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Sensory pudendal nerve stimulation increases bladder capacity through sympathetic mechanisms in cyclophosphamide-induced cystitis rats

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Aims: Interstitial cystitis and bladder pain syndrome is a prevalent health concern with inadequate treatments. Neuromodulation has emerged as a therapeutic option to treat patients refractory to standard care. The objective of this study was to determine the efficacy and mechanism(s) of sensory pudendal nerve stimulation on bladder function in cystitis rats.

Methods: Female rats were administered saline ($n = 8$) or cyclophosphamide (CYP, 150 mg/kg IP, $n = 16$) and single-trial cystometry experiments were conducted under urethane anesthesia 48 h after injection. Electrical stimulation (0.02-0.22 mA, 10-20 Hz) was delivered to the sensory branch of the pudendal nerve and its effect on the bladder and external urethral sphincter were measured. Stimulation trials were also conducted following bilateral hypogastric nerve transection (HGNT) or pharmacological inhibition of beta-adrenergic receptors (propranolol, 1 mg/kg IV) to determine the mechanisms of bladder inhibition.

Results: CYP-induced cystitis decreased bladder capacity ($P = 0.0352$) and bladder compliance ($P = 0.024$) by up to 38% of control. Electrical stimulation of the sensory pudendal nerve increased bladder capacity ($P < 0.0001$) in control and CYP rats by up to 51-52% of their respective baselines. HGNT did not influence bladder inhibition generated by sensory pudendal nerve stimulation in control rats, whereas HGNT and propranolol decreased the efficacy of electrical stimulation in CYP rats.

Conclusions: Sympathetic reflex activity mediates sensory pudendal nerve stimulation in CYP treated but not control rats. These studies demonstrate an alternative approach to neuromodulation in cystitis and establish mechanistic changes during stimulation that may enable the development of novel therapeutics.

KEYWORDS
electrical stimulation, hypogastric nerve, inflammation, propranolol, pudendal nerve, urinary bladder

Martin Michel led the peer-review process as the Associate Editor responsible for the paper.

1 | INTRODUCTION

Interstitial cystitis (IC) and bladder pain syndrome (BPS) is estimated to affect 3-8 million U.S. women and 1-4 million U.S. men age 18 or older.[1] IC/BPS is defined as unpleasant sensations and lower urinary tract symptoms lasting longer than 6 weeks and in the absence of other clinical causes.[2] Despite the considerable patient population, the pathophysiology of IC/BPS is not clear and is likely multifactorial. This unknown pathophysiology results in diverse treatment options that do not reliably resolve symptoms, and a wide range of dietary, behavioral, and pharmacological interventions have been used without consistent benefit.[2] There are only two treatments, dimethyl sulfoxide and pentosan polysulfate sodium, that are FDA-approved for IC and the efficacy for a patient is unpredictable.[2]

There is therefore a need to develop approaches to treat patients refractory to conventional interventions. Sacral neuromodulation is a fourth-line treatment to improve symptoms in IC/BPS,[2] and is associated with improvement in pain, symptom scores, daytime frequency, nocturia, urgency, and voided volume.[3] Additionally, sacral neuromodulation decreased micturition frequency[4] and reduced the frequency of voiding and non-voiding contractions in preclinical bladder irritation models.[5] These data suggest neuromodulation may provide additional benefit over the established approaches.

An alternative to sacral neuromodulation that has not yet been explored for IC/BPS is sensory pudendal nerve stimulation. The pudendal nerve is a distal branch of S2-4 and pudendal nerve stimulation inhibits the micturition reflex and increase bladder capacity in animals[6,7] and humans.[8] Patients with voiding dysfunction and pudendal nerve stimulation demonstrated greater improvement in pelvic pain, urinary frequency, and urgency when compared to sacral neuromodulation.[8] The objective of this study was to investigate the efficacy of sensory pudendal nerve stimulation in a rat model of cystitis. We sought to quantify effects on bladder capacity in cystitis rats and determine the mechanisms of bladder inhibition with neuromodulation. By targeting the pudendal nerve in a preclinical model of cystitis, we establish a foundation for alternative approaches to neuromodulation in IC/BPS.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult female Wistar rats (200-250 g) purchased from Charles River (Boston, MA) were housed two per cage and maintained in standard laboratory conditions. Prior to experimentation, rats received an intraperitoneal (ip) injection of saline (1 mL, $n = 8$) or cyclophosphamide (150 mg/kg, $n = 16$, CYP, Sigma-Aldrich, St. Louis, MO). Rats then underwent terminal cystometry 48 h after the injection. CYP is well established to increase voiding frequency, decrease bladder capacity, and increase somatic sensitivity.[9]

