Non-continuous neuromodulation in awake, unrestrained felines increases bladder capacity

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Abstract

Objective. Neuromodulation is a standard therapy for bladder symptoms such as overactive bladder. Previous studies have demonstrated that non-continuous stimulation can increase bladder capacity, and that bladder pressure can be estimated from dorsal root ganglia (DRG) neural activity in anesthetized animal models. Our goal is to determine if non-continuous neuromodulation elicits similar bladder capacity effects as continuous neuromodulation and if bladder pressure can be estimated from DRG signals in an awake, unrestrained animal model. *Approach.* We performed aseptic, chronic implant surgeries with seven adult, male felines. Three animals were used to establish procedures, three for experimental testing, and one did not yield data. Bipolar stimulating electrodes were placed on the ipsilateral pudendal nerve and sacral nerve. Microelectrode arrays were inserted in two ipsilateral sacral DRG. Two single-lumen catheters were implanted in the bladder dome for recording bladder pressure and infusing saline. Fixed-sequence, repeated bladder fills were performed in three awake test felines to evaluate the bladder capacity during no-stimulation, non-continuous stimulation, and continuous stimulation at either the pudendal or sacral nerve. Non-continuous stimulation was performed based on increases in bladder pressure estimated from DRG recordings or when 50% of the average no-stimulation bladder capacity was reached. *Main results.* We observed similar bladder capacity increases for non-continuous stimulation (122 ± 32% of no-stimulation control) as for continuous stimulation (121 ± 33%) in the three test animals. Non-continuous stimulation paradigms reduced stimulation time by 44% on average. Median correlation coefficients of 0.46 and 0.64 (maximum 0.93) between neural activity and bladder pressure were obtained for awake trials with bladder units in two animals. *Significance.* This study demonstrated the feasibility of using non-continuous neuromodulation to increase bladder capacity in awake, unrestrained felines and for decoding bladder pressure from DRG recordings. Further studies are needed to optimize non-continuous stimulation timing for clinical translation.

Keywords: bladder, closed-loop, neuromodulation, dorsal root ganglia, pudendal nerve

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1. Introduction

Overactive bladder (OAB) is a common dysfunction that affects women and men [1]. The prevalent symptom is urinary urgency sensation with or without incontinence, even when urine volume is low. The first line of treatment for OAB typically consists of behavioral changes such as pelvic floor muscle training and fluid intake modification [2]. Anticholinergic and β-adrenergic drugs are standard second-line treatments. However, many patients with OAB discontinue these treatments within one year due to poor efficacy or side effects [3]. Clinicians may offer chemodenervation via intradetrusor injection of OnabotulinumtoxinA (Botox), posterior tibial nerve stimulation (PTNS), or sacral neuromodulation (SNM) as third-line treatments [4].

SNM has a greater reduction in urinary incontinence episodes and voiding frequency, as compared to PTNS and Botox, while Botox is associated with a higher risk of urinary tract infections and urinary retention [5], [6]. In SNM, a four-contact stimulation lead is placed by a sacral nerve, typically S3. This spinal level includes primary innervation of the pelvic viscera and floor, including the bladder, urethral sphincter, and pelvic floor muscles, via the pelvic and pudendal nerves. Distal neuromodulation directly at the pudendal nerve, which is comprised of fibers from sacral nerves S2-S4, has also been studied for the treatment of OAB and other bladder dysfunctions [7], [8].

Some SNM patients have a relapse of symptoms or efficacy loss after a few years [9]. The default setting for the SNM device is continuous stimulation at 14 Hz [10]. In other applications, prolonged nerve exposure to continuous stimulation can induce habituation [11]. Sacral nerve habituation may contribute to unsuccessful SNM outcomes, though it has not been proven [12]. Clinical studies have demonstrated that cycling SNM (e.g., 16 seconds on and 8 seconds off) provides similar therapeutic efficacy to continuous SNM [10], suggesting that continuous SNM may not be necessary to elicit equivalent therapy efficacy. Pre-clinical and clinical studies have demonstrated that triggering pudendal or sacral stimulation on a specific part of the bladder filling cycle or during detrusor activity can increase bladder capacity [13], [14], [15], [16]. This prior work suggests that stimulation that takes into consideration the bladder state could be a promising next step for the therapy and may prolong clinical outcomes by stimulating only when needed. However, further studies are required to compare the efficacy of continuous stimulation with that of bladder activity-related closed-loop stimulation.

Implementing a closed-loop system involves new challenges: identifying reliable biomarkers and developing algorithms to control stimulus timing. One potential biomarker are bladder sensory signals that can be observed with microelectrodes at sacral-level dorsal root ganglia (DRG) [17], [18]. We previously demonstrated that DRG signals can be used to estimate bladder pressure [13], [19] and act as a biomarker for closed-loop neuromodulation in anesthetized settings [13], [14]. In typical anesthetized studies, stimulation intensity is delivered at one to six times the lowest intensity that evokes motor responses [20]. Intensities above the motor threshold are not normally used clinically, as subsensory stimulation is preferred [10], [21], and can be as effective as suprasensory stimulation [22]. Moving beyond anesthetized studies and using clinically relevant stimulation is critical for translating neuromodulation therapies.

The primary objective of this study was to compare the effects of non-continuous and continuous neuromodulation on the bladder in awake, unrestrained felines using clinically-relevant stimulation intensities. The secondary objective was to estimate bladder pressure in real-time from sacral-level DRG recordings to trigger closed-loop stimulation in awake felines. We hypothesized that non-continuous stimulation would yield similar bladder capacity increases as continuous stimulation but would significantly reduce the applied stimulation time, as has been observed under anesthesia [14]. We performed sequential bladder fills during continuous, non-continuous, or no stimulation in a set of awake, unrestrained test felines. Non-continuous stimulation was performed when non-voiding contractions were detected using neural activity from the DRG or on a fixed timing at the latter part of the bladder fill cycle. We also studied the effect of stimulation on peak bladder pressure, bladder compliance, and voiding efficiency. Finally, we looked for a potential carry-over effect of stimulation between experimental sessions based on litterbox activity.

2. Methods

2.1 Animals

All animal care and experimental procedures were approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC), following the National Institute of Health's guidelines for the care and use of laboratory animals. Procedures were conducted in 7 purpose-bred adult, neurologically-intact male felines (1.29 ± 0.19 years old, 4.80 ± 0.57 kg at surgery). Shortly after implant recovery, Animal 7 had a health issue and subsequently was removed from the study. Animals were free-range housed in a 6.7 m by 6.4 m room with controlled temperature (19-21 °C) and relative humidity (35-60%), food and water available ad-lib, and a 12-hour light/dark cycle. Animals received enrichment via staff interaction and toys.

