Neuromodulation in a rat model of the bladder micturition reflex

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Su X, Nickles A, Nelson DE. Neuromodulation in a rat model of the bladder micturition reflex. Am J Physiol Renal Physiol 302: F477–F486, 2012. First published November 2, 2011; doi:10.1152/ajprenal.00515.2011.—A rat model of bladder reflex contraction (BRC) was used to determine the optimal frequency and intensity of spinal nerve (SN) stimulation to produce neuromodulation of bladder activity and to assess the therapeutic mechanisms of this neuromodulation. In anesthetized female rats (urethane 1.2 g/kg ip), a wire electrode was used to produce bilateral stimulation of the L6 SN. A cannula was placed into the bladder via the urethra, and the urethra was ligated to ensure an isovolumetric bladder. Saline infusion induced BRC. Electrical stimulation of the SN produced a frequency- and intensity-dependent attenuation of the frequency of bladder contractions. Ten-herz stimulation produced maximal inhibition; lower and higher stimulation frequency produced less attenuation of BRC. Attenuation of bladder contraction frequency was directly proportional to the current intensity. At 10 Hz, stimulation using motor threshold pulses (Tmot) produced a delayed inhibition of the frequency of bladder contractions to 34 ± 11% of control. Maximal bladder inhibition appeared at 10 min poststimulation. High current intensity at 0.6 mA (∼6 × Tmot) abolished bladder contraction during stimulation, and the inhibition was sustained for 10 min poststimulation (prolonged inhibition). Furthermore, in rats pretreated with capsaicin (125 mg/kg sc), stimulation produced a stronger inhibition of BRC. The inhibitory effects on bladder contraction may be mediated by both afferent and efferent mechanisms. Lower intensities of stimulation may activate large, fast-conducting fibers and actions through the efferent limb of the micturition reflex arc in SN neuromodulation. Higher intensities may additionally act through the efferent limb.

A stimulation frequency range (1–20 Hz) was tested at Tmot intensity. The response to 10 Hz was measured at Tmot, 6 × Tmot, 3 × Tmot, and 0.6 mA (∼6 × Tmot). The response to 10 Hz was measured at Tmot, 2 × Tmot, 3 × Tmot, and 0.6 mA (∼6 × Tmot). When the stimulation frequency range was 1–20 Hz, the response to stimulation was proportional to the current intensity.

Spinal nerves are known to contain a mixture of large and small fiber types. It is also known that these fibers have different sensitivities to electrical stimulation. Therefore, it is likely that different stimulation parameters will activate different populations of SN fibers and therefore would have different consequences on BRC. The axons carryingafferent signals from structures of the lower urinary tract are contained primarily in pelvic, hypogastric, and pudendal nerves. The cell bodies of these nerves are found in dorsal root ganglia. Most mechano-sensitive afferent fibers, which respond to bladder distension, a natural mechanical stimulus to evoke sensations such as fullness, urgency, and pain, pass through L6 SN in the rat. Half of these are primary C fibers. At the cellular level using isolated dorsal root ganglion neurons suggest that ~50% of visceral sensory neurons are C-fiber-type neurons. This is based on their sensitivity to capsaicin, a vanillyl amide, which activates, at a low dose, and desensitizes, at a high dose, primary afferent C fibers. C-fiber sensory afferents have been suggested to mediate urgency-induced sensation and may represent a target for neuromodulation. We measured the inhibitory effect of SN stimulation on the micturition reflex in the BRC model in rats in which the primary afferent C fibers had been desensitized by chronic pretreatment with a high dose of capsaicin. Comparison of the effects in capsaicin-treated and control rats should help to evaluate the role of afferent fibers in the control of bladder activity in this model.

Our study described, for the first time, the effects of different parameters of electrical stimulation of the SN on the micturition reflex in a rat model of isovolumetric bladder contraction. A stimulation frequency range (1–20 Hz) was tested at Tmot intensity. A broader range (0.01–100 Hz) was tested at 0.6 mA (∼6 × Tmