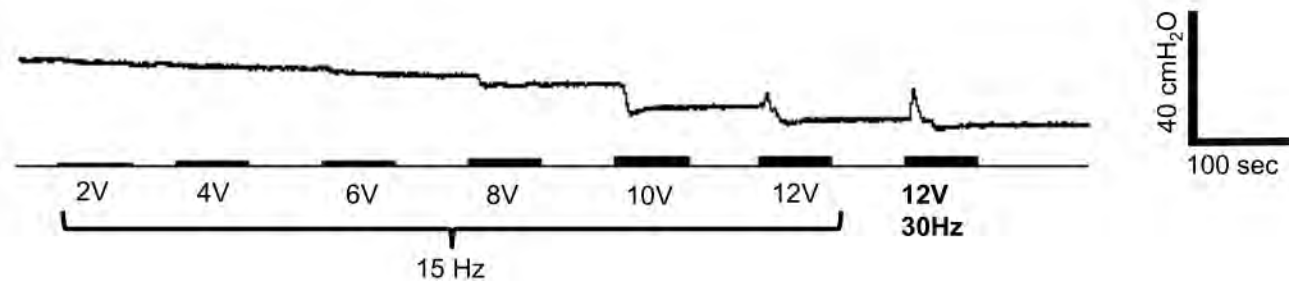
(b)(4)

of S2/S3 Ventral Root

S2 Ventral Root



S3 Ventral Root

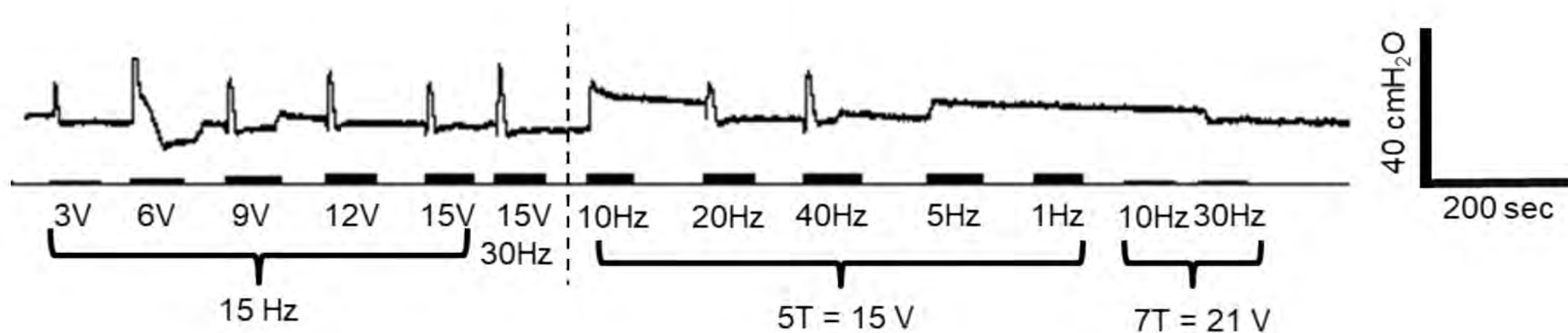


(b)(4)



Task 1.2.3

(b)(4)



(b)(4)

