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Variable Patterned Pudendal Nerve Stimuli Improves Reflex Bladder Activation

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Abstract

We evaluated variable patterns of pudendal nerve (PN) stimuli for reflex bladder excitation. Reflex activation of the bladder has been demonstrated previously with 20–33 Hz continuous stimulation of PN afferents. Neuronal circuits accessed by afferent mediated pathways may respond better to physiological patterned stimuli than continuous stimulation. Unilateral PN nerve cuffs were placed in neurologically intact male cats. PN stimulation (0.5–100 Hz) was performed under isovolumetric conditions at bladder volumes up to the occurrence of distension evoked reflex contractions. Stimulus evoked reflex bladder contractions were elicited in eight cats. Across all experiments, bursting of 2–10 pulses at 100–200 Hz repeated at continuous stimulation frequencies evoked significantly larger bladder responses than continuous (single pulse) stimulation (52.0 ± 44.5%). Bladder excitation was also effective at 1 Hz continuous stimuli, which is lower than typically reported. Variable patterned pulse bursting resulted in greater evoked reflex bladder pressures and increased the potential stimulation parameter space for effective bladder excitation. Improved bladder excitation should increase the efficacy of neuroprostheses for bladder control.

Keywords

Functional electrical stimulation (FES); neuroprosthesis; pudendal nerve; stimulation pattern; urinary system

I. Introduction

FOLLOWING spinal cord injury or other neurological disorders, loss of lower urinary tract control may occur. The resultant impact upon quality of life includes health problems and severe medical costs [1]–[3]. Typical management strategies for bladder care include medication, catheterization, and surgery [3]–[6]. These methods, when successful, can often have undesirable side effects such as bladder and urinary tract infections [3], [5], [6]. In individuals with spinal cord injury, a Brindley sacral root stimulator may be implanted to provide bladder control [4], [7], [8]. However, it is an invasive procedure which does not lead to physiologic voiding patterns [7] and usually requires a dorsal rhizotomy [4], [9] which results in loss of reflex erection and defecation [4], [5].

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The pudendal nerve (PN) contains afferent fibers which initiate reflex bladder voiding and continence reflexes [10]–[21]. Nerve mapping has shown that afferent fibers in the pudendal nerve trace to the sacral roots used most often for sacral root stimulation [22], [23]. This reflex activation of the bladder travels through two systems: reflex circuitry contained within the sacral spinal cord and within the parasympathetic pelvic ganglion.

The use of peripheral nerve stimulation for bladder emptying offers advantages over sacral root stimulation. A surgical procedure to access the PN can be done in less time than for a sacral root electrode [13], with sufficient space for a multielectrode nerve cuff [24]. Access to the PN is also feasible with placement of a tined lead [13], [25] or needle electrode [17]. A rhizotomy is unnecessary, when using PN afferent stimulation to evoke bladder contractions [18], [26]. Furthermore, minimally invasive intraurethral stimulation to excite the bladder, activating urethral afferent fibers which feed into the pudendal nerve, has been clinically shown to be a convenient diagnostic tool for an implanted device [27], [28]. One current disadvantage of PN afferent stimulation, however, is that voiding is not as efficient as the use of sacral root stimulation following a rhizotomy [26]. Improving PN-evoked bladder contractions may reduce this deficiency.

Research has shown that applying continuous stimulation at 20-40 Hz to the PN can activate reflex voiding pathways in cats [12], [14]–[18], [26]. Animal research on PN stimulation has indicated success at eliciting contractions for bladder levels both below and above volumes which result in distension evoked contractions, although these studies focused on trials performed at bladder levels above distension evoked volumes [14]–[16], [26]. The basis for reflex pathways responding to 20-40 Hz stimulation is not fully understood, although it has been hypothesized to relate to peak firing rates during fluid flow through the urethra [14], indicating that improved stimulus patterns may be possible.

Many sensory neurons employ structured temporal codes to transfer information [29], [30]. Bursting of action potentials in neurons has been observed in a variety of settings, in mammalian spinal cord neurons [31], intestinal neurons [32], and electroreceptors [33]. Furthermore, bursting has been shown in nonmicturition related afferents of the PN [34]–[36] but has not yet been indicated in urethral afferents. The intraburst frequency, the burst duration and the interburst interval can all convey information about the input stimulus. There are indications that, in many situations, bursts of action potentials transfer signals more reliably because transmitter release is facilitated [37]. Enhanced responses to burst stimulus has been reported in the rat submandibular glands [38] and in colonic vasculature [39]. Both of these issues are pertinent in afferent stimulation evoked bladder response. Neuronal bursting may specifically mimic afferent signals to the sacral centers, which could increase outputs to the pelvic ganglion, enhancing transmitter release to the second order neurons.

The purpose of this study was to evaluate PN stimulus patterns for reflex bladder excitation that imitate neuronal bursting activity, at functional isovolumetric bladder volumes. Matching neuronal signaling processes may result in improved efficacy of PN evoked bladder contractions, which will enhance the performance of neuroprosthetics for restoration of bladder function.

II. Methods

A. Animal Model

This was a prospective study utilizing mature, neurologically and urologically intact, purpose-bred male cats. All animal studies were approved by the Case Western Reserve University Institutional Animal Care and Use Committee prior to initiation.

B. Experimental Preparation

Animals were initially sedated with an intramuscular injection of ketamine (35 mg/kg). Anesthesia was induced (65 mg/kg) and maintained (15 mg/kg) intravenously with a dose of α-chloralose. During surgical preparation, anesthesia was augmented with isoflurane (0.25%–2%). In five cats, buprenorphine (0.01 mg/kg) was administered every 12 h to ensure analgesia. Vitals were monitored (V9204 Advisor Monitor, Surgivet, Waukesha, WI) for the duration of the experiment. A port for fluid infusion was introduced into a cephalic vein and connected to an IV pump (Flo-Gard 6200, Baxter Healthcare, Deerfield, IL) with a glucose-saline drip. The bladder was exposed via a midventral line incision. A dual-lumen 6 Fr catheter (DLC-6D, Life-Tech, Stafford, TX) was placed through the dome of the bladder and held in place with a purse-string suture. Bladder pressure was recorded through one lumen of the bladder catheter, which was connected to a disposable pressure transducer (Deltran I, Utah Medical Products, Midvale, UT). Bladder infusion of room temperature saline through the other lumen of the bladder catheter was performed using a syringe pump (Genie, Kent Scientific, Torrington, CT).