2.2 Surgical procedure

For each implant surgery, anesthesia was induced by an intramuscular administration of a mixture of Ketamine (6.6 mg/kg), Butorphanol (0.66 mg/kg), and Dexmedetomidine (0.011 mg/kg). Animals were intubated and transitioned to 1-4% isoflurane anesthesia during surgical procedures. Heart rate, O2 perfusion, temperature, and blood pressure were monitored, and an intravenous line was placed for saline infusion (10-20 ml/hr). A laparotomy was performed to insert two 1-mm inner diameter Silastic catheters into the dome of the bladder, which were secured with a purse-string suture and tunneled to a midline incision in the lower back. A postero-lateral incision was used to access the left pudendal nerve to place a bipolar cuff electrode (2-mm diameter, 2-2.5 mm wire separation). The electrode wires (32 AWG-AS 636, Cooner Wire, Chatsworth, CA) were also tunneled

to the back incision. The skin and muscle were reflected in layers at the back incision to expose the L7-S3 vertebrae. The dorsal process and lamina of the S1 and S2 vertebrae were removed to expose the DRG. These spinal levels are equivalent to S2 and S3 in humans and are common SNM targets in feline studies [14], [23]. Wire electrodes (32 AWG-AS 636, Cooner Wire, Chatsworth, CA) were secured in place on the top of the right S1 spinal nerve just distal to the DRG for SNM with stay sutures secured to nearby muscle tissue. Microelectrodes (4x8 layout, IrOx 1 mm shanks, Blackrock Microsystems, Salt Lake City, UT) were inserted with a pneumatic inserter in S1 (left side in all Animals) and S2 (left side in Animals 1-2, 4-7; right in Animal 3) DRG. A four-post stainless-steel base plate was secured to the iliac crests below the skin, with the posts inserted through the skin. A 3D-printed chamber, or backpack, was attached to the baseplate posts and used to hold catheter ports and electrode connectors within secured locations [24], [25]. After completing all surgical steps, standard post-op care and analgesia were provided while the animal recovered. Experimental testing was not performed for at least one week after surgery.

2.3 Experiment setup and data collection

Bladder pressure was monitored with pressure transducers (DPT-100, Utah Medical Products, Midvale, UT) connected to each implanted catheter and an amplifier (TBM4M, World Precision Instruments, Sarasota, FL). The bladder pressure signal was recorded at 1 kHz, using a Grapevine Neural Interface Processer (NIP; Ripple, Salt Lake City, UT). Neural data from the microelectrodes were sampled at 30 kHz and band-passed filtered (250 Hz to 7.5 kHz) with the NIP and Grapevine Trellis software. During each bladder infusion trial, 0.9% warm saline (36°C) was infused (2 or 5 mL/min) into the bladder with a syringe pump (AS50 Infusion Pump, Baxter International, Deerfield, IL, or Model NE-1000, New Era Pump Systems, Inc., Farmingdale, NY). The stimulation electrodes were connected to a pulse stimulator (Model 2100 Isolated Pulse Stimulator, A-M Systems, Sequim, WA).

Unsorted DRG spikes were used for bladder pressure estimation for closed-loop trials. The spikes were detected using a dual-threshold crossing at 3–5.5 times the root-mean-square value of the signal on each microelectrode channel. The pressure signal from the non-infusion catheter and neural spike times were streamed to a previously-developed online Kalman filter decoder in a MATLAB Graphic User Interface (MathWorks, Natick, MA) [13], [14]. If the non-infusion catheter failed, the pressure signal from the infusion catheter was used. First, the decoder was trained on a no-stimulation bladder infusion, specific to the animal and the day of the experiment. The firing rates of threshold-crossings on each channel were calculated using a 2-second window average and each was correlated to the 2-second window average of the bladder pressure. The Kalman filter was trained using channels with a firing rate correlation coefficient (CC) to pressure greater than a threshold of 0.7. If no channel had a CC greater than 0.7, the threshold was decreased in 0.1 steps until a channel met the criteria. If no channel had a CC greater than 0.2, closed-loop stimulation was not performed. The Kalman filter generated a pressure estimate every 2 seconds. Nerve stimulation was triggered when the estimated pressure surpassed a pressure change threshold during a set time window. Stimulation was ceased after a set period. The pressure change threshold, time window, and stimulation period were determined per animal.

2.4 Experimental sessions

The first three animals (Animals 1-3) were used to establish the experimental procedures. Animals 1 and 2 were primarily used to test the decoding algorithms in sedated test sessions. Animal 3 was mainly used to establish awake logistics and procedures for repeated test sessions. Finally, Animals 4 through 6 were used for repeated neuromodulation sessions to evaluate the bladder capacity during no-stimulation, non-continuous stimulation, and continuous stimulation at either the pudendal or sacral nerve.

2.4.1 Sedated test sessions

Animals were sedated at regular intervals (once every 1-2 weeks). Dexmedetomidine IM (0.01-0.04 mg/kg) was used to sedate the animals prior to maintenance with a continuous intravenous infusion of Alfaxalone (24-250 μg/kg/min) [26] until the end of testing. The primary goal of sedated test session was to evaluate stimulation electrode and DRG recording microelectrode functionality prior to awake testing. Motor thresholds (MT) for the sacral and/or pudendal nerve electrodes were obtained at 1 Hz and 210 μs pulse width, and strength-duration curves with other pulse widths were obtained in some sessions. Impedances at 1 kHz were measured for DRG microelectrodes. During some sedated test sessions, bladder fills (2 or 5 ml/min) were performed for evaluating the effect of nerve stimulation on the bladder capacity, and to assess DRG neural signals and train a bladder pressure decoding algorithm for closed-loop neuromodulation. The MT was defined as the lowest stimulation amplitude that induced anal sphincter twitching. Additional sedation sessions were performed for backpack inspection and cleaning as needed.

2.4.2 Awake, unrestrained test sessions

For an awake, unrestrained test session, the bladder catheters and electrodes were either connected while awake or during an immediately preceding sedation session. Testing for awake sessions were completed in a clear-walled 40 x 70 x 50 cm plexiglass chamber (Figure 1). The chamber allowed felines to freely move around in a controlled space, with cables draped outside the chamber and a scale underneath to collect urine voided through the meshed floor. Awake, unrestrained test sessions were only completed if the animal did not show anxiety and continued to engage with treats, toys, or wet food after being connected.

2.4.3 Preparatory test sessions

Across animals, we developed procedures to habituate animals to the testing chamber and experimental paradigm (Supplement Part 1). In Animal 2, various SNM patterns were applied during sedated and awake sessions for examining an animal's response to the paradigms. In Animal 3, continuous sacral and pudendal neuromodulation trials at or above MT were performed during test sessions which had concurrent bladder filling. Real-time estimation of bladder pressure from DRG recordings in different experimental conditions was also performed in Animal 3 to establish optimal stimulation and recording conditions for the following animals. In Supplement Part 1 we discuss some lessons learned from these experiments.

2.4.4 Awake repeated neuromodulation sessions

In Animals 4-6, at least seven awake, unrestrained test sessions were performed for each animal. Each session consisted of at least three sequential bladder fills for each of three stimulation paradigms: no stimulation, non-continuous stimulation, and continuous stimulation. The per-session objective was to first perform bladder fills with no stimulation to train the DRG-signal decoder prior to performing bladder fills with closed-loop and then continuous stimulation. A fixed-order, non-randomized fill sequence was used to address potential stimulation carry-over effect on subsequent periods and a cumulative effect of stimulation. Due to failures of the DRG microelectrodes for some animals, non-continuous stimulation was performed in two paradigms: using neural signals to perform closed-loop stimulation (Animal 4) or by initiating stimulation when the bladder reached an average of half capacity of the no-stimulation fills (Animals 5 and 6). In closed-loop trials, nerve stimulation was applied for 15 seconds when the estimated pressure increased at least 5 cmH_2O in a 4 second window. Stimulation was applied at 5 Hz and 1 MT, with a 210 μs pulse width, a stimulation setting previously shown to inhibit bladder contraction in anesthetized felines [27]. Feline behavior was observed for signs that nerve stimulation was uncomfortable (e.g. stop engaging with wet food or responding to sensation). The number of trials per session, stimulation location, and stimulation paradigm varied per animal due to electrode performance and/or animal behavior changes.

2.5 Monitoring for neuromodulation carry-over effect

A video camera system (TV-DVR104K, TRENDnet, Torrance, CA) was used to monitor the litterboxes of the feline housing room. For each animal, the number of visits to a litterbox per 24-hour period were counted prior to the surgery, during surgery recovery, and before and after each test session. Some animals were kept in a recovery cage after surgery, either to recover further from surgery or to isolate from other animals, and did not have data collected during those periods.

2.6 Study completion

After completion of all testing for each animal, a final surgery was performed for a final assessment of implant functionality and to explant DRG microelectrode arrays, simulation electrodes, backpack, and bladder catheters. Animals were euthanized with a dose of intravenous sodium pentobarbital (36 mg/kg) while under deep isoflurane anaesthesia.

2.7 Data analysis

The primary data analysis focused on analyzing the effect of neuromodulation on bladder capacity during the repeated neuromodulation sessions of Animals 4-6. For each bladder fill, the bladder capacity was measured and normalized to the control (no-stimulation) group average in each session. The bladder capacity for each fill sequence was calculated as the greater of the amount infused and the voided urine. Residual fluid in the bladder after voiding, if the infused volume was greater than the voided volume, was not removed to avoid the potential for pulling a vacuum inside the bladder. Voiding efficiencies for each fill sequence were calculated by dividing the voided volume by the bladder capacity. The peak bladder pressure increase was calculated as the 1-second average of the maximum pressure during voiding minus the average pressure during the first two seconds of the trial. Bladder compliance for each fill was calculated as the ratio between the bladder capacity and the peak bladder pressure. A one-way ANOVA was used to compare the effects of the different stimulation paradigms on each data

measure, followed by Tukey HSD post hoc intra-group tests for multiple comparisons. Linear regression was used for the average no-stimulation bladder capacities over time.

For bladder fills with neural decoding from DRG signals, the correlation coefficient (R) between the measured pressure and estimated pressure was calculated to evaluate the performance of the decoding algorithm. Neural recordings were sorted offline for animals with functioning DRG microelectrodes during bladder fills. Bladder units were defined as having a firing rate correlation coefficient to the bladder pressure greater than 0.2. A Mann-Whitney Rank Sum Test was used to compare the bladder pressure and bladder unit firing rate correlation between sedated and awake sessions for these units. For all tests, a significance level of 0.05 was used. When relevant, results are presented as mean ± standard deviation.

3. Results

We performed successful implant surgeries with six animals. The first three animals were used to establish procedures, and the remaining three animals were to evaluate the effect of neuromodulation across repeated test sessions. From Animal 1 to Animal 3, the complexity of the test sessions was increased towards establishing a target paradigm for the Repeated Neuromodulation sessions. Table 1 has a summary of the experiments.

3.1 Electrode functionality

3.1.1 Stimulation electrodes

Across Animals 1-6, three of the sacral electrodes (39.2 ± 29.2 average days of use) and five of the pudendal nerve electrodes (52.3 ± 32.0 average days of use) were able to evoke anal twitching for the duration of the experimental period (Figure S1). Several implanted electrodes failed within 2-3 weeks. These failures were typically due to an unintended strain placed on the wires during early animal handling, except for the sacral electrode in Animal 4, for which the wires withdrew under the skin shortly after the surgery. There was a slight increase in visual anal twitch thresholds over time across experiments for electrodes (Figure S1). The average in-surgery pudendal electrode threshold at 210 μs was 153.5 ± 92.6 μA, and across all post-surgery sessions was 254.4 ± 157.9 μA (median 230 μA, 100–660 μA range). The average in-surgery sacral electrode threshold was 137.3 ± 98.5 μA and across all post-surgery sessions was 439.9 ± 868.8 μA (median 127 μA, 70–4102 μA range). Chronaxies for strength duration curves obtained from pudendal nerve and sacral nerve electrodes varied over time (Figure S1 C-D).

3.1.2 DRG recording microelectrodes

We observed DRG signals from 4 out of 5 animals that had awake settings. In Animal 6, the microelectrode cables were accidentally cut during the first test session due to poor surgical routing. Figure S2 summarizes microelectrode functionality and impedances after implant for other animals.

3.2 DRG bladder units

Bladder units were detected during awake, unrestrained activities in three animals in offline analysis (Animals 2-4), with other units observed in four total animals (Table S1). Figure S3 shows three representative bladder units during a no-stimulation bladder fill in Animal 3. Animals 3 and 4 had average bladder unit correlations of 0.41 ± 0.14 (range 0.20-0.74) and 0.49 ± 0.21 (0.21-0.92), respectively, between bladder unit firing rates and bladder pressure. A wide variability in sorted unit correlation to pressure and duration of tracking was observed across Animals 3 and 4 (Figure S4). Each Animal had one unit that was identifiable for the duration of testing, with considerable variability in its correlation over time. In Animal 4, there were no differences (Welch's t-test, p-values > 0.08) in the firing characteristics of bladder units during no-stimulation and continuous stimulation trials (Figure S5). Continuous stimulation was not performed in Animals 2 and 3.

3.3 Awake bladder pressure decoding

We utilized DRG recordings to estimate bladder pressure during awake bladder fills in Animals 3 and 4. In Animal 3, there was significant variability in the number of specific channels identified for decoding across sessions and trials (Figure S6A). For 38 total real-time decoding trials across 11 sessions, the average correlation coefficient was 0.45 ± 0.43 (median: 0.51, range: -0.63 – 0.90). Thirty of these trials used a single channel for the decode, and a total of 26 different channels were used at least once. Decoding of the bladder pressure during 5 ml/min bladder fills (correlation coefficient = 0.33 ± 0.45, n = 23) was significantly lower (p = 0.03) than for 2 ml/min fills (0.63 ± 0.33, n = 15) in Animal 3. In Animal 4, the decoding performance had an overall lower performance (Figure S6B). For 56 total real-time decoding trials (28 closed-loop trials, 25 no-stimulation trials, and 2 continuous stimulation trials when real-time decoding was accidentally left on) across 7 sessions, the average correlation coefficient was 0.32 ± 0.39 (median: 0.39, range: -0.75 – 0.93). Thirty-five of these trials used a single channel for

the decoding, and a total of 11 channels were used at least once. Offline analysis showed that the bladder units identified during sedated trials were not always present during awake bladder fills. These inconsistencies resulted in some models being trained on channels without bladder units present during awake bladder fills, leading to decoding driven by noise or neural signals unrelated to bladder pressure (n = 22 trials in Animal 3; n = 36 trials in Animal 4). For trials in which real-time decoding was performed on channels with at least one bladder unit present during an awake trials, Animal 4 had a correlation coefficient of 0.46 ± 0.37 (median: 0.44, range: -0.31 – 0.93, n = 20), while Animal 3 had a higher and more consistent performance with a correlation coefficient of 0.64 ± 0.27 (median: 0.74, range: 0.14 – 0.90, n = 16).

3.4 Awake DRG-based closed-loop stimulation

We performed DRG-based closed-loop stimulation in Animals 3 and 4. Nerve stimulation was applied for 15 seconds when the estimated bladder pressure increased by at least 5 cm H_2O within a 4-second window. In both animals, pudendal nerve stimulation was performed at 5 Hz, 1 MT. In Animal 3, 14 closed-loop trials were completed. The average bladder capacity increased from (32.8 ± 3.1 mL, n=12) during no-stimulation trials to (37.9 ± 5.0 mL, n=14) during closed-loop stimulation, an 16% average increase. In Animal 4, 28 closed-loop trials were completed. The average bladder capacity was 54.7 ± 7.5 mL for closed-loop stimulation trials, a 29.9% average increase over no-stimulation trials (42.1 ± 7.0 mL, n = 25). We also performed continuous stimulation in Animal 4, discussed in the next section. Figure 3A & B show a representative no-stimulation trial used to train a bladder pressure model and a set of closed-loop, continuous, and no-stimulation trials for a later Animal 4 session.

3.5 Awake repeated neuromodulation sessions

In Animals 4 through 6, seven awake, unrestrained sessions were performed with each animal, each with a sequence of at least three no-stimulation, three non-continuous stimulation, and three continuous stimulation bladder fills. In Animal 4, a previously developed closed-loop DRG-based algorithm was used to initiate pudendal nerve stimulation at 5 Hz, 1 MT when the algorithm detected a bladder contraction [14]. Continuous (144 ± 24% of control capacity) and closed-loop stimulation (131 ± 21%) of the pudendal nerve significantly increased bladder capacity in Animal 4 (Table 2, Figure 3A), with an average duty cycle of 50 ± 21% in the closed-loop trials.

In Animals 5 and 6, with DRG signals not usable for closed-loop control, non-continuous stimulation was performed. We initiated stimulation at approximately half of the no-stimulation bladder capacity. In both animals, sessions with sacral and pudendal nerve stimulation at 5 Hz 1 MT were performed. In Animal 5, stimulation did not affect capacity as non-continuous (90 ± 28%), and continuous (96 ± 31%) stimulation across both nerve locations did not yield different capacities than no-stimulation trials. In Animal 6, non-continuous stimulation (148 ± 46 %, n=8) and continuous (128 ± 26 %, n=6) stimulation on the pudendal nerve significantly increased bladder capacity. Similar bladder increases were achieved using non-continuous (129 ± 24 %, n=19) and continuous (128 ± 22 %, n=18) stimulation on the sacral nerve. The average duty cycle for non-continuous trials was 46 ± 20% and 63 ± 17% for Animals 5 and 6, respectively.

Post hoc comparisons with a Tukey HSD test identified statistically significant differences in bladder capacity for Animals 4 and 6 and across all three test Animals pooled together (Table 2, Figure 3). We also identified significant differences in bladder compliance in Animal 4 and across all three test animals pooled together (Table 2, Figure 3). We did not observe significant differences for peak bladder pressure changes or for voiding efficiencies (Table 2, Figure 3).

3.6 Stimulation effect on bladder function outside of sessions

We did not observe a carry-over effect of stimulation sessions on bladder capacity based on tracking litterbox usage (Figure 4A, Table 3). In general, the animals had a consistently low count of litterbox visits prior to surgery, which increased in the recovery period after surgery. During weeks with experimental sessions, litterbox visits were significantly higher across animals than before surgery ($p < 0.001$), however there were no differences between pre-session and post-session visit counts within or across animals. The increase in litterbox visit counts varied across animals, from a 1.7x increase (Animal 4) up to an 8.6x increase (Animal 1) and there was not a trend of pre- or post-session measures being higher across animals (Table 3). Across Animals 4 through 6, there was not a cumulative increase in bladder capacity across the test sessions. While Animal 4 had a small, non-significant capacity increase over time, Animals 5 and 6 each had a significant ($p < 0.01$), linear decrease in bladder capacity across sessions (Figure 4B).

4. Discussion

In this study, we showed that non-continuous neuromodulation can yield similar bladder capacity increases as continuous stimulation in an awake, unrestrained feline model. Furthermore, we demonstrated the feasibility of decoding bladder pressure from DRG recordings in awake, unrestrained sessions in two animals. To our knowledge, this is the first study to show real-time closed-loop stimulation from neural recordings in an awake, unrestrained medium-sized animal model towards restoring bladder function. This animal model provides a closer representation of the clinical setting than the standard use of anesthetized animals. Clinical and pre-clinical studies have shown that non-continuous peripheral nerve stimulation during non-voiding contractions or latter parts of a bladder fill can significantly increase bladder capacity [14], [28], [29], [30], [31], [32]. In our study, neuromodulation was effective in two of three test animals (Figure 3, Table 2). In the three test animals, non-continuous stimulation on the pudendal nerve elicited a similar effect on bladder capacity as continuous stimulation (Table 2), which aligns with a prior study with anesthetized animals [31]. Stimulation was also performed on the sacral nerve in two of these animals. In contrast to some clinical studies [7], [33], we did not observe significant differences between the stimulus location and efficacy of stimulation (Table 2). The duration of stimulation in non-continuous trials for responding test animals averaged 44% less than for continuous stimulation, with capacity increased by 32.5% (Table 2). These results are similar to a previous study of subjects with spinal cord injury that detected bladder contractions with a urethral catheter to control the stimulation of the dorsal genital nerve [28]. In that study, continuous and conditional stimulation increased bladder capacity by $36 \pm 24\%$ and $51 \pm 37\%$, respectively, with conditional stimulation decreasing the stimulation time by $27 \pm 17\%$. Pre-clinical studies in anesthetized animals have shown that stimulation timing plays an essential role in therapy efficiency; optimal stimulation timing may be during non-voiding bladder contractions [29] or in the second half of bladder fills [32]. There is a correlation between these timings, as non-voiding bladder contractions became more frequent above half-bladder capacity [14]. Here, we obtained bladder capacity increases for stimulation during estimated bladder pressure increases (e.g., Figure 2B) and for fixed periods approaching a full bladder (e.g., Figure 2D). Further studies investigating optimal stimulation timings and the relevance of stimulation paradigms across sedated and awake animal models are needed.