Task 1.2.3 Sustained Penile Response to S1 Root Stimulation

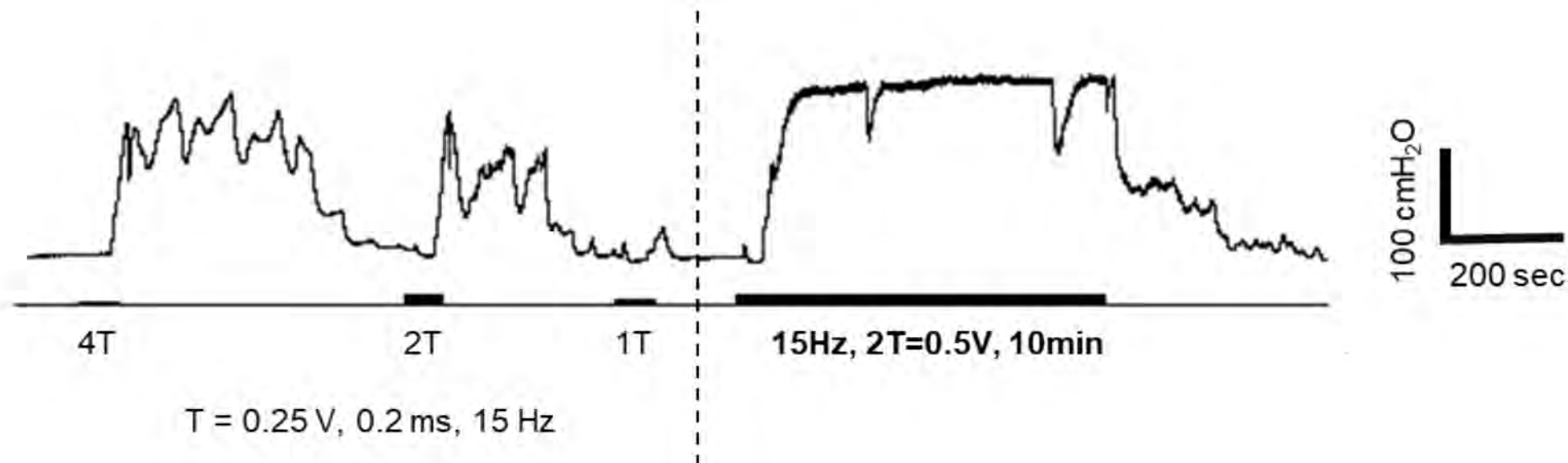


Figure 5. Stimulation of S1 root (dorsal + ventral) induced sustained increase in penile pressure lasting more than 10 minutes.

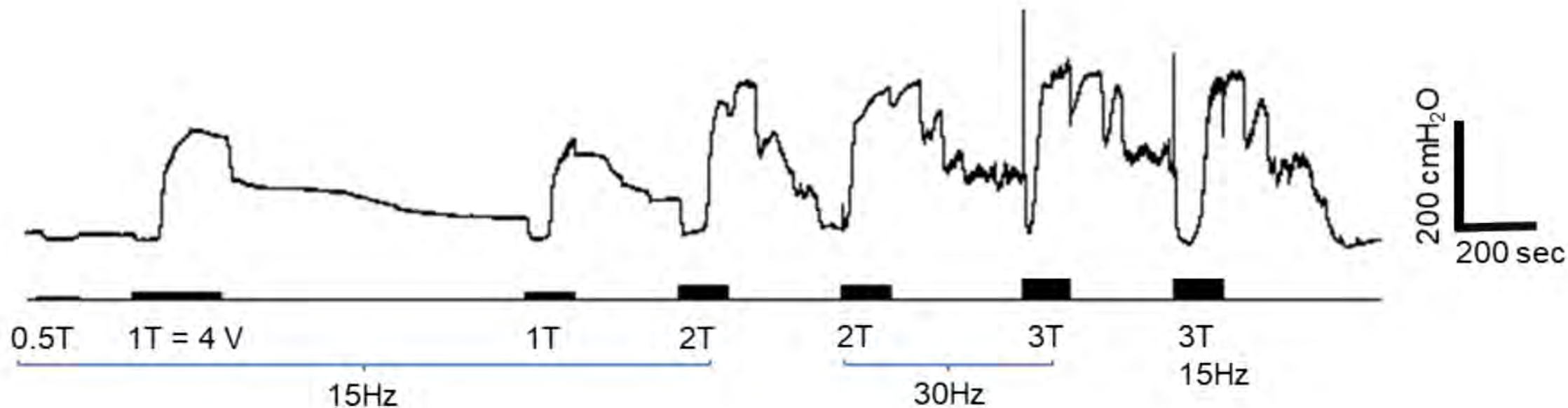


Figure 6. Penile pressure induced by S1 root stimulation after a complete transection of the spinal cord at the T9/T10 level.



(b)(4)

and component manufacturing

(b)(4)

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(b)(4)

development and manufacturing

(b)(4)

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information Act.