The pudendal nerve was accessed via a postero-lateral gluteal approach on one side. A tripolar silicone nerve cuff (0.5 mm × 3 mm contact size; 1 mm intercontact distances) was placed on the pudendal nerve trunk proximal to the branching location of the distal rectal and deep perineal branches. Prior to initiation of experimental procedures, the cat was positioned prone on a foam platform, arranged such that the hips and upper torso were supported with the abdomen hanging freely.

C. Experimental Setup

Bladder pressure transducer outputs and TTL output signals from the stimulator (Pulsar 6bp-as, FHC, Bowdoinham, ME) were sampled at 100 Hz by a custom designed LabVIEW (version 7.1, National Instruments, Austin, TX) display, after being conditioned by a strain gauge and a feed-through module (NI SCC-SG24 & NI SCC-FT01, National Instruments), respectively, in a signal conditioning connecting block (NI SC-2345, National Instruments) connected to a DAQ-card (NI DAQ 6024E, National Instruments). Custom-designed electronics were used to extend the duration of each TTL pulse prior to the DAQ-card to eliminate under-sampling. The LabVIEW display provided real-time data viewing and stored data in text files on a laptop (Inspiron 8600, Dell, Round Rock, TX). Pressure and stimulus data were also written to strip chart paper with a chart recorder (TA11, Gould Instrument Services, Valley View, OH).

Starting at 50 μA, single, cathodic, current-controlled, 100-μs biphasic pulses were applied to the PN cuff to determine the threshold for fiber activation. The current level was incremented in 10–50 μA steps until visual activation of the external anal sphincter was observed. The threshold current for this motor activation of the pudendal nerve was defined in each experiment as the PN_{TH}.

D. Stimulus Definitions

Stimuli for reflex bladder activation were given using cathode-leading, current-controlled balanced biphasic pulses with a fixed pulse width (pw) of 100 μs. Stimulus patterns (see Fig. 1) were arranged such that one or more pulses ($\#_P$) were repeated at a pulse frequency (f_{PU}, in a manner similar to bursting. A train of pulses was repeated at a train rate frequency (f_{TR}, which is analogous to the single pulse continuous frequency used by others. Stimulus durations (t_{STIM}) were approximately 20 s, varying between 15 and 60 s. Stimulus amplitudes were set at 2–3 times the PN_{TH}. The stimulus pattern nomenclature used in these experiments is $A\#_P$ x B f_{PU} @ f_{TR}. In this manner, 5 $\#_P$ at 200 Hz f_{PU} repeated every 1 s is

represented as 5 × 200 Hz @ 1 Hz. Equivalent representations of a continuous 33 Hz train of pulses are 33 × 33 Hz @ 1 Hz and 1 × 33 Hz @ 33 Hz.

E. Experimental Protocol

Upon completion of the surgical prep, a cystometrogram was performed to examine the excitability of the bladder, filling at 0.5–2 mL/min. During filling, at 1–5 mL increments, the PN was stimulated to evaluate whether reflex evoked contractions could be elicited. Throughout this initial excitability evaluation, the stimulus pattern was alternated between a continuous 33 Hz train and a low f_{TR} pulse bursting (e.g., 5 × 200 Hz @ 1 Hz). The bladder volume was recorded for the first reflex contraction elicited by PN stimulation (V_{STIM})and for the first distension evoked contraction (V_{TH}). Stimulus evoked contractions were defined as bladder pressure increases above 15 cm H_2O from baseline and reaching a plateau by the end of the applied stimulus. Distension evoked contractions were defined in the absence of stimulation as bladder contractions evoking 15 cm H_2O above baseline for at least 10 s.

At or above V_{STIM}, the bladder was maintained near isovolumetric either via a 3.5 or a 5 Fr catheter occluding the urethra or by replacing any volume voided after each trial. If the bladder passed beyond V_{TH} into an over excitable state, due to added fluid from urine generation, the bladder was emptied to reset the system. The time duration of a bladder fill cycle in which the bladder was infused with saline and then eventually emptied was tracked. The urine generation rate for each fill cycle time duration was estimated based on the net volume removed beyond the infused amount. The bladder volume for a given stimulation trial was estimated using the net infused volume and urine generated assuming a constant urine rate per fill cycle.

Stimulation trials were performed with two objectives: evaluate continuous pulse trains in the frequency range from 0.5 to 100 Hz, focusing on 0.5, 1, 5, 10, 20, 33, and 50 Hz, and to evaluate bursting of pulses in the same continuous frequency range (f_{TR}), using 1—25 $\#_P$ repeated at 33–1000 Hz f_{PU}, focusing on 1, 5, and 10 $\#_P$ at 100 and 200 Hz f_{PU}. In five experiments, balanced and randomized predetermined sets of parameter combinations were utilized, to increase the rigor of the study. In two experiments, $\#_P$ was varied among 1, 5, and 10, f_{PU} was varied between 100 and 200 Hz, and f_{TR} was varied among 0.5, 1, and 3 Hz. In three experiments, $\#_P$ was varied between 1 and 5 f_{PU} for kept constant at 200 Hz while f_{TR} was varied among 0.5, 1, 5, 10, 20, 33, and 50 Hz. Any repeat of these balanced sets, whether within an experiment or across experiments, was rerandomized.