2.2 | Surgical procedures

Rats were anesthetized with urethane (1.2 g/kg sc, and supplemented as needed) 1 h prior to surgical procedures for acute terminal experiments. Urethane is the preferred anesthetic agent to use in urodynamic studies because the micturition reflex is preserved.[10] Body temperature was monitored and adjusted to 37°C by a thermostatically controlled warming pad (ATC2000, World Precision Instruments, Sarasota, FL). Heart rate and arterial blood oxygen saturation levels were monitored by a pulse oximeter (2500A, Nonin Medical Inc., Plymouth, MN).

The sensory branch of the pudendal nerve contains nerve fibers that innervate the urethra, genitalia, and perigenital skin and stimulation can generate bladder inhibition.[7] The sensory pudendal nerve was accessed using a posterior approach with the animal in a prone position as described previously.[11] Briefly, the gluteus muscles were incised, the ilium and sacrum were separated, and the pudendal nerve was isolated from connective tissue and vasculature. A bipolar cuff electrode (2 mm length, 0.2 mm inner diameter, CorTec, Freiburg, Germany) was placed on the sensory pudendal nerve for electrical stimulation. The leads were secured, the incision was closed, and the animal was turned to a supine position for cannulation of the jugular vein and/or implantation of the bladder catheter.

For experiments with intravenous drug administration, the skin was incised adjacent to the trachea, the jugular vein was isolated from connective tissue, and a PE-50 catheter was inserted into the lumen and secured. The urinary bladder was then exposed through a lower midline abdominal incision and a flared PE-60 catheter was inserted through the bladder dome into the lumen and secured. The bladder catheter was connected to an infusion pump (PHD 4400, Harvard Apparatus, Holliston, MA) and to a pressure transducer and amplifier (ETH-255, CB Sciences Inc., Dover, NH) to measure intravesical pressure. A bipolar paddle electrode (Micro-Leads, Boston, MA) was placed between the pubic symphysis and the external urethral sphincter (EUS) and connected to an amplifier to measure EUS electromyogram (EMG). Pressure and EMG signals were amplified, filtered, and sampled at 400 or 4000 Hz, respectively, by a PowerLab 8/30 recording unit (AD Instruments, Colorado Springs, CO) and displayed by LabChart 7 Pro (v7.3.7, AD Instruments) for off-line analysis. Following experimentation, animals were euthanized with Euthasol (250 mg/kg, ip) and bilateral thoracotomy.

2.3 | Cystometry

The urinary bladder recovered from instrumentation during 45 min of continuous infusion of physiological saline (1-8 mL/h) and the urethra open. The infusion rate was adjusted in each animal to achieve ~10 min intermicturition intervals. Voided volumes were collected and measured with a 1 mL syringe after each voiding event.

Following the recovery period, the bladder was emptied and the current threshold to evoke a reflex response in the EUS from electrical stimulation of the sensory pudendal nerve was determined at 1 Hz. Single-trial cystometry then began by filling (1-8 mL/h) the bladder until a voiding event, stopping the infusion pump, capturing infused, residual, and voided volumes, and waiting 3 min before the next trial. Regulated current, biphasic stimulus pulses were applied with a pulse width of 0.1 ms, frequency between 10 and 20 Hz, and one and two times the EUS reflex threshold amplitude (0.02-0.22 mA). These parameters were selected because they generate increased bladder capacity in control and overactive bladder rats[7] and do not inhibit initiation of bladder contractions as observed with higher amplitudes. Electrical stimulation was applied continuously throughout the filling phase and terminated prior to the voiding event to preserve bladder emptying efficiency. Parameters were randomized and each pair had at least two replicates that were averaged within animals for a total of $n = 98$ control trials and $n = 195$ CYP trials.