Across awake sessions, stimulation was performed at 1 MT and there was no sign of pain or anxiety. However, when stimulation was turned on some animals groomed the pelvic area, indicative of perception. At 1 MT, we did not observe a decrease in voiding efficiency during stimulation trials (Table 2, Figure 3). The peak pressure during the voiding phase was not significantly different within or across animals though there were differences in compliance across responding animals. The significant increase in compliance with stimulation for Animal 4 (Figure 3B) aligns with a clinical neuromodulation study that observed detrusor inhibition without changes in urethral resistance (no differences in peak pressure) or detrusor contractility during the voiding phase, which resulted in higher bladder compliance [34]. However, in Animal 6 there was a non-significant increase in peak pressure (Figure 3C) that resulted in no changes in bladder compliance (Figure 3B), suggesting that in this animal neuromodulation may have affected the spinal nerves that modulate bladder contractions or activated efferent fibers to the sphincter. In contrast to studies with anesthetized animals [13], [14], we were unable to consistently decode bladder pressure from unsorted DRG spikes in awake animals (Figure S6). The primary factor was noise during awake recordings. The noise was typically caused by movement of the backpack and cables during position changes. Spike detection using dual-threshold crossings was not sufficient to reject noise consistently. Another factor that affected our decoding performance was that units were not consistently present between sedated and awake testing, as we determined from offline spike sorting analysis. This variation led to channels selected for decoding for awake-testing bladder trials that often did not contain bladder-related activity, contributing to the high variability and low correlation coefficient in bladder pressure decoding (Figure S6). We hypothesize that microelectrode micromotion had a role in the variability of the correlation for tracked bladder units (Figure S4). Also, our use of sedated bladder fills to train the decoding algorithm may have overfit the model. For the preparatory animals we evaluated using awake neural recordings for decoding, however, the recordings were inconsistent, foreshadowing our challenges in the test animals. Ultimately, model training during non-sedated bladder fills will be necessary to provide the most realistic scenario for translation of this approach. Also, reducing or eliminating recording noise during awake sessions will be essential for maintaining the spike detection algorithm as simple as possible. A sophisticated spike detection algorithm that considers waveform properties may be needed to reject noise [35], [36]. An adaptive decoder [37] that uses data from multiple bladder fills and incorporates bladder neuron response characteristics [18], [38] may also increase the accuracy of the decoder by accommodating the variability of the neural recordings.

We used microelectrodes that are not specifically designed for DRG, as in prior studies [13], [14], [39]. DRG units were observed for most animals (Table S1) and could be tracked over time across sedated and awake sessions (Figure S4), however functionality slightly decreased over time (Figure S2B) and we did not observe bladder units in all animals (Table S1). Mounting these rigid microelectrode arrays to small, curved DRG can be challenging, leading to arrays shifting over time [24], which may have contributed to the instability of sorted units (Figure S4, S5) and decoding (Figure S6). Previous research has

demonstrated that neural cell body densities are higher at the outer regions of the DRG [40], [41]. Flexible electrode arrays that can conform to the curved DRG surface to target these regions [42] or penetrating thin-film arrays with a high density of electrode sites along the shank [43] may have better success for identifying and tracking units over time.

In DRG-based closed-loop trials, nerve stimulation was applied for 15 seconds when the estimated bladder pressure increased by at least 5 cm H_2O within a 4-second window. This stimulation protocol assumes that non-voiding contractions, characteristic of preclinical models of OAB, are present. In preclinical models, researchers typically induce hypersensitivity or bladder inflammation to study OAB and its treatments [44]. OAB symptoms are elicited through the intravesical infusion of external substances like PGE2 or acetic acid [13], [45]. These methods reduce the bladder capacity and sometimes increase the frequency of non-voiding contractions during bladder fills. In this study, we used healthy felines and did not directly seek to create OAB conditions. However, most animals had large increases in their rate of litterbox usage after the implant surgery (Table 3, Figure 4A), suggesting an increase in urgency-like sensations. Additionally, two test animals had a significantly decreasing trend over time in their bladder capacity (Figure 4B), which may have been caused by mechanical irritation of the implanted bladder catheters. Contrary to findings from sedated preclinical OAB models [13] and sedated bladder fills in this study, the pressure traces during awake session bladder fills did not show non-voiding contractions (Figure 2). This discrepancy raises questions regarding the relevance of the stimulation protocol in awake animals. Future studies should examine the presence of non-voiding contractions during awake bladder fills in a preclinical model to ascertain whether the stimulation algorithm used here remains applicable for the treatment of OAB. Thus, in this study, the effects of stimulation were not a result of inhibiting non-voiding contractions; rather, the stimulation may have mitigated the effects of irritation during sessions. However, there was no evidence that multiple stimulation sessions per week had a long-term functional impact (Figure 4). Chronaxies for strength-duration curves obtained from pudendal nerve and sacral nerve electrodes varied over time (Figure S1C-D). The sacral nerve electrodes had more threshold variability than the pudendal nerve electrodes (Figure S1A-B), likely due to the structural differences between the electrodes. The pudendal nerve electrode's motor threshold values were similar to previously reported thresholds [25]. We argue these chronaxie and threshold observations are more likely attributable to changes in electrode stability (e.g., scar tissue formation, electrode movement) rather than intrinsic changes in nerve excitability (e.g., nerve damage, neuroplasticity) and did not have an impact on bladder outcome measures.

In this study, stimulation carry-over effects were not observed (Figure 4). Bladder capacity increases were only observed during stimulation within sessions. This contrasts with clinical studies, which have shown maintained improvements in bladder symptoms with intermittent neuromodulation (8 hours on and 16 hours off) [46] or sustained effects for days after cessation of neuromodulation [47]. Those carry-over effects were present after longer post-implant stimulation periods, suggesting that neuromodulation may need to be applied for a longer induction period before persistent effects are observed. In contrast with a prior study which had suggested that bladder units can desensitize during continuous stimulation in sedated bladder fills [14], our results did not have a consistent sensitivity trend during awake bladder fills with and without stimulation (Figure S5). Unfortunately, we did not perform sedated bladder fills with continuous stimulation for a direct comparison to the prior study. A study with an implanted stimulator that can apply continuous or conditional stimulation in an animal's housing space, such as previously developed with sheep [48], may provide further insights into long-term efficacy and carry-over effects.

Neuromodulation to control bladder function in pre-clinical models is generally performed under anaesthesia [49]. Awake testing with rodent models is growing in prevalence, facilitated by the relative ease of tunnelling implant catheters and wires to connectors mounted on the skull, wireless stimulators and metabolic cages suited to rodent space requirements, and immobilization techniques that are better tolerated by small species [49], [50], [51]. Awake, unrestrained testing with medium-sized animals like felines brings new challenges (discussed in part in Supplement Part 1), including stress induced by handling, connecting implant monitoring system to highly mobile animals, and performing bladder fills in a controllable yet comfortable space. Several limitations of this study include a lack of stimulation type randomization within stimulation sessions due to the need for verifying non-continuous simulation algorithms during no-stimulation trials and variations in animal behavior during trials (e.g., eating, walking or playing with toys). Longer-term studies with untethered implants that utilize the animals' natural bladder filling may allow for improved study design.

5. Conclusion

This study demonstrated that non-continuous neuromodulation can yield observable, significant bladder capacity increases without affecting peak pressures or voiding efficiency in a pre-clinical awake animal model. There is a clear promise for clinical use of non-continuous or even closed-loop neuromodulation; advances in recording electrode interfaces or approaches are needed. DRG may be a promising location for monitoring bladder pressure-related signals, as indicated by the large number of bladder units identified across Animals 2-4. We expect an electrode array specifically designed for DRG will have better long-term performance and help drive this therapeutic approach towards clinical utility. Future studies investigating non-continuous

stimulation at different intervals during bladder fills are needed to maximize the therapy outcome while minimizing the energy required. Finally, it appears that the presence of the catheters in the bladder led to overactive bladder-like symptoms, in which some animals had reductions in functional bladder capacity over time and visited the litterbox at a significantly greater frequency than prior to surgery. Future studies with improved DRG interfaces that monitor bladder activity using complex decoders under awake, urestrained conditions and without test chambers are needed.

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TABLES

Table 1. Summary of experiments.

#	Goal(s)	DRG bladder units	Sacral Neuromodulation Sedated / Awake testing	Pudendal Neuromodulation Sedated / Awake testing	Non-Con Stim type	Litterbox tracking
1	Bladder decoding during sedation; habituation protocol	Yes	Yes / No	Yes / No	None	Yes
2	Bladder decoding during sedation; habituation protocol	Yes	Yes/ Yes	No / No	Half volume	No
3	Awake testing logistics	Yes	Yes / Yes	No / Yes	Closed loop	Yes
4	Repeated neuromodulation	Yes	No / No	No / Yes	Closed loop	Yes
5	Repeated neuromodulation	No	No / Yes	No / Yes	Half volume	Yes
6	Repeated neuromodulation	No	No / Yes	No / Yes	Half volume	Yes

Non-Con Stim = non-continuous stimulation

Table 2. Summary of Repeated Neuromodulation Sessions across Animals 4-6. Sample sizes per animal, stimulation location, and trial type are given as (N). Bold values are significantly different ($p < 0.05$) from No-Stimulation. NA indicates not performed. Total is the average of all trials performed in an animal – pudendal and sacral trials.

	Pudendal Nerve			**Sacral Nerve**			**Total**		
Exp.	**No-Stim**	**Non-Con-Stim**	**Con-Stim**	**No-Stim**	**Non-Con-Stim**	**Con-Stim**	**No-Stim**	**Non-Con-Stim**	**Con-Stim**
Bladder Capacity (mL)									
4	42.1 ± 7.0 (25)	**54.7 ± 7.5 (28)**	**58.8 ± 6.6 (26)**	NA	NA	NA	42.1 ± 7.0 (25)	**54.7 ± 7.5** (28)	**58.8 ± 6.6 (26)**
5	21.6 ± 6.5 (13)	23.5 ± 5.4 (6)	19.6 ± 5.7 (11)	26.0 ± 12.7 (26)	26.2 ± 12.5 (12)	25.9 ± 13.5 (20)	24.5 ± 11.2 (39)	25.3 ± 10.5 (18)	23.6 ± 11.6 (31)
6	10.1 ± 5.5 (8)	**12.9 ± 3.3 (8)**	10.0 ±4.2 (6)	10.5 ± 2.5 (21)	**14.2 ± 4.4 (19)**	**13.4 ± 4.0 (18)**	10.4 ± 3.5 (29)	**13.8 ± 4.1** (27)	**12.5 ± 4.3** (24)
Maximum bladder pressure increase (cm H_2O)									
4	55.0 ± 9.8	53.7 ± 9.5	52.8 ± 11.0	NA	NA	NA	55.0 ± 9.8	53.7 ± 9.5	52.8 ± 11.0
5	58.0 ± 18.5	58.6 ± 5.6	63.8 ± 16.2	71.7 ± 17.4	60.7 ± 10.7	60.6 ± 14.2	66.1 ± 18.8	59.8 ± 8.6	62.0 ± 14.8
6	Data corrupted			45.7 ± 15.7	51.0 ± 20.7	51.9 ± 16.2	45.7 ± 15.7	51.0 ± 20.7	51.9 ± 16.2
Bladder compliance (mL/cm H_2O)									
4	0.79 ± 0.22	**1.06 ± 0.27**	**1.20 ± 0.31**	NA	NA	NA	0.79 ± 0.22	**1.06 ± 0.27**	**1.20 ± 0.31**
5	0.52 ± 0.59	0.41 ± 0.12	0.31 ± 0.07	0.32 ± 0.14	0.33 ± 0.16	0.36 ± 0.20	0.40 ± 0.39	0.36 ± 0.15	0.34 ± 0.16
6	Pressure data corrupted			0.26 ± 0.11	0.38 ± 0.40	0.37 ± 0.46	0.26 ± 0.11	0.38 ± 0.40	0.37 ± 0.46
Voiding Efficiency (%)									
4	96 ± 11	95 ± 11	98 ± 3	NA	NA	NA	96 ± 11	95 ± 11	98 ± 3
5	89 ± 23	96 ± 4	96 ± 5	86 ± 17	73 ± 29	93 ± 14	87 ± 18	83 ± 24	94 ± 12
6	94 ± 8	88 ± 17	90 ± 19	93 ± 10	89 ± 12	91 ± 12	93 ± 9	88 ± 14	91 ± 14

Table 3. Summary of litterbox visits across animals per 24-hour periods. Animal 2 did not leave the recovery cage, and Animals 4 and 5 stayed in the cage longer during recovery. Statistical differences across groups are indicated in Figure 4.

Animal	Pre-Surgery	Post-Surgery	Pre-Session	Post-Session	Animal Total
1	4.4 ± 1.3 (7)	63.8 ± 38.8 (4)	40.3 ± 21.7 (8)	35.6 ± 14.7 (6)	36.1 ± 13.5
3	3.7 ± 0.8 (7)	9 (1)	15.4 ± 6.3 (7)	16.4 ± 5.4 (7)	11.1 ± 2.8
4	2.0 ± 0.8 (7)	*in cage*	7.1 ± 3.7 (6)	7.0 ± 3.2 (5)	7.0 ± 3.3
5	2.1 ± 0.7 (7)	*in cage*	7.6 ± 8.3 (5)	8.4 ± 8.3 (5)	6.1 ± 3.6
6	3.7 ± 1.3 (7)	5.4 ± 2.4 (5)	43.9 ± 24.3 (16)	41.5 ± 15.3 (16)	23.6 ± 9.5
Per-Animal Summary	3.2 ± 1.1 (5)	---	22.2 ± 18.7 (5)	21.0 ± 16.8 (5)	

FIGURES

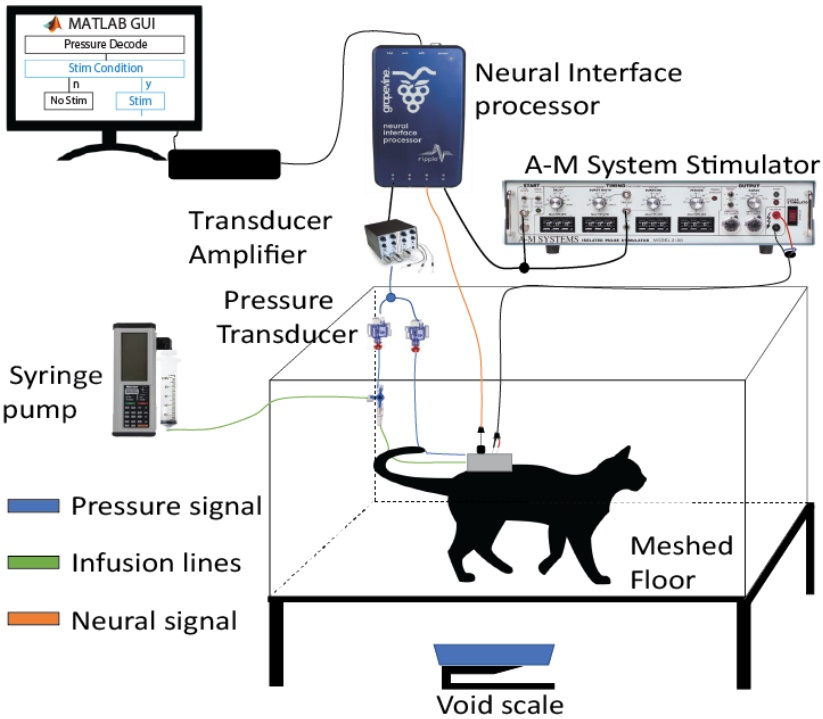

Figure 1. Experimental test setup for awake, unrestrained sessions. The animal was placed in the test chamber and connected to the infusion pump, pressure transducers, data acquisition system, and the stimulator.

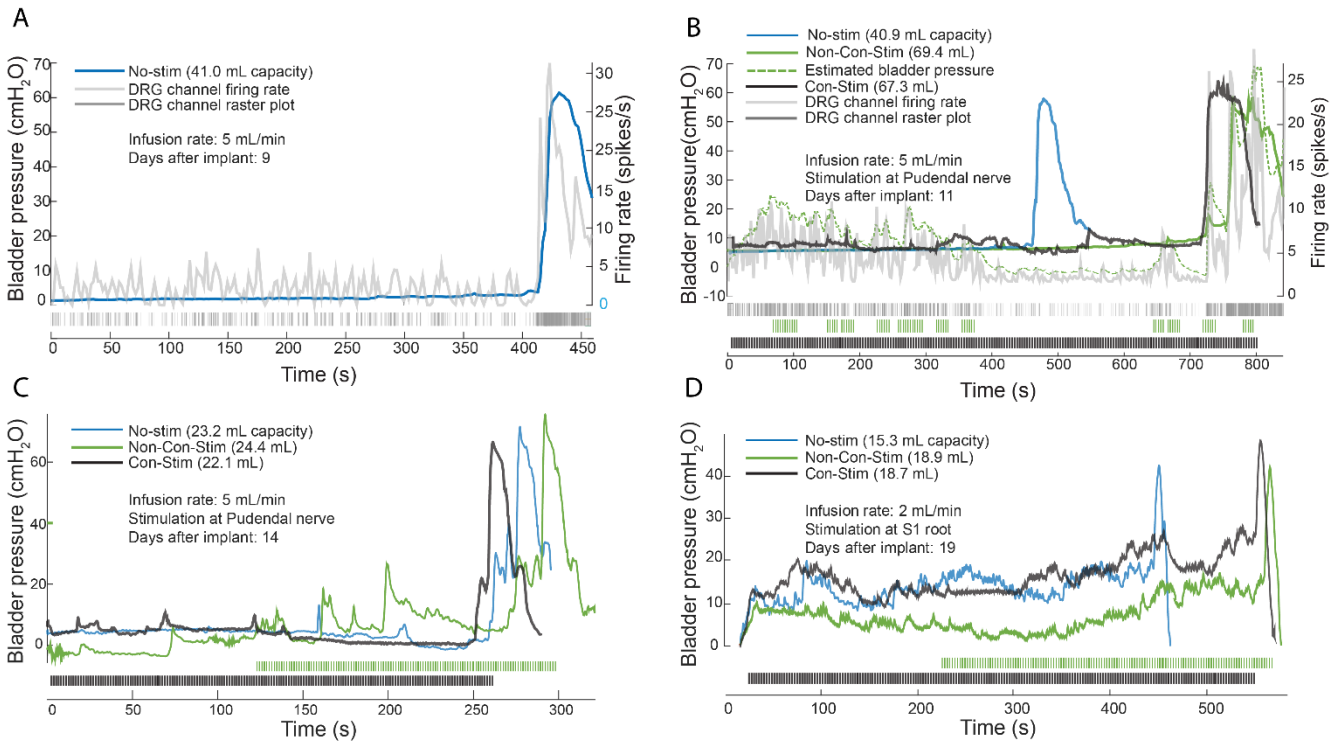

Figure 2. Representative trials across three animals with repeated testing. A. No-stimulation bladder fill in Animal 4 used to train a bladder pressure model from one DRG channel. B. Representative bladder fill trials and closed-loop stimulation trial in Animal 4 using model of part A. C. Representative bladder fill trials from Animal 5. D. Representative bladder fill trials from Animal 6.