F. Data Analysis

A sample evoked bladder contraction and measured parameters are shown in Fig. 2. During all trials in which the bladder was excitable, each applied stimuli and the corresponding bladder response were examined. The baseline pressure prior to initiation of the stimulus was defined by the 3 s average ($P_{BASE–AVE}$) and standard deviation ($PBASE–SD$) of the bladder pressure. After the start of the stimulus, the evoked bladder pressures were analyzed while they exceeded an evaluation threshold ($EVAL_{TH}$), determined by $P_{BASE–AVE} + 3 \times P_{BASE–SD}$. During the pressure evaluation period, the average (P_{AVE}) and maximum (P_{MAX}) pressures were recorded. Average ($P_{AVE–EV}$) and maximum ($P_{MAX–EV}$) evoked pressures were calculated as the difference between P_{AVE} and $P_{BASE–AVE}$ and P_{MAX} and $P_{BASE–AVE}$, respectively.

The data recorded during each experiment was analyzed using Excel (Office 2007, Microsoft, Redmond, WA) and JMP 6 (SAS, Cary, NC). Maximum and average evoked pressures were accumulated for each stimulus pattern within and across experiments. Each animal was categorized as a lower or a higher continuous frequency (f_{TR}) responder using

the responses for 1 and 33 Hz continuous stimuli. The linear trendline slope fit to the average $P_{AVE–EV}$ at 1 Hz and 33 Hz f_{TR} for $\#_P$ of 1 was used to differentiate between groups. A negative $f_{TR} - P_{AVE–EV}$ slope for an experiment corresponded to lower stimulus frequencies exciting the bladder at greater levels than higher frequencies, resulting in that cat being categorized as a lower frequency responder. A positive $f_{TR} - P_{AVE–EV}$ slope for an experiment corresponded to higher frequencies exciting the bladder at greater levels than lower frequencies, resulting in that cat being categorized as a higher frequency responder. Across experiments, blocks of trials from the two predetermined parameter set combinations were separately combined and analyzed with a multifactor main effects ANOVA after screening for interactions, with $\#_P$, f_{PU} and f_{TR} as factors as well as t_{STIM} and the bladder volume. Additionally, all trials performed under excitable conditions, and subgroups of lower frequency responders and higher frequency responders, were analyzed in the same manner with an ANOVA. Student's t-test was used for comparison testing of pooled groups of trials. The t-test for two dependent samples was used for comparison testing of subgroups within experiments pooled across experiments. For evaluations of statistical significance, α was set to 0.05.

III. Results

A. Animals

PN reflex excitability was evaluated in thirteen male cats (4.2 ± 0.7 kg, 3.6–5.6 kg; 1.7 ± 0.3 years old, 0.7–2.0 years old). The nerve cuff was placed on the PN such that both the deep perineal and caudal rectal branches were included. In two cats, the nerve cuff was placed closer to the spinal cord, such that the urethral sensory nerves were also included. Across all experiments, the average PN_{TH} was 273 ± 117 μA (90–500 μA). In eight experiments (three cats on buprenorphine), bladder contractions were evoked by afferent PN stimulation. In the remaining experiments, afferent PN excitation modulated bladder responses but did not initiate contractions. Only the results from the eight fully excitable cats are presented here.

B. Excitable Bladder Volume

Stimulus evoked contractions were obtained below V_{TH} in all cats. The average V_{STIM} was 28.1 ± 17.2 mL (22.6 mL median). Of the 47 PN stimulus responsive bladder fill cycles, 85.1% (n = 40) had V_{STIM} below V_{TH}. In six fill cycles (12.8%), V_{STIM} was equal to V_{TH}. In one (2.1%) cycle, V_{STIM} was above V_{TH} (102.3%). Across experiments, for bladder fill cycles in which both PN stimulation evoked and distension evoked contractions occurred (23 cycles), the average V_{STIM}/V_{TH} ratio was 81.0 ± 23.7% (median = 88.0%). If cycles without the occurrence of a distension evoked contraction are included in the analysis, by using the maximum infused volume for V_{TH}, then the average V_{STIM}/V_{TH} ratio across experiments was 84.2 ± 10.7% (median = 83.1%).

C. Responses to Continuous Stimulation

For continuous, single pulse stimulation, evoked bladder responses were averaged across all cats. Pooled $P_{AVE–EV}$ and $P_{MAX–EV}$ results across all trials performed at key frequencies are given in Table I. $P_{AVE–EV}$ results across experiments and experimental averages are shown in Fig. 3. Bladder excitation for 5 Hz stimuli was significantly lower ($p < 0.05$) than that at 0.5, 1, 20, and 50 Hz. In addition, comparing results at 5 Hz to results at 33 Hz and comparing 10 Hz results to all other frequencies except for 5 Hz indicated significant difference at $\alpha < 0.10$. Limited trials at other continuous frequencies gave lower evoked pressures for frequencies between and outside the two peaks seen in Fig. 3 while matching excitability within the second peak (nonexcitable frequencies: 2, 3, 60, 75, and 100 Hz; excitable frequencies: 25 and 40 Hz). Accumulation of $P_{MAX–EV}$ results across stimulus

trials indicated the same trends as for $P_{AVE–EV}$. For simplicity, only $P_{AVE–EV}$ data are presented.

Bladder contractions were evoked by stimulation at both lower (0.5–1 Hz) and higher (20–50 Hz) continuous frequencies. Five cats (including both cats with PN cuff placements that contained urethral sensory fibers) were categorized as lower frequency responders (average $f_{TR} - P_{AVE–EV}$ slope = –0.31 ± 0.17) and three were categorized as higher frequency responders (average $f_{TR} - P_{AVE–EV}$ slope = 0.87 ± 0.43). These two groups were significantly different from each other ($p < 0.05$). In the lower frequency responding cats, 1 Hz continuous stimuli evoked significantly greater $P_{AVE–EV}$ than 33 Hz continuous stimuli (11.0 ± 3.8 cm H_2O, 4.2 ± 3.6 cm H_2O, $p < 0.001$). In the higher frequency responding cats, 33 Hz continuous stimuli evoked significantly greater $P_{AVE–EV}$ than 1 Hz continuous stimuli (16.9 ± 8.1 cm H_2O, 1.7 ± 3.2 cm H_2O, $p < 0.01$).