Upon completing the baseline stimulation parameter replicates in single-trial cystometry, a subgroup of rats ($n = 8$ control, $n = 8$ CYP) underwent transection of the hypogastric nerves (HGNT). Briefly, the hypogastric nerves were isolated through the lower midline abdominal incision at the level of the inferior mesenteric ganglion and transected bilaterally. A separate subgroup of rats ($n = 8$ CYP) with intact hypogastric nerves were administered propranolol hydrochloride (1 mg/kg/h iv, Sigma-Aldrich) after completing baseline single-trial cystometry. Propranolol is a non-selective beta-adrenergic antagonist that blocks inhibition of the bladder produce by pudendal nerve stimulation following acetic acid irritation.[12] Propranolol has a distribution half-life of 22 min and an elimination half-life of 85 min.[13] Randomized cystometry trials were conducted 15 min after initial propranolol administration and rats were re-dosed every hour to maintain steady state. After either HGNT or propranolol and similar to the design of the previous block, electrical stimulation was applied continuously throughout the filling phase and each parameter pair had at least two replicates that were averaged within animals for a total of $n = 101$ control trials and $n = 202$ CYP trials.

2.4 | Data analysis

As described previously,[11] cystometrograms were analyzed using the following parameters: pressure at volume threshold (cmH_2O), defined by the user at the first deflection of pressure that occurred during a voiding event; filling pressure (cmH_2O), the mean value of the data points between the start of the infusion pump and the user-defined pressure at volume threshold; peak micturition pressure (cmH_2O), the value of the largest data point during a voiding contraction; volume threshold (mL), the volume instilled into the bladder from the start of the infusion pump to the user-defined pressure at volume threshold; bladder compliance (mL/cmH_2O), calculated by dividing the infusion rate by the slope of bladder pressure versus time; EUS bursting time (s), the average time of EUS bursting activity per single-trial cystometrogram; and voided percentage (%), expressed as the percentage of voided volume divided by the sum of the voided volume and residual volume.

All values are reported mean ± standard deviation. The results of electrical stimulation of the sensory branch of the pudendal nerve on bladder function were compared with repeated measures analysis of variance (ANOVA). The remaining data, such as reflex threshold amplitude and baseline analyses, were compared with two-tailed Student's unpaired or paired *t*-test as appropriate. When *F*-test statistic exceeded the critical value at $\alpha = 0.05$, the Bonferroni multiple comparisons test was used to compare group means.

3 | RESULTS

3.1 | CYP-induced cystitis decreased bladder capacity and bladder compliance

Rats maintained similar body weight 48 h after saline or CYP injection (227 ± 22 and 219 ± 19 g respectively, $P = 0.3949$). During single-trial cystometry, CYP rats had decreased volume threshold ($P = 0.0352$) compared to control rats, as well as decreased bladder compliance (0.21 ± 0.03 and 0.13 ± 0.09 mL/cmH_2O, $P = 0.024$) (Tables 1 and 2). Unlike the changes in bladder capacity and compliance, intravesical pressures ($P \geq 0.05$), voided percentage ($P = 0.1453$), and EUS bursting time (3.23 ± 2.1 and 3.65 ± 1.47 s, $P = 0.5956$) were similar in control and CYP rats (Tables 1 and 2).

3.2 | Electrical stimulation of the sensory pudendal nerve increased bladder capacity

The minimum current amplitude to evoke an EUS reflex from stimulation of the sensory branch of the pudendal nerve was similar in control and CYP rats (0.05 ± 0.02 and 0.07 ± 0.02 mA, respectively, $P = 0.0844$). Electrical stimulation also yielded similar increases in bladder capacity in control (20-52%) and CYP (25-51%) rats and we did not observe carry-over effects between trials.

Control: Sensory pudendal nerve stimulation increased volume threshold ($P < 0.0001$) across amplitudes (1T and 2T)

TABLE 1 Cystometrogram measurements for control rats

	Average filling pressure, cmH_2O	Pressure at volume threshold, cmH_2O	Peak micturition pressure, cmH_2O	Volume threshold, mL	Voided percentage, %
Control					
Baseline	4.06 ± 0.5	6.65 ± 0.9	26.3 ± 2.5	0.76 ± 0.19	23 ± 10
Pudendal *n*., sensory					
1T, 10 Hz	4.44 ± 0.5	7.55 ± 1.2	27.3 ± 2.7	0.98 ± 0.22***	20 ± 12
1T, 20 Hz	3.95 ± 0.7	7.43 ± 1.2	26.6 ± 2.6	0.93 ± 0.3*	20 ± 9.2
2T, 10 Hz	4.58 ± 0.7	9.63 ± 3.6	28.8 ± 3.1	1.2 ± 0.32**	17 ± 12
2T, 20 Hz	4.51 ± 0.7	8.89 ± 2.1*	27.5 ± 2.3*	1.1 ± 0.34**	17 ± 11
Baseline, HGNT	4.18 ± 0.5	7.13 ± 1	24.1 ± 2.8****	0.75 ± 0.15	24 ± 11
Pudendal *n*., sensory					
1T, 10 Hz, HGNT	4.05 ± 0.4	6.85 ± 1	24.4 ± 2.8	0.98 ± 0.21**	22 ± 9.8
1T, 20 Hz, HGNT	4.0 ± 0.4	7.38 ± 1.4	24.2 ± 2.7	0.94 ± 0.19**	20 ± 10**
2T, 10 Hz, HGNT	4.39 ± 0.5	8.67 ± 2.2	25.1 ± 3	1.1 ± 0.27**	20 ± 9
2T, 20 Hz, HGNT	4.09 ± 0.7	7.87 ± 2.1	25 ± 3	1 ± 0.28*	22 ± 7.8