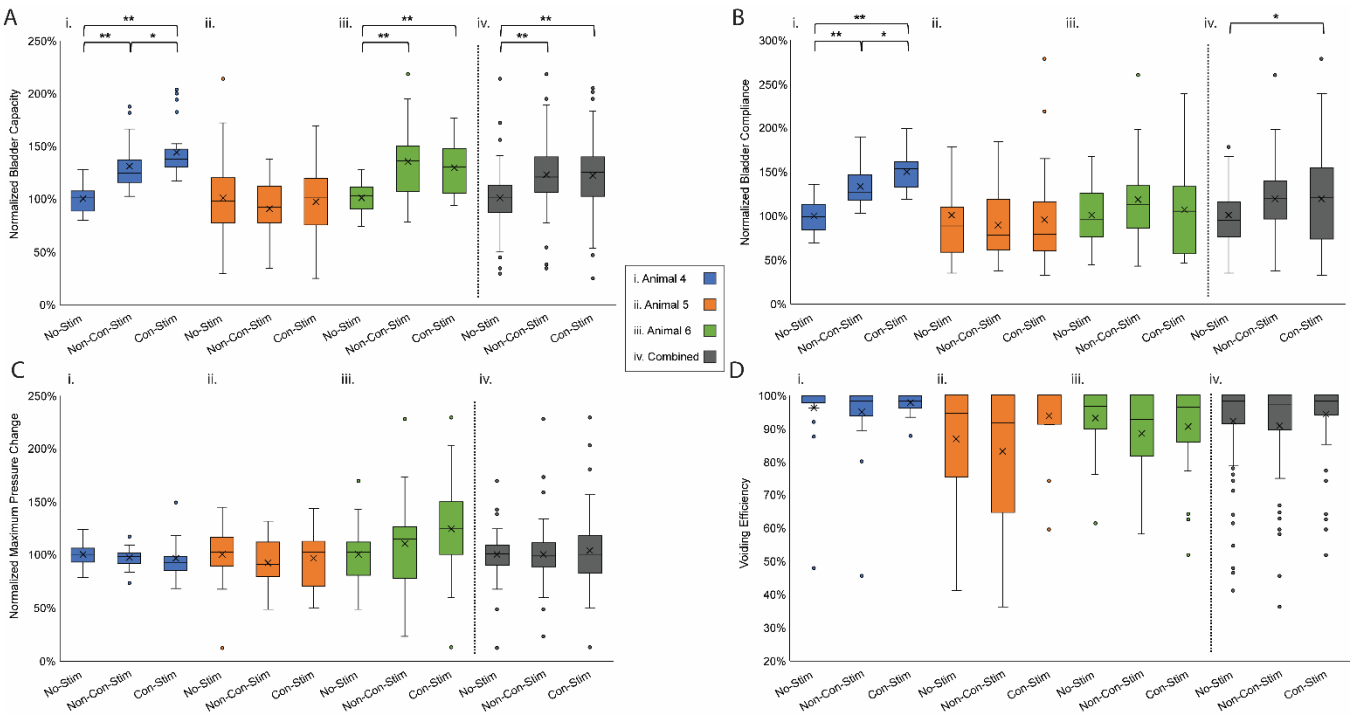

Figure 3. Normalized data across repeated neuromodulation sessions. A. Normalized bladder capacity. B. Normalized bladder compliance. C. Normalized maximum bladder pressure change. D. Voiding efficiency. Animals i. 4. ii. 5. iii. 6. iv. Animals 4-6 combined. Boxes represent the interquartile range (IQR), with the line in the middle of each box representing the median and X representing the mean. Tips of box whiskers represent 1st quartile – 1.5*IQR and 3rd quartile + 1.5*IQR. (* $p < 0.05$. ** $p < 0.01$)

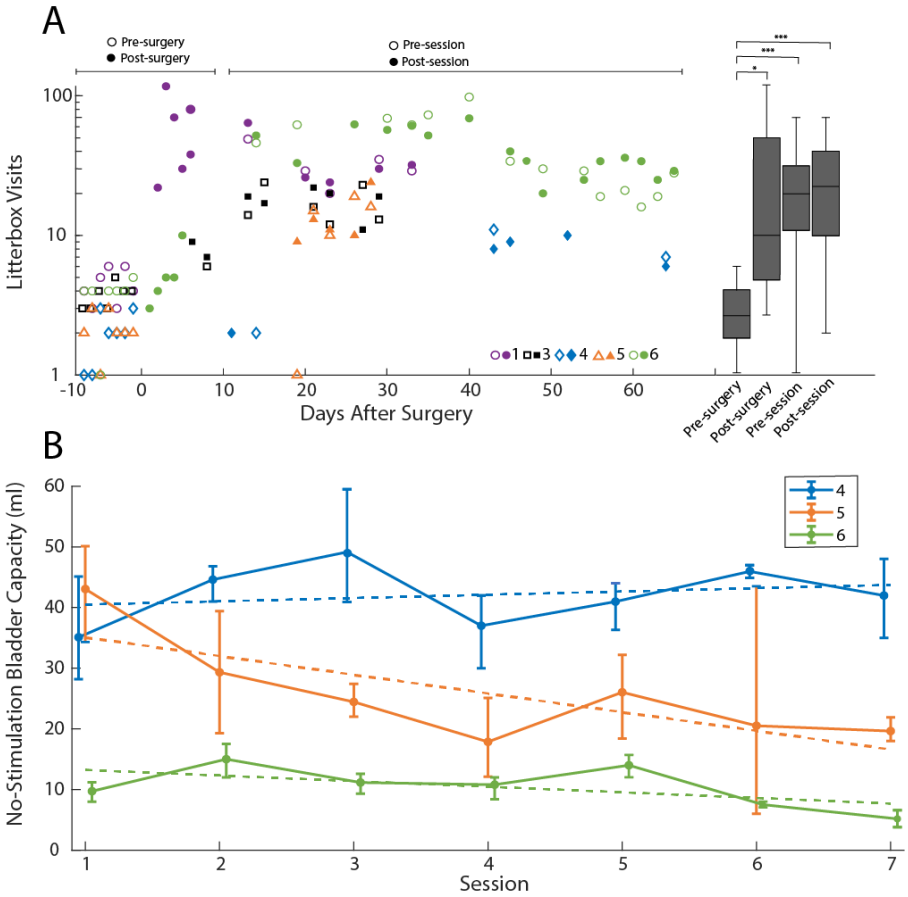

Figure 4. A. Analysis of feline litterbox use during study. The scatterplot contains 24-hour interval counts of litterbox usage before surgery (open icons), within ~1 week after surgery (closed icons), and before (open icons) and after (closed icons) test sessions. The boxplots summarize the litterbox visits per period across animals and are organized as in Fig. 2. Animals 3 and 4 spent most of the time within a recovery cage in the post-surgery period. Animal 2 spent his entire implant time in the cage and is omitted here. Some pre- and post-session periods for Animal 4 had no litterbox visits. (* $p < 0.05$. *** $p < 0.001$) B. Average no-stimulation bladder capacities for animals with repeat test sessions. Dashed lines are linear regression fits (p-values: Animal 4 = 0.73, Animal 5 = 0.001, Animal 6 = 0.002).