D. Responses to Pulse Bursting

The use of variable patterned pulse bursting resulted in increased evoked pressures over single pulse continuous stimulation in all experiments (Fig. 4). The best burst patterned stimulus provided an average increase of 52.0 ± 44.5% ($p < 0.004$) over the dominant single pulse continuous stimulation frequency (1 Hz or 33 Hz). Lower frequency responders increased 56.4 ± 46.6% ($p < 0.008$) and higher frequency responders increased 44.6 ± 49.6% ($p > 0.10$, nonsignificant due to small n of three experiments) from single pulse stimuli to the most favorable bursting patterned stimuli. For two experiments, the absence of error bars in Fig. 4 indicates that only a single trial was run for a stimulus parameter combination.

The effect of the train rate frequency (f_{TR}) and number of pulses ($\#_P$) on evoked bladder pressures ($P_{AVE–EV}$) is shown in Fig. 5. Regions of improved $P_{AVE–EV}$ are evident on the surface plots for both lower and higher frequency responding animals. The largest evoked pressures occurred for bursting stimulation patterns with greater than one pulse, as opposed to the commonly used single pulse, continuous stimulation patterns. Pulse frequencies (f_{PU}) used in Fig. 5 were 100 and 200 Hz for lower frequency responders and 200 Hz for higher frequency responders.

The most effective bursting stimulus patterns in lower frequency responding cats were consistent, leading to a robust increase in the evoked pressure over the nominal single pulse stimuli. The best bursting stimulus patterns in lower frequency responders were 5/10 × 100/200 Hz @ 1 Hz, depending on the cat. Across all trials with these parameters, significantly greater $P_{AVE–EV}$ than 1 Hz single pulse continuous stimuli was evoked (14.3 ± 4.7 cm H_2O, 11.0 ± 3.8 cm H_2O, $p < 0.002$). Without pooling, 5 and 10 pulses × 100/200 Hz @ 1 Hz waveforms evoked significantly greater $P_{AVE–EV}$ than 1 Hz continuous stimuli (14.2 ± 4.8 cm H_2O, $p < 0.004$; 15.4 ± 4.0 cm H_2O, $p < 0.003$) while not being significantly different from each other ($p > 0.35$).

The most effective bursting stimulus patterns were not consistent in higher frequency responding cats, leading to a moderate increase in the evoked pressure over the nominal single pulse stimuli. In higher frequency responding cats, a bursting pattern could always be selected to generate greater pressures than the nominal single pulse stimuli, but not at a regular f_{TR}. The best bursting stimulus patterns were 2 × 200 Hz @ 20/25/33 Hz, depending on the cat. Across all trials with 2 $\#_P$ and 20, 25, and 33 Hz f_{TR}, significantly greater $P_{AVE–EV}$ than 33 Hz single pulse continuous stimuli was not evoked (16.6 ± 7.0 cm H_2O, 16.9 ± 8.0 cm H_2O, $p > 0.90$). If only the best bursting patterned stimulus from each higher frequency responding cat is pooled together using parameters given in Fig. 4, for comparison to 33 Hz single pulse continuous stimuli, a greater but not significant difference was observed (20.2 ± 8.4 cm H_2O, 16.9 ± 8.0 cm H_2O, $p > 0.30$).

Although increasing $\#_P$ initially improved $P_{AVE–EV}$, further increasing $\#_P$ led to a fall-off of the evoked bladder pressure response (Fig. 5). This decrease was observed before stimulation became essentially continuous, the limit of the parameter space. The evoked bladder pressure fell to about 25% of the maximum $P_{AVE–EV}$ when the number of pulses was 51.8 ± 9.3% (higher frequency responders: for 10, 20, 33, and 50 Hz f_{TR}) and 17.6 ± 9.8% (lower frequency responders: for 0.5 and 1 Hz f_{TR}) of the amount that would result in a continuous stimulus at a f_{PU} of 200 Hz.

The f_{PU} space was not thoroughly explored, however f_{PU} of 100 and 200 Hz ($\#_P = 5$, $f_{TR} = 1$ Hz) were not significantly different (15.6 ± 3.9 cm H_2O, 13.7 ± 5.0 cm H_2O, $p > 0.10$) from each other. For lower frequency responding animals, variations to f_{PU} indicated regions of low and high evoked responses, with approximately 75 Hz separating the two regions. Pulse frequencies of 33 and 50 Hz were significantly lower than both 100 and 200 Hz ($p < 0.02$) but were not significantly different from each other ($p > 0.60$), for $\#_P = 5$, $f_{TR} = 1$ Hz. In two cats, limited trials of f_{PU} at 500 and 1000 Hz were not different than 100 and 200 Hz ($p >$, 0.35), for $\#P = 5$, $f_{TR} = 1$ Hz.

E. ANOVA Analyses

Across all data pooled together and data subgroups of the lower and higher frequency responders and pooled trials of the balanced sets, most significant experimental factors were consistent. Any variations found in the analyses were expected based on the balanced set designs or shifts in viewpoint due to analyzing a fraction of the overall data set. The train rate frequency (f_{TR}) was always a significant factor ($p < 0.05$). The number of pulses ($\#_P$) was only significant ($p < 0.05$) in the ANOVA analysis of the pooled data ($p > 0.05$ otherwise). This was expected as subgroups of the pooled data allowed for nonlinear interactions between f_{TR} and $\#_P$ to balance out the effect of pulse bursting at a few $\#_P$ values. The pulse frequency (f_{PU}) followed a similar pattern of testing significance when limited trials at extreme values, as described above, had an increased effect depending on the data subset being analyzed ($p < 0.05$ only for lower frequency responder group). Other factors, such as t_{STIM} and the bladder volume, were consistently significant ($p < 0.001$, $p <$ 0.05). Thus, using the randomized pre-determined sets or all data pooled together did not change the conclusions of this work that pulse bursting results in greater evoked bladder pressures.

IV. Discussion

In all cats, bursting of PN stimuli evoked greater reflex bladder contractions than continuous single pulse stimuli. Whereas the higher frequency responding cats had similar optimal single pulse stimuli (20 Hz, 33 Hz) as that reported elsewhere [14]–[17], evidence of responsiveness to low frequency (0.5–1 Hz) PN stimuli was also obtained. The use of low frequency stimuli with pulse bursting while operating at lower bladder volumes may result in improved efficacy of clinically implemented neuroprostheses for restoration of bladder function.