Electrical stimulation of the sensory branch of the pudendal nerve increased volume threshold and the effects were preserved after hypogastric nerve transection (HGNT). HGNT decreased peak micturition pressure relative to baseline but this had no impact on voided percentage. All values represent mean ± SD. $n = 8$, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ compared to paired control baseline. ****$P \leq 0.01$ compared to paired pre-treatment control baseline.

and frequencies (10 and 20 Hz) in control rats (Figure 1A, Table 1). Additionally, stimulation increased pressure at volume threshold ($P = 0.03$) and peak micturition pressure ($P = 0.0105$) relative to baseline (Table 1). Other cystometric parameters, including filling pressure ($P = 0.0548$), voided percentage ($P = 0.2270$), and EUS bursting time (3.23-3.55 s, $P = 0.622$) were unchanged with electrical stimulation of the sensory branch of the pudendal nerve (Table 1).

CYP: Sensory pudendal nerve stimulation increased volume threshold ($P < 0.0001$) across amplitudes and frequencies in CYP rats (Figure 2A, Table 2). Filling pressure ($P < 0.0001$), pressure at volume threshold ($P < 0.0001$), and peak micturition pressure ($P < 0.0001$) were also increased following electrical stimulation (Table 2). Although we observed decreased EUS bursting time in all parameters except 1T 20 Hz stimulation (2.74-4.25 s, $P = 0.0048$), electrical stimulation did not alter voided percentage ($P = 0.0938$) in CYP rats (Table 2).

3.3 | Sympathetic deactivation decreased the efficacy of electrical stimulation in CYP but not control rats

Control: Bladder function, apart from decreased peak micturition pressure ($P = 0.0026$), was not significantly changed in control rats after bilateral HGNT (Table 1). Sensory pudendal nerve stimulation continued to increase volume threshold ($P = 0.0002$) following HGNT in control rats (Figure 1B, Table 1). Increased peak micturition pressure ($P = 0.0489$) was also preserved, however the increase in pressure at volume threshold mediated by electrical stimulation was not ($P = 0.0810$) (Table 1). Similar to baseline cystometric parameters, electrical stimulation did not alter filling pressure ($P = 0.1162$), voided percentage ($P = 0.3027$), or EUS bursting time (2.31-3.32 s, $P = 0.1964$) following HGNT.

CYP: Comparable to control rats, bilateral HGNT in CYP rats decreased peak micturition pressure ($P = 0.0042$), as well as increased filling pressure ($P = 0.0079$) (Table 2). Sensory pudendal nerve stimulation, however, failed to increase volume threshold ($P = 0.2485$) or peak micturition pressure ($P = 0.1566$) following HGNT in CYP rats (Figure 2B, Table 2). HGNT did not block all the effects of electrical stimulation as we still observed increased filling pressure ($P = 0.0357$) and increased pressure at volume threshold ($P = 0.0233$) (Table 2). The other cystometric parameters, including voided percentage ($P = 0.4885$) and EUS bursting time (3.38-4.68 s, $P = 0.4068$), remained unchanged (Table 2).

To complement our studies of HGNT in CYP rats, we administered intravenous propranolol to determine the role of postsynaptic beta-adrenergic receptors. In this subgroup of CYP rats, propranolol increased filling pressure ($P = 0.0062$) and volume threshold ($P = 0.0180$) relative to baseline

TABLE 2 Cystometrogram measurements for CYP rats

	Average filling pressure, cmH_2O	Pressure at volume threshold, cmH_2O	Peak micturition pressure, cmH_2O	Volume threshold, mL	Voided percentage, %
CYP-induced cystitis					
Baseline	4.95 ± 1.6	8.63 ± 2.9	24.5 ± 3.4	0.5 ± 0.3	17 ± 7.4
Pudendal *n*., sensory					
1T, 10 Hz	5.6 ± 1.8***	10.6 ± 3.4****	26.1 ± 3.2***	0.64 ± 0.34****	16 ± 10
1T, 20 Hz	5.24 ± 1.8*	9.53 ± 3.2	25.2 ± 2.9	0.6 ± 0.32**	16 ± 10
2T, 10 Hz	5.97 ± 2.1****	11.2 ± 3.2****	26.8 ± 3.3**	0.71 ± 0.38****	13 ± 7.5**
2T, 20 Hz	5.42 ± 1.9**	10 ± 3.8*	25.5 ± 3.3	0.61 ± 0.32****	13 ± 9.7
Baseline, propranolol	6.7 ± 2.7******	12.1 ± 3.6	22.9 ± 3.2	0.52 ± 0.33*****	17 ± 12
Pudendal *n*., sensory					
1T, 10 Hz, propranolol	6.84 ± 3	11.6 ± 2.6	23.4 ± 4	0.48 ± 0.32	22 ± 21
1T, 20 Hz, propranolol	6.85 ± 2.5	11.3 ± 3.4	23.9 ± 3.8	0.48 ± 0.29	16 ± 8.9
2T, 10 Hz, propranolol	7.33 ± 3	12 ± 2.6	23.8 ± 3.2	0.48 ± 0.28	20 ± 11
2T, 20 Hz, propranolol	7.19 ± 3	11.7 ± 3	24 ± 3.4	0.46 ± 0.28	24 ± 14
Baseline, HGNT	4.93 ± 1.2******	7.54 ± 1.3	22.5 ± 3.7******	0.61 ± 0.36	19 ± 13
Pudendal *n*., sensory					
1T, 10 Hz, HGNT	4.97 ± 1.5	7.65 ± 1.6	22.9 ± 4	0.59 ± 0.29	15 ± 13
1T, 20 Hz, HGNT	5.07 ± 1.6	8.1 ± 1.8	22.6 ± 4	0.61 ± 0.31	17 ± 12
2T, 10 Hz, HGNT	5.44 ± 1.6	8.74 ± 1.7	23.6 ± 4.2	0.66 ± 0.34	17 ± 10
2T, 20 Hz, HGNT	4.99 ± 1.5	8.06 ± 1.6	22.6 ± 4.1	0.61 ± 0.33	19 ± 12

Electrical stimulation of the sensory branch of the pudendal nerve increased intravesical pressures and volume threshold. Both propranolol and hypogastric nerve transection (HGNT) decreased the efficacy of electrical stimulation, however the intervention(s) also had effects on baseline pressure and volume threshold. All values represent mean ± SD. $n = 8$-16, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$ compared to paired CYP-induced cystitis baseline. *****$P \leq 0.05$, ******$P \leq 0.01$ compared to paired pre-treatment CYP-induced cystitis baseline.

cystometry (Table 2). Propranolol blocked the effects of sensory pudendal nerve stimulation on volume threshold ($P = 0.3874$), intravesical pressures ($P \geq 0.05$), voided percentage ($P = 0.3047$), and EUS bursting time (2.41-2.98 s, $P = 0.7531$) (Figure 2C, Table 2).

4 | DISCUSSION

These studies demonstrate that electrical stimulation to the sensory branch of the pudendal nerve is a novel therapeutic approach to improve bladder function in an animal model of cystitis. We determined that electrical stimulation to the sensory branch of the pudendal nerve increased bladder capacity by up to 51% in CYP-induced cystitis rats. We also found that sympathetic reflex activity mediated the inhibition of sensory pudendal nerve stimulation in CYP, but not control, rats. Together, these results suggest a functional reorganization of reflex activity after inflammation and that pudendal neuromodulation may be an alternative approach to manage bladder capacity in IC/BPS.

Many animal models have been developed to determine the pathophysiology of lower urinary tract symptoms in IC/BPS, and inflammation is one potential contributor to these chronic symptoms. We chose to irritate the bladder with CYP as this is an established model, but the effects of neuromodulation had not yet been investigated therein. While CYP results in greater inflammatory responses than IC/BPS, the model reliably produces functional alterations including increased voiding frequency, decreased bladder capacity, and increased somatic sensitivity.[9] CYP administration produces the pathophysiological hallmarks of IC/BPS including increased voiding frequency, decreased bladder capacity, and increased somatic sensitivity.[9] We confirmed that CYP decreased bladder capacity, as well as bladder compliance. Decreased compliance is associated with decreased capacity and suggests a loss of accommodation with an increase in intravesical pressure during filling.[14] This may have been initiated by bladder wall fibrosis and decreased elasticity that develops in bladder tissues after CYP. Since we were unable to demonstrate nociceptive behavior and somatic sensitivity in CYP rats, due to the limitations of our experimental