Task 1.8

(b)(4)

PCBA Development Process

(b)(4)

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Task 1.8

(b)(4)

PCBA and Enclosure

(b)(4)



Task 1.8 Investigator Controller

(b)(4)

(b)(4)



Task 1.9 Patient controller

(b)(4)

[Redacted content]

(b)(4)

[Large redacted content area]



(b)(4)

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1595



Work Plan for Next 4 Weeks (b)(4)

- Task 1.2.3: Restore sexual function (b)(4)

Restoring penile erection in 2 cats by S1 spinal root stimulation (b)(4)

- Task 1.4: (b)(4)

(b)(4)

(b)(4)

- Task 1.6: External pulse generator (b)(4)

(b)(4)

- Task 1.7: (b)(4)

First prototypes of (b)(4) delivered for test (b)(4)

- Task 1.8: (b)(4)

(b)(4)

(b)(4)

- Task 1.9: Patient controller (b)(4)

Firmware features complete (b)(4)



Work Plan for Next 4 Weeks

(b)(4)

- Task 1.11: GLP animal study

(b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol

(b)(4)

Incorporating feedback from FDA

(b)(4)

- Task 1.16: FDA IDE submission

(b)(4)

Begin to prepare IDE application

(b)(4)



Monthly Financial Overview

(b)(4)

WCWP v DoD, No. 23-3264 DARPA 22-F-1285 1598



Administrative issues

- Nothing to report



- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4), (b)(6)

Reporting Period: [9/1/2021] – [9/30/2021]

9/30/2021





Overview – Technical Update

- Penile erection study was performed in 2 cats. (b)(4) board was successfully tested in a cat experiment.
- (b)(4)
- The current (b)(4) schematic was reduced as much as possible with the existing components. (b)(4)
- (b)(4)
- (b)(4)
- Additional (b)(4) were conducted. (b)(4)
- (b)(4)
- The physician/investigator and patient GUIs are fully defined and completed (b)(4)
- (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.



Overview – Financial Update

- University of Pittsburgh has been distributing funds monthly to (b)(4) without any issue.



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among

(b)(4)



4 Week Work Plan from Previous Report

- Task 1.2.3: Restore sexual function (b)(4)
Restoring penile erection in 2 cats by sacral root stimulation (b)(4)
- Task 1.4: (b)(4)
(b)(4)
(b)(4)
- Task 1.6: (b)(4)
(b)(4)
- Task 1.7: (b)(4)
First prototypes of (b)(4) delivered for test (b)(4)
(b)(4)
- Task 1.8: (b)(4)
(b)(4) and initial evaluation complete (b)(4)
(b)(4)
(b)(4)
- Task 1.9: Patient controller for (b)(4)
(b)(4)



4 Week Work Plan from Previous Report

- Task 1.11: GLP animal study (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol (b)(4)

Revising protocol based on feedback from FDA (b)(4)

- Task 1.16: FDA IDE submission (b)(4)

Begin to prepare IDE application for (b)(4)



COVID related updates

- Current MSR period – (b)(4) (9/1/2021-9/30/2021)
 - (b)(4)



Big Wins

Nothing to report.



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 9/30/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.2 Animal studies to develop the external system and the implantable system (b)(4)							(b)(4)
1.2.3 Restore sexual function (b)(4)	(b)(4)	(b)(4)				Milestone 1.2.3(1-4) – Deliverable 1.2.3	
1.3 Test Bed Environment (b)(4)							
1.3.4 (b)(4)	(b)(4)	(b)(4)				Milestone 1.3.4	
1.4 (b)(4)							
1.4.3 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.3 – Deliverable 1.4	
1.4.4 (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.4 – Deliverable 1.4	
1.4.5 Full integration and test completed for initial prototype (b)(4)	(b)(4)	(b)(4)				Milestone 1.4.5 – Deliverable 1.4	
1.6 External pulse generator (b)(4)	(b)(4)	(b)(4)					
1.6.2 (b)(4)	(b)(4)	(b)(4)				Milestone 1.6.2	
1.7 (b)(4) electrodes for human use (b)(4)							
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)	(b)(4)				Milestone 1.7.3 – Deliverable 1.7	