A. Responses to Pulse Bursting

The variable patterned stimuli used here are analogous to action potential burst timings, with $\#_P$ related to the number of spikes, f_{PU} to the intraburst frequency, and $1/f_{TR}$ to the inter-burst interval. Neural recordings have indicated action potential patterns similar to the stimuli used in this study, including hippocampal spike timings (2-5 × ~ 200 Hz @ 40 Hz) [37] and the firing rates of neurons near intact intestinal mucosa responding to mucosal stimulation (3.8 ± 0.3 × 39 ± Hz) [32]. PN afferents have shown bursts of 4–16 spikes in response to scrotal temperature changes [36], bursting (8–10 spikes up to 400 Hz) initiated

by mechanostimulation of the vaginal wall [34], and action potential firing rates increasing up to 400 Hz in reaction to clitoral tapping [35]. Comparable variable patterned stimuli have been used for nonbladder related afferent fiber activation, such as for improved release of "brain-derived neurotrophic factor" in the dorsal horn when using bursting (4 × 100 Hz @ 2 Hz) in comparison to constant frequency stimuli [40], enhanced reciprocal inhibition between ankle flexor and extensor muscles (10 pulses × 100 Hz @ 0.67 Hz) while continuous stimulation was ineffective [41], activation of visceral pain responses in the bowel (5 × 200 Hz @ 2 Hz) [42], and reduction of soleus muscle spasticity in hemiplegics by superficial peroneal stimulation (5 × 250 Hz @ 0.3/0.7/2/4 Hz) [43]. Bursting patterned stimulation may increase recruitment of bladder activating post-ganglionic pelvic nerve fibers as has been shown to occur for increased frequency of preganglionic stimulation [44]. The occur-rence of burst patterns during sensory stimuli and their effectiveness in synaptic transmission strongly suggests a possible role in evoking reflex bladder contractions. Our results in exploring burst pattern parameters ($\#_P$ and f_{TR}) support this role.

The present study defines the effective bursting parameter regions for afferent mediated bladder excitation. At least two studies have used bursting stimulation for bladder excitation. A 3 × 100 Hz @ 1 Hz patterned stimulus has been used to test reflex bladder discharges [12]. The use of 3 × 300 Hz @ 0.3 Hz and 3 × 300 Hz @ 3 Hz patterned stimuli were included with the use of continuous 10 Hz, 20 Hz, and 50 Hz stimulation for PN-evoked excitation of the bladder in decerebrate and spinalized cats [11]. Whereas those studies alluded to the possibility of using lower frequency stimuli coupled with bursting of pulses for reflex bladder activation, they did not evaluate a wider spectrum of parameters as was done here. These examples are consistent with our results in evoking bladder contractions using pulse bursting and they used stimulus parameters near the most effective lower frequency range observed in this study (Fig. 5).

The use of pulse bursting increases the potential parameter space for generating bladder contractions with PN stimuli. This extra dimension allows for optimal stimulus patterns for reflex bladder excitation to be explored, which may lead to improved voiding efficiencies closer to that obtained when using sacral stimulation following dorsal rhizotomy. However, the increased parameter space is not unlimited. Combined increases in $\#_P$ and f_{TR} result in continuous or near-continuous stimuli that can approach or exceed f_{PU}, which are not effective. Responsiveness of the system to these patterned stimuli, though, falls off well before the total pulses per second become f_{PU}, (Fig. 5).

B. Lower Frequency Excitation

Most PN-afferent bladder excitation studies have focused on the use of 20–33 Hz continuous stimuli [12], [14]–[18], [26]. The higher frequency responding cats had similar effective single pulse stimuli (20 Hz, 33 Hz). This study provides evidence of responsiveness to low frequency (0.5–1 Hz) PN stimuli, though less than that evoked by higher frequency stimuli. Although 2 Hz has been used for reflex bladder excitation via intraurethral stimulation in cats [27] and humans [27], [28], PN excitation has not focused on lower frequencies. In general, PN stimulation with continuous stimulation frequencies below 10 Hz are thought to primarily induce continence reflexes, not bladder excitation [15], [17], [18]. Researchers have performed limited trials of PN stimulation at lower frequencies, suggesting the potential to evoke contractions at 0.5 Hz [16], 1 Hz [10], 2 Hz [14], and below 10 Hz [17]. In the present work, lower frequency responders were effectively activated by continuous single pulse 0.5 and 1 Hz stimuli. Limited trials outside that range indicated a quick reduction in excitability (1.25 Hz excitable; 0.25 Hz and 2 Hz not consistently excitable; 3 Hz inhibitory) (Fig. 5).

In lower frequency responding cats, 5 and 10 $\#_P$ repeated at 1 Hz f_{TR} were consistently the best burst stimuli. However, the optimal f_{TR} for burst patterned stimuli was not consistent in higher frequency responding cats, with pooled results not significantly greater than for single pulse 33 Hz continuous stimuli. The lack of a consistent best f_{TR} is due to the broader range of higher frequencies. The variability in optimal frequencies (20–33 Hz) in higher frequency responding cats is consistent with the variability in preferred frequencies observed by different research groups (20 Hz [16], [18] versus 33 Hz [14], [15], [17]).

The lack of a consistent dominant frequency range across all experiments has been reported elsewhere [17]. Other research has indicated excitation for both lower and higher frequency PN stimulation but has not discussed whether the preferred frequency was consistent across experiments [14], [16]. The preferential excitation of the PN reflex pathway using lower or higher frequency stimulation patterns among experiments and the varying evoked pressure levels are not well understood but may be linked to the activation of different afferent fiber classes within the PN [17], anatomical variations among PNs [17], and varying effects of the anesthetic agents, surgical procedures, and other experimental procedures performed.