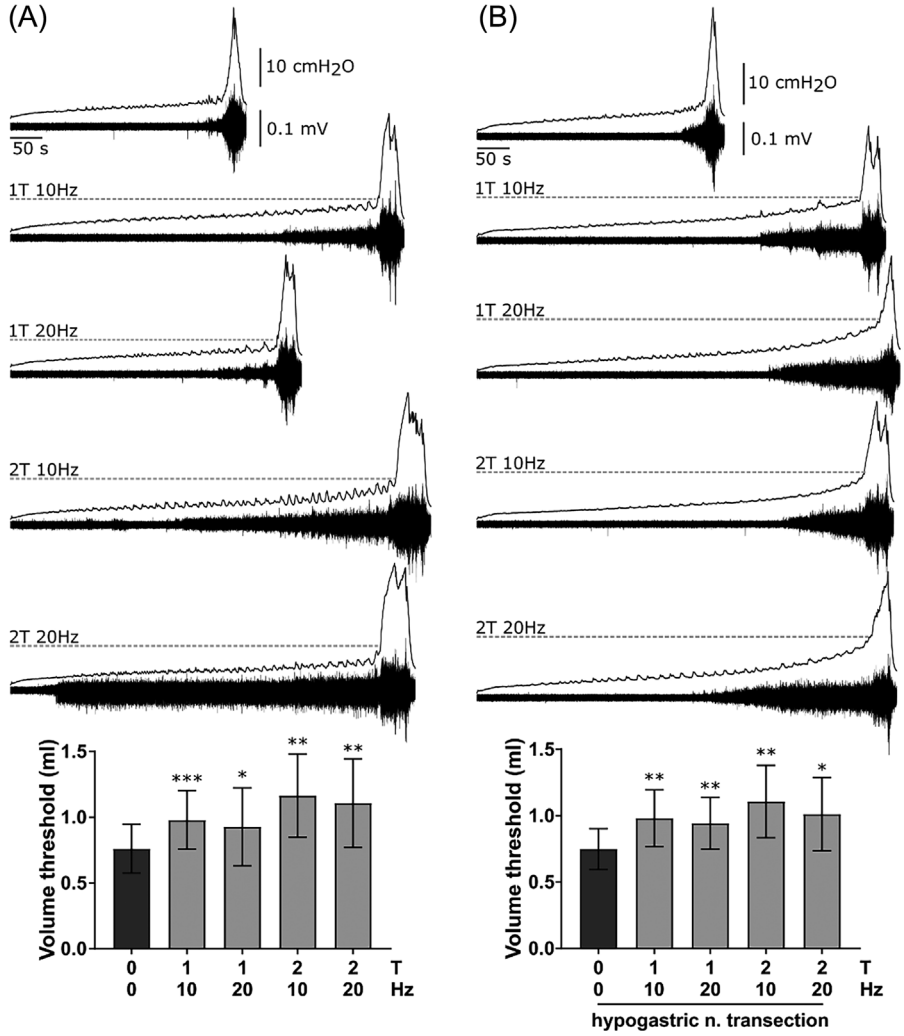

FIGURE 1 Electrical stimulation of the sensory pudendal nerve increased bladder capacity and this effect persisted following hypogastric nerve transection (HGNT) in control rats. Representative cystometrogram traces of intravesical pressure and external urethral sphincter (EUS) EMG activity before (A) and after HGNT (B) demonstrating increased volume threshold with electrical stimulation. $n = 8$, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, one-way repeated measures ANOVA, post hoc comparisons to baseline with Bonferroni correction

procedures, we directed our therapeutic outcome to the recovery of bladder capacity.

Sympathetic mechanisms without stimulation. Propranolol increased bladder capacity in CYP rats suggesting paradoxical off target effects on bladder function. Beta-adrenergic receptors are expressed in the rat bladder and urethra,[15] as well as on capsaicin sensitive afferent nerve terminals in the rat spinal cord.[16] Beta-adrenergic receptor activation classically relaxes the smooth muscle of the bladder, however increased bladder capacity has been demonstrated in CYP rats with desensitization of capsaicin sensitive afferent nerves.[17] Thus, the increased bladder capacity in the current studies may have emerged from inhibition of capsaicin sensitive nerves rather than postsynaptic adrenoceptors on the bladder smooth muscle. In addition, propranolol may have attenuated the inflammatory response through genetic or non-genetic mechanisms to increase bladder capacity.[18] Similar to previous observations,[19] we expected propranolol not to affect bladder capacity in control rats as there is no inflammatory response and the primary capsaicin afferents are quiescent. We did not pursue these studies because sensory pudendal nerve stimulation did not rely on sympathetic reflex activity in control rats.

Propranolol, as well as HGNT, also increased filling pressure in CYP rats. This was previously demonstrated in control rats and attributed to the removal of sympathetic tone during urine storage.[20] Along with changes in filling pressure, HGNT decreased peak micturition pressure in control and CYP rats. While the changes in peak micturition pressure may result from decreased smooth muscle contraction or increased urethral relaxation, the change had no functional impact on bladder emptying as voided percentage was comparable before and after transection. These studies together suggest

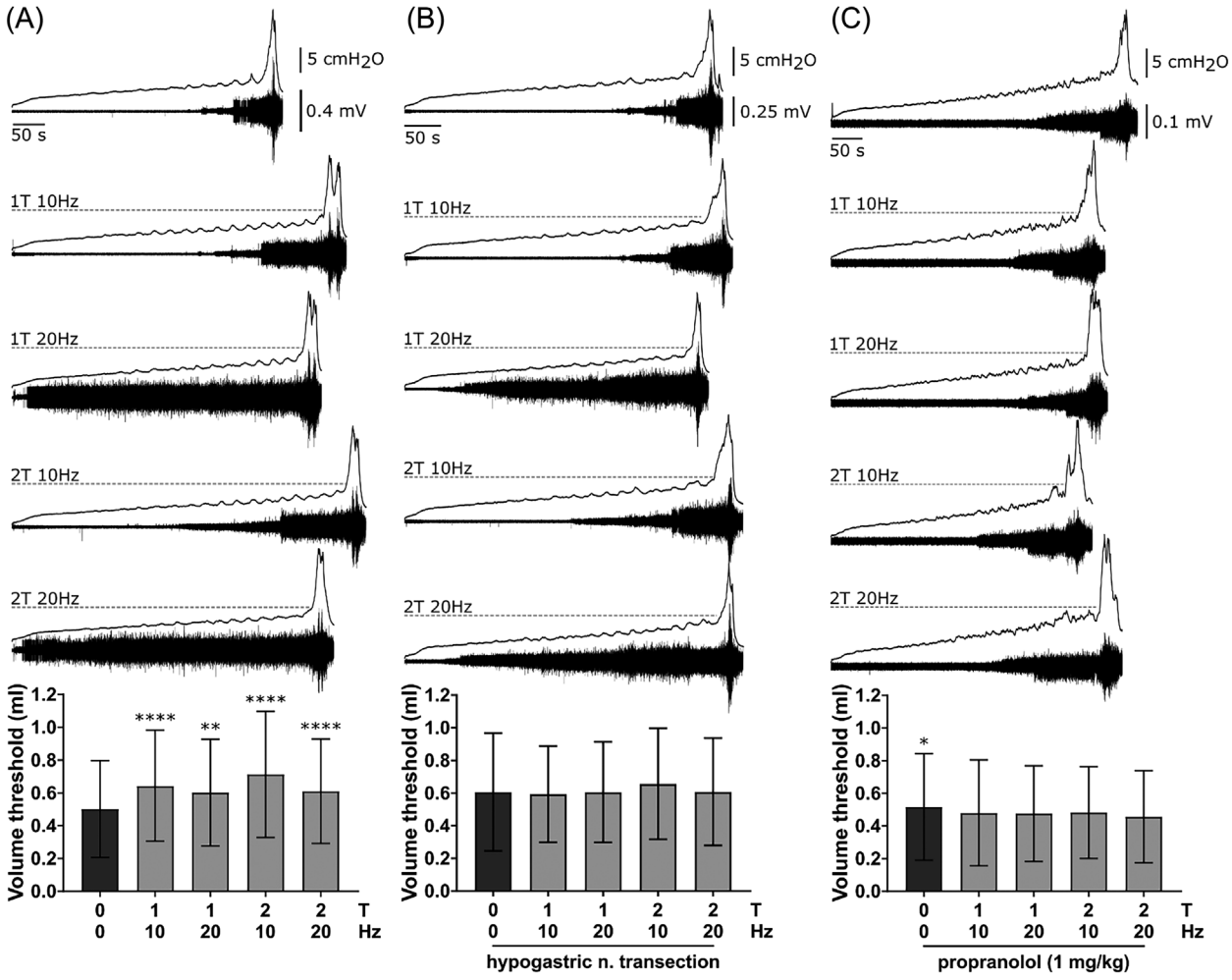

FIGURE 2 Electrical stimulation of the sensory pudendal nerve increased bladder capacity and this effect was blocked by hypogastric nerve transection (HGNT) or propranolol in CYP-induced cystitis rats. Representative cystometrogram traces of intravesical pressure and external urethral sphincter (EUS) EMG activity before (A) and after HGNT (B) or propranolol (C) demonstrating decreased efficacy of electrical stimulation following sympathetic deactivation. Propranolol (C) alone also increased volume threshold relative to baseline. $n = 8$-16, $^{**}P \leq 0.01$, $^{****}P \leq 0.0001$, one-way repeated measures ANOVA, post hoc comparisons to baseline with Bonferroni correction, $^{*}P \leq 0.05$, two-tailed Student's paired *t*-test relative to no stimulation baseline

that sympathetic deactivation may alter intravesical pressures but only affect bladder function (ie, volume threshold) following pathological insults. Further work is needed to determine the functional role of sympathetic activity in CYP rats, however this was beyond the scope of this study.

Sympathetic mechanisms with stimulation. Sacral neuromodulation is a fourth-line treatment in IC/BPS that decreases micturition frequency[4] and decreases the frequency of voiding and non-voiding contractions in preclinical bladder irritation models.[5] The limitations of sacral neuromodulation such as reoperation rates, late failures, and adverse events, however suggest that improvements are needed.[8] The current studies demonstrate an alternative approach to sacral neuromodulation with similar preclinical efficacy (~50%) in increasing bladder capacity with cystitis. Sensory pudendal nerve stimulation also increased bladder capacity at parameters (ie, 10-20 Hz, sensory threshold) comparable to those used in the clinical practice of sacral neuromodulation. Consistent with preclinical experiments,[7] the current studies show frequency dependent effects whereby larger increases in bladder capacity at each amplitude were observed at 10 Hz than at 20 Hz. This suggests that sensory pudendal nerve stimulation may provide similar benefits to patients with IC/BPS and should be further explored as a therapeutic option.