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 9/30/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller (b)(4)							(b)(4)
1.8.2 (b)(4)		(b)(4)				Milestone 1.8.1	
1.8.3 (b)(4)						Milestone 1.8.1	
1.9 Patient controller (b)(4)							
1.9.1 (b)(4)		(b)(4)				Milestone 1.9.1 – Deliverable 1.9	
1.9.2 Verification testing completed (b)(4)						Milestone 1.9.2 – Deliverable 1.9	
1.16 FDA IDE Submission (b)(4)							
1.16 FDA IDE application for the (b)(4) is submitted		(b)(4)				Milestone 1.16 – Deliverable 1.16	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)		(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies to restore sexual function are performed in 2 cats and the (b)(4) board is successfully tested in 1 cat.
- (b)(4)
- The (b)(4) boards have firmware and can be controlled (b)(4). The current (b)(4) schematic was reduced as much as possible with the existing components. A new (b)(4) enclosure size was designed to fit the smaller (b)(4) board size (b)(4).
- With new and smaller components, the smaller (b)(4) schematic has begun to be reduced even further to (b)(4) size. New quotes were requested for (b)(4) IPG cans from additional suppliers.
- (b)(4)
- Additional (b)(4) characterization studies were conducted. More fixtures and tools were created to aid in the construction (b)(4) manufacturing processes have continued to be validated.
- The physician/investigator and patient GUIs are fully defined and completed (b)(4)
- (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.

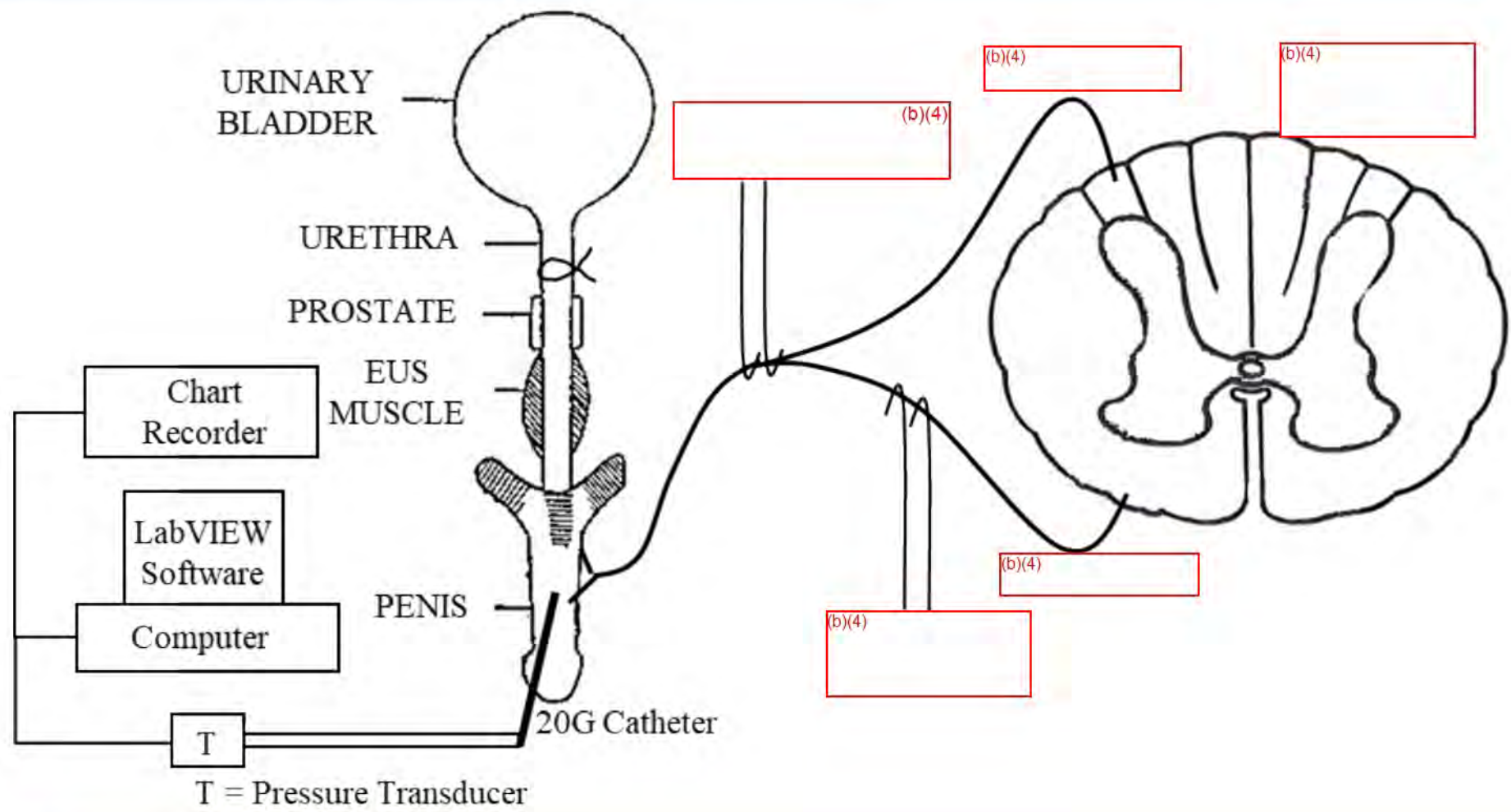


Figure 1.