C. Clinical Relevance

Pulse bursting increases the potential parameter space for bladder contractions evoked by PN stimuli, thereby providing a wider range of utility for clinical applications. As the optimal stimulus parameters for humans are unknown [28], having a larger parameter set to choose from, both for bursting and low frequency stimuli, may increase the prospect of clinical effectiveness and will yield more customization options for individual neuroprosthetic users. Although the magnitude of the evoked pressure increases with bursting seen here was not large (3.3 cm H_2O in both lower frequency and higher frequency responders), the percentage increase seen (30% and 19.5%, respectively) may have a clinical impact.

For higher frequency responders, using an intermittent on/off pattern for a bursting patterned stimuli may result in greater bladder drive and more effective voiding than simple intermittent trains of continuous stimuli. The effective stimulus duty cycle for low frequency bursting ($\#_P \cdot f_{TR}/f_{PU}$ = 2.5-10% for 5 × 200 Hz @ 1 Hz and 10 × 100 Hz @ 1 Hz) is well below that used in intermittent PN stimulation for voiding (77%–83%) [26] and sacral root motor drive (33%–67%) [7], [8], [26]. The smaller duty cycles and lower aggregate stimulation levels from lower frequency stimulus bursting may reduce the potential for habituation, which can limit the effectiveness of afferent-mediated approaches. The extended stimulus off periods in bursting stimulation allow more time for voiding and may lead to more physiological voiding patterns by allowing a nearly continuous stream of urine. The potential for improved voiding may allow PN stimulation to reach voiding efficiencies obtained by sacral stimulation after dorsal rhizotomy. Additionally, stimulator power requirements are decreased for lower frequency bursting when compared to the use of continuous stimulation or bursting at higher frequencies due to the lower number of total pulses per second.

As has been discussed elsewhere [15], [16], [18], PN stimulation for bladder excitation may be coupled with inhibitory PN stimulation such that continence could be induced until a time that a neuroprosthetic user was ready to empty their bladder. This bladder neuroprosthetic will be particularly useful for an individual with spinal cord injury who has a hyperreflexive bladder that contracts at low volumes. After reducing bladder contractions by using an inhibitory stimulus for continence, the use of a pulse bursting stimulus may provide improved bladder excitation during the voiding phase of bladder control. Future translational studies should include voiding trials, tests of efficacy after spinal transection and evaluations in clinical settings.

D. Bladder Volume

A minimum bladder volume is necessary before either dis-tension-evoked (V_{TH}) or stimulation-evoked (V_{STIM}) bladder contractions can be elicited [15], [21], [28]. The majority of PN afferent stimulation studies that indicated the ability to evoke reflex contractions below V_{TH} have focused on trials above V_{TH} [14]–[16], operating at V_{STIM}/V_{TH} of 125%–200% [14]–[16], [26]. The present work indicated that consistent, effective PN stimulation for reflex excitation of the bladder at functional (below "full") volumes is feasible. Ratios of V_{STIM}/V_{TH} of 66 ± 17% [14], 78 ± % [15], less than 70% [16], and 65 ± 17% [45] have been observed, using slightly different threshold criteria. The present work utilized bladder volumes below V_{TH} the majority of the time, with an average V_{STIM}/V_{TH} ratio of 81.0 ± 23.7%. This V_{STIM}/V_{TH} ratio is likely overestimated due to a more relaxed bladder contraction threshold definition. Extending the 24 fill cycles that did not result in a distension evoked contraction to the point where V_{TH} was reached would have resulted in a lower V_{STIM}/V_{TH} ratio. Focusing research on reflex bladder excitation at volumes below that resulting in distension evoked contractions will give greater autonomy to a potential neuroprosthetic user, providing a greater bladder volume margin in which a user would be able to empty their bladder before undesired contractions could lead to incontinence.

E. Study Limitations

The unbalanced evaluation of stimulus parameters across experiments was a limitation of this study. In each experiment, if preferential responsiveness to lower or higher f_{TR} stimuli was observed then the parameter space in that region was tested further. Aside from the limited use of factorial designs, a complete evaluation of the parameter space in all cats was not performed. However, as is indicated in the results, ANOVA analyses of the randomized predetermined sets and the overall data set indicated similar significant factors for cases where partitioning the data by taking a data subset did not distort the analysis. This indicates that the data was not unreasonably biased in any direction.

Several factors may have contributed to the minimal excitability seen in the five cats that were excluded, including possible shifts in spinal reflexes or damage to lower urinary tract innervation due to surgical procedures and other experimental protocols performed, the possibility of bypassing a tight V_{STIM}/V_{TH} range for evoking contractions, or possible anesthetic interference [46]. It may also be possible that some cats do not respond to neuromodulation for bladder control using afferent pathways. Clinical trials investigating the use of neuromodulation for nonfunctional bladders have indicated a subset of their subjects (20%) that were nonresponders [13].

V. Conclusion

Variable or bursting stimulus patterns evoke greater bladder contractions than that elicited by single pulse continuous stimuli. Bursting increases the effective stimulation parameter space for generating bladder contractions. This work also provides evidence that PN-evoked bladder contractions can be elicited with lower frequency stimuli than typically used and that consistent reflex bladder excitation at functional bladder volumes is feasible. Improved bladder excitation should increase the efficacy of PN afferent-based neuroprostheses for restoration of bladder function.

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Biography

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Narendra Bhadra received graduate medical and postgraduate orthopedic degrees from University of Calcutta, India, in 1978 and 1983, respectively, and the Ph.D. degree in bioengineering from Case Western Reserve University, Cleveland, OH, in 2001. He completed his residency and postgraduate training at Calcutta Medical College and University College of Medicine, Calcutta.

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Dr. Gustafson received an Excellence in Neural Engineering Award from the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society, in 2002.