Pudendal nerve stimulation may inhibit bladder function through hypogastric nerve activation, inhibition of parasympathetic ganglionic transmission, direct smooth muscle relaxation, or other central reflex mechanisms.[6,12,21] Sympathetic reflex mechanisms are suggested to contribute to the effects of pudendal nerve stimulation because activation of the pudendal nerve by dorsal nerve of the penis[21] or intravaginal[22] electrical stimulation increased hypogastric nerve activity.

However, HGNT, as well as propranolol, failed to block the effects of pudendal nerve stimulation in healthy cats.[6] Despite the lack of inhibition under control conditions, sympathetic activity has been demonstrated to modulate pudendal nerve stimulation following nociceptive insult.[12] The current studies are consistent with these past observations that sympathetic efferent pathways have a role in sensory pudendal nerve inhibition of nociceptive reflex activity.

The increased role of sympathetic reflex activity with bladder irritation may occur through convergence on or unmasking of efferent signaling. Increased convergence may arise from pelvic[23] and pudendal[21] afferents eliciting reflex firing in the hypogastric nerve. The increased multiunit afferent activity previously observed in the pelvic nerve of CYP rats[24] may therefore increase the role of sympathetic reflexes after delivery of sensory pudendal nerve stimulation. In addition to increased convergence, bladder irritation may also unmask suppressed sympathetic reflexes. Chemical bladder irritation increases hypogastric afferent nerve activity[25] and subsequent transection of the hypogastric nerve increased intercontraction intervals.[26] While the current studies do not support a functional role for hypogastric afferent nerves in CYP rats, the increased hypogastric activity may influence the effects produced by sensory pudendal nerve stimulation.

5 | CONCLUSIONS

Sensory pudendal nerve stimulation increased bladder capacity in CYP-induced cystitis rats. Sympathetic reflex activity mediated the effects of pudendal nerve stimulation in CYP-treated but not in control rats. These studies demonstrate an alternative approach to neuromodulation in cystitis and establish mechanistic changes during stimulation that may enable the development of novel therapeutics.

ETHICS STATEMENT

Animal care and experimental procedures were approved by the Duke University IACUC and experimentation was in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

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