(b)(4)

(b)(4)



Task 1.2.3

(b)(4)

(b)(4)



Task 1.2.3

(b)(4)

(b)(4)



Testing the (b)(4) board in a cat experiment



Figure 4. The (b)(4) board successfully generated the required stimulation signals for bladder inhibition or excitation, and for (b)(4) nerve block.



(b)(4)

(b)(4)



Task 1.4.3 Integrate pulse generator circuit into can

(b)(4)

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Regulatory Strategy for (b)(4)

Figure 8

(b)(4)

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Safety Limits

(b)(4)

Figure 9

(b)(4)



Thermaquil EFS Stimulation Parameters

(b)(4)

Figure 10

(b)(4)



Work Plan for Next 4 Weeks

(b)(4)

- Task 1.2.3: Restore sexual function (b)(4)

Restoring penile erection in 2 cats by (b)(4) stimulation (b)(4)

- Task 1.4: Implantable pulse generator with (b)(4)

Integrate pulse generator circuit into can (b)(4)

Final PC test application with wireless board connectivity (b)(4)

Full integration and test completed for initial prototype (b)(4)

- Task 1.7: (b)(4) for human use (b)(4)

First prototypes of leads delivered for test (b)(4)

- Task 1.8: Physician/Investigator controller (b)(4)

Evaluation board fabricated/assembled and initial evaluation complete (b)(4)

Integration with a simple enclosure with display and firmware (b)(4)

- Task 1.9: Patient controller (b)(4)

Verification testing completed (b)(4)



Work Plan for Next 4 Weeks (b)(4)

- Task 1.11: GLP animal study (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol (b)(4)

Incorporating feedback from FDA from (b)(4)

- Task 1.16: FDA IDE submission (b)(4)

Continue to prepare IDE application (b)(4)



Monthly Financial Overview

(b)(4)

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Administrative issues

- Nothing to report



- Nothing to report

University of Pittsburgh BG+ Monthly Status Report

(b)(4)

(b)(4); (b)(6)

Reporting Period: [10/1/2021] – [10/31/2021]

10/31/2021





Overview – Technical Update

- Penile erection study was performed in 2 cats. (b)(4) board was successfully tested in 2nd cat experiment.
- The association of GUI commands with (b)(4) commands has been completed.
- The current (b)(4) schematic continued to be reduced to a smaller (b)(4). As the new/smaller components were identified, the smaller (b)(4) schematic has continued to be reduced even further to (b)(4) size.
- New quotes were received for (b)(4) cans from a (b)(4).
- (b)(4)
- Additional (b)(4) characterization studies were conducted.
 - It was determined that (b)(4)
 - Materials have been purchased to develop (b)(4) out of both material possibilities.
 - (b)(4)
- IQs PQs and OQs were conducted to verify the (b)(4) manufacturing processes.
 - Additional measuring equipment may be needed to accurately measure (b)(4) (b)(4) repeatedly.
- Thermaquil is drafting IDE (b)(4)
- Clinical protocol has been updated in response to FDA feedback (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.



Overview – Financial Update

- University of Pittsburgh has been distributing funds monthly to (b)(4) and Thermaquil without any issue.



Overview – Administrative Update

- Administrative/progress meetings and design meetings are hold weekly to coordinate the activity among University of Pittsburgh, (b)(4) and Thermaquil.



4 Week Work Plan from Previous Report

- Task 1.2.3: Restore sexual function (b)(4)
Restoring penile erection in 2 cats by (b)(4) stimulation (b)(4)
- Task 1.4: Implantable pulse generator with (b)(4)
Integrate pulse generator circuit into can (b)(4)
Final PC test application with wireless board connectivity (b)(4)
Full integration and test completed for initial prototype (b)(4)
- Task 1.7: (b)(4) electrodes for human use (b)(4)
First prototypes of (b)(4) delivered for test (b)(4)
(b)(4)
- Task 1.8: Physician/Investigator controller for (b)(4)
Evaluation board fabricated/assembled and initial evaluation complete (b)(4)
(b)(4)
Integration with a simple enclosure with display and firmware (b)(4)
- Task 1.9: Patient controller (b)(4)
Verification testing completed (b)(4)



4 Week Work Plan from Previous Report

- Task 1.11: GLP animal study (b)(4)

FDA clarified their position (b)(4)

(b)(4)

- Task 1.13: Early feasibility human clinical protocol (b)(4)

Incorporating feedback from FDA (b)(4)

- Task 1.16: FDA IDE submission (b)(4)

Continue to prepare IDE application for (b)(4)



COVID related updates

- Current MSR period – (b)(4) (10/1/2021-10/31/2021)
 - (b)(4)



Big Wins

(b)(4)

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Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 10/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
(b)(4) 1.2 Animal studies to develop the external system and the implantable system (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.2.3 Restore sexual function (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.2.3(1-4) – Deliverable 1.2.3	(b)(4)
(b)(4) 1.3 Test Bed Environment (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
Integration of (b)(4) in enclosure	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.3.4 with firmware and (b)(4) connectivity (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.3.4	(b)(4)
1.4 Implantable pulse generator with (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.4.3 Integrate pulse generator circuit into can (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.4.3 – Deliverable 1.4	(b)(4)
Final PC test application with	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.4.4 wireless board connectivity (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.4.4 – Deliverable 1.4	(b)(4)
Full integration and test	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.4.5 completed for initial prototype (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.4.5 – Deliverable 1.4	(b)(4)
1.6 External pulse generator (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
Integration of the external pulse generator components with	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.6.2 firmware and the enclosure (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.6.2	(b)(4)
(b)(4) 1.7 (b)(4) electrodes for human use (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
1.7.3 First prototypes of (b)(4) delivered for test (b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	Milestone 1.7.3 – Deliverable 1.7	(b)(4)



Milestones and Deliverables Summary

[University of Pittsburgh] Active Task Status – Past Month (b)(4)

Date: 10/31/2021

SOW Task #	Planned Start	Actual Start	Due Date	Finish	Status (%)	Associated Milestone and Deliverable	Issues
1.8 Physician/Investigator controller (b)(4)							(b)(4)
Evaluation board							
1.8.2 fabricated/assembled and initial evaluation complete (b)(4)		(b)(4)				Milestone 1.8.1	
Integration with a simple							
1.8.3 enclosure with display and firmware (b)(4)						Milestone 1.8.1	
1.9 Patient controller (b)(4)							
1.9.2 Verification testing completed (b)(4)		(b)(4)				Milestone 1.9.2 – Deliverable 1.9	
1.16 FDA IDE (b)(4)							
1.16 FDA IDE application for the (b)(4) is submitted		(b)(4)				Milestone 1.16 – Deliverable 1.16	
1.13 Early feasibility human clinical protocol for (b)(4)							
1.13 Early feasibility human clinical protocol for (b)(4)		(b)(4)				Milestone 1.13 – Deliverable 1.13	
1.11 GLP animal study (b)(4)							
1.11 GLP animal study						Milestone 1.11 – Deliverable 1.11	



Technical Progress Summary

- Animal studies to restore sexual function are performed in 2 cats and the (b)(4) board is successfully tested in 2nd cat.
- (b)(4)
- (b)(4)
- The (b)(4) schematic was updated with smaller components and further arranged into the (b)(4) layout.
- The GUI application is undergoing wireless testing with the (b)(4) hardware.
- The integration of the (b)(4) sized PCB into an (b)(4) will be completed once can samples arrive and the boards have been respun (b)(4)
- (b)(4) manufacturing processes are currently under validations (IQ / OQ and PQ) and testing. Additional measuring equipment may be needed to validate (b)(4) sizes.
- (b)(4) The (b)(4) GUI and firmware will be loaded onto the controller as soon as the (b)(4) GUI has been converted to (b)(4)
- Thermaquil is incorporating specific FDA feedback in IDE, clinical trial protocol, informed consent, and (b)(4)
- Continued identifying user needs and voice of customer to inform device requirements.