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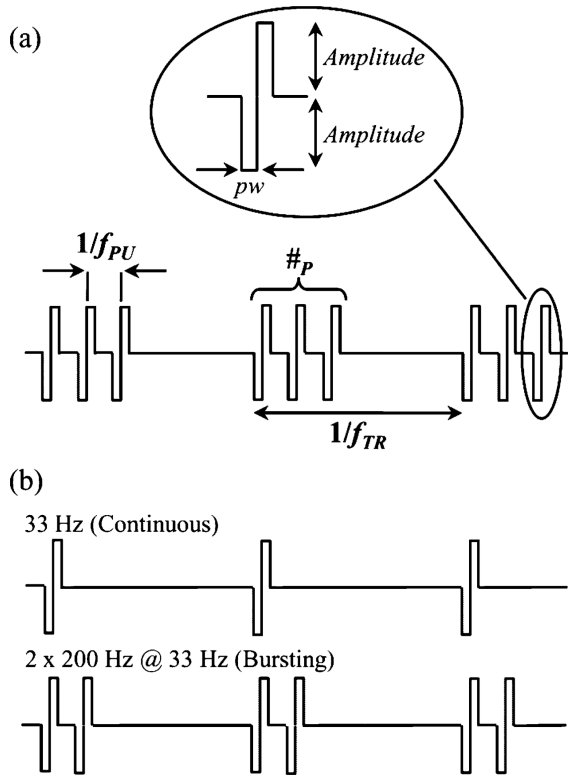

Fig. 1.

(a) Schematic representation of variable "burst" stimulus pattern. Nomenclature: pw = pulse width; $\#_P$ = number of pulses; f_{PU} = pulse frequency; f_{TR} = train rate frequency. (b) Example traces for continuous (e.g., 33 Hz) and burst (e.g., 2 × 200 Hz @ 33 Hz) stimulation.

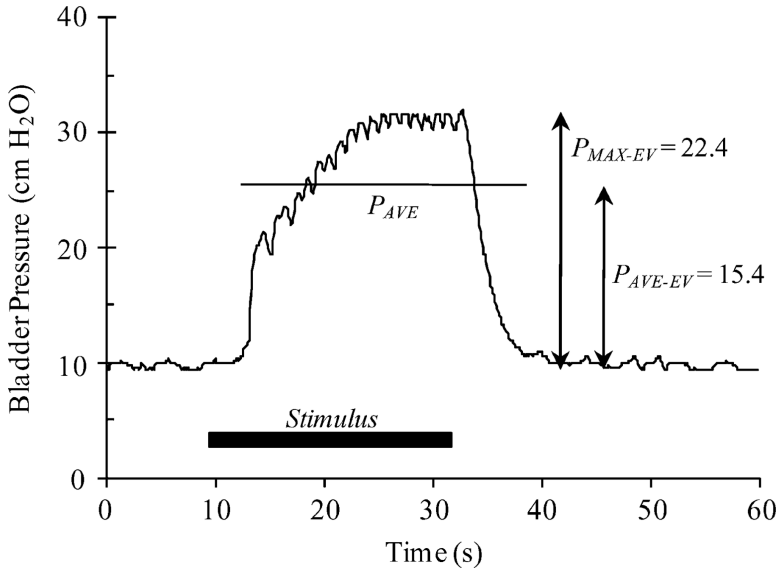

Fig. 2.

Example of stimulus evoked bladder contraction, for a low-frequency bursting stimulus pattern (5 × 200 Hz @ 1 Hz). Measured parameters: $P_{BASE–AVE}$ = 3 s pressure average prior to stimulus initiation; $P_{BASE–SD}$ = 3 s pressure standard deviation prior to stimulus initiation; $EVAL_{TH} = P_{BASE–AVE} + 3 \cdot P_{BASE–SD}$; P_{AVE} = av-erage pressure while above $EVAL_{TH}$(duration noted by horizontal line in figure); P_{MAX} = maximum pressure while above $EVAL_{TH}$; $P_{AVE–EV}$ $P_{AVE} - P_{BASE–AVE}$; $P_{MAX–EV} = P_{MAX} - P_{BASE–AVE}$.

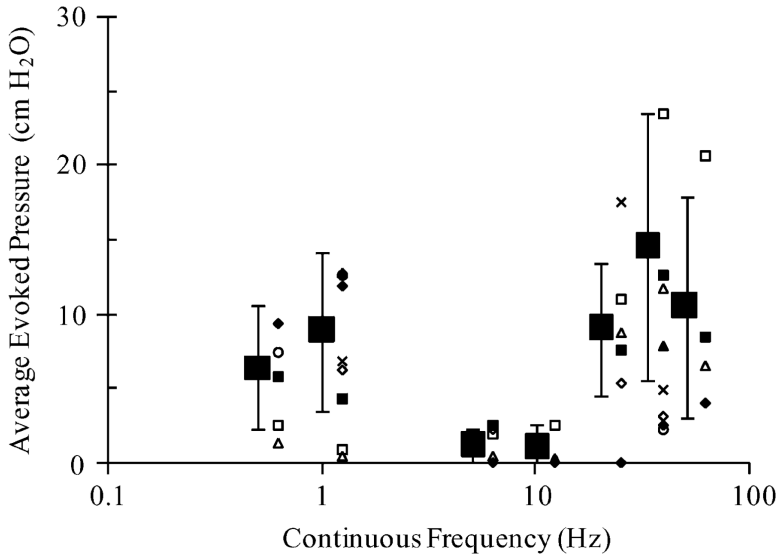

Fig. 3.

Effective continuous stimulation frequencies for bladder activation. Average evoked pressures ($P_{AVE–EV}$) at 0.5, 1, 5, 10, 20, 33, and 50 Hz train rate frequencies (f_{TR}), for one pulse ($\#_P$). Large squares indicate results averaged across all trials performed, with pooled standard deviations as error bars. Smaller icons at each frequency represent averages from individual experiments.