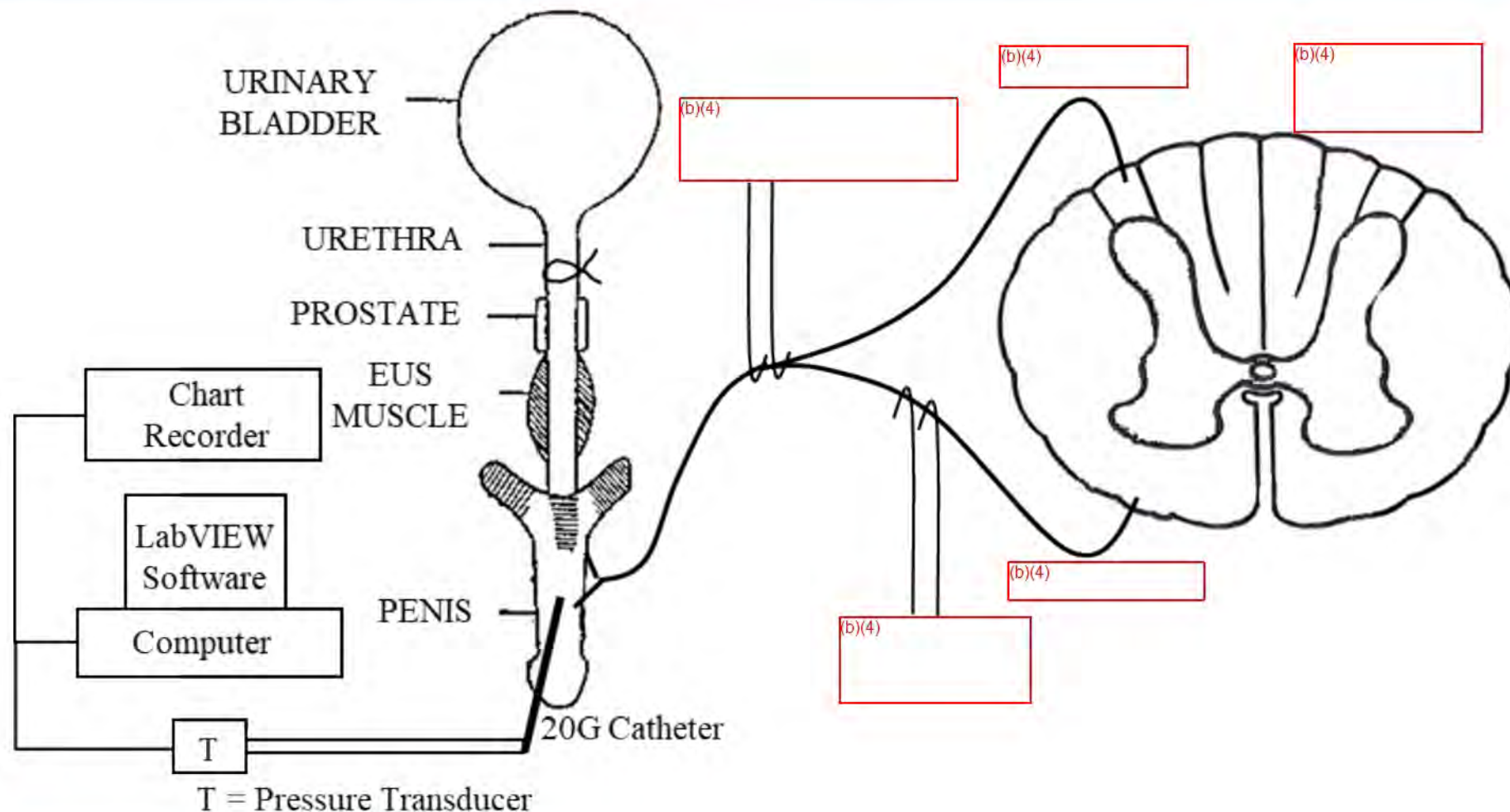


Figure 1.

(b)(4)

(b)(4)



Task 1.2.3

(b)(4)

(b)(4)



Task 1.2.3 Sustained Penile Response to (b)(4) Stimulation





Testing the (b)(4) board in the 2nd cat experiment



Figure 4. The (b)(4) board successfully generated the required stimulation signals for bladder inhibition or excitation, and for (b)(4) nerve block.



(b)(4)

(b)(4)

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Task 1.13: Early feasibility human clinical protocol for

(b)(4)

(b)(4)