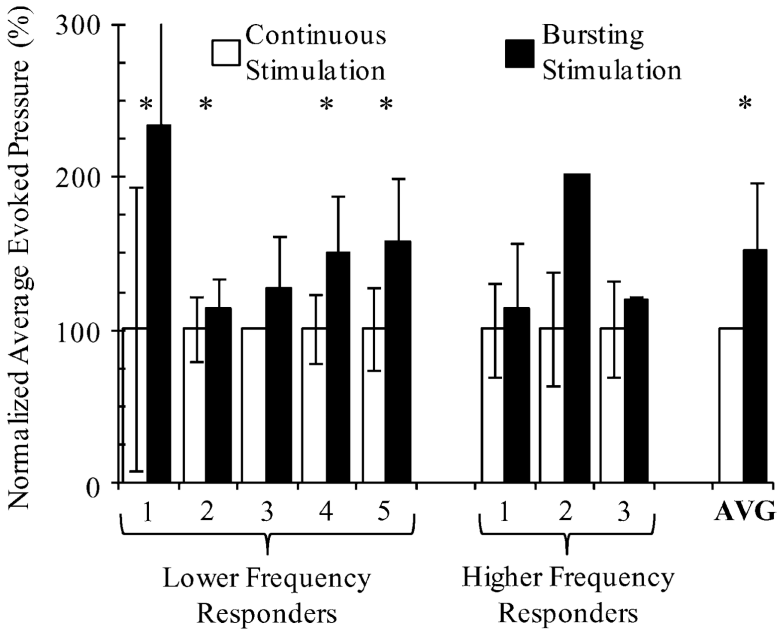

Fig. 4.

Pulse bursting increased evoked pressure over continuous single pulse stimuli in every experiment. The multipulse stimulus parameter combination in each experiment that resulted in the largest evoked pressure was used for comparison. In each experiment, the average and standard deviation (error bars) of the average evoked pressure for single and multiple pulse stimulus patterns are normalized to the mean average evoked pressure for the single pulse stimulus pattern. Continuous stimulation frequencies were 1 Hz for lower frequency responders and 33 Hz for higher frequency responders. Bursting stimulation patterns were 5 × 100/200 Hz @ 1 Hz for lower frequency responders 1, 2, 3, and 5, and 10 × 100/200 @ 1 Hz for lower frequency responder 4. Bursting stimulation patterns were 2 × 200 Hz @ 20, 25, and 33 Hz for higher frequency responders 1, 2, and 3, respectively. Asterisks indicate experiments in which average pressures were significantly different ($p < 0.05$), using student's t-test. The evoked pressure increase average across experiments was also significantly different ($p < 0{:}005$), according to a t-test for two dependent samples. The standard deviation for bursting stimulation in lower frequency responder #1 was 123%.

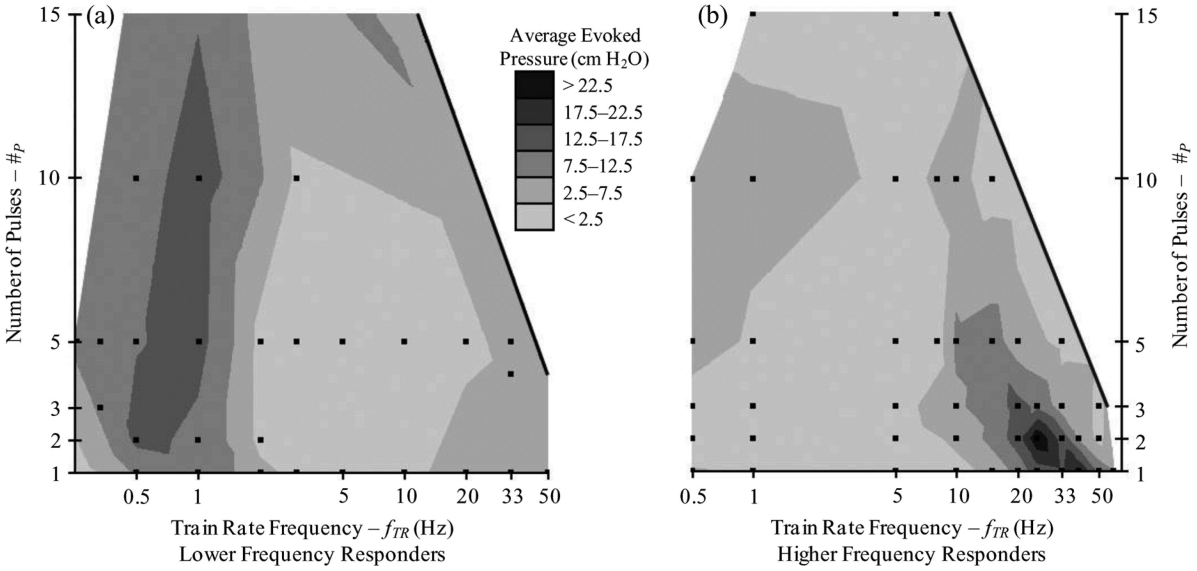

Fig. 5.

Contour plots of average evoked pressure ($P_{AVE–EV}$) against number of pulses ($\#_P$) and train rate frequency (f_{TR}), for (a) lower frequency responding and (b) higher frequency responding experiments. Preferred ranges indicate that the addition of pulses results in greater evoked pressures than that evoked by continuous frequency stimulation at $\#_P = 1$. The dots indicate locations of stimulus parameter combinations that were included; 280 trials across 27 stimulus parameter combinations in (a) and 293 trials across 47 stimulus parameter combinations in (b). The region outside the solid line indicates the limit of the parameter space where adding pulses leads to a continuous stimulation frequency, equal to or greater than the pulse frequency (f_{PU}), for a pulse frequency of 200 Hz.

TABLE I

Evoked Pressures for Single Pulse Continuous Frequencies

f_{TR} (Hz)	$P_{AVE\text{-}EV}$ (cm H_2O) Average ± S.D.	$P_{MAX\text{-}EV}$ (cm H_2O) Average ± S.D.	Trials (Cats)
0.5	6.4 ± 2.2	11.9 ± 7.6	22 (5)
1	8.9 ± 5.4	15.1 ± 8.5	30 (8)
5	1.1 ± 1.1	1.9 ± 1.4	9 (5)
10	1.0 ± 1.7	1.7 ± 2.1	9 (3)
20	9.0 ± 4.5	17.0 ± 8.3	18 (6)
33	14.5 ± 9.0	27.9 ± 17.3	109 (8)
50	10.5 ± 7.4	17.9 ± 13.3	11 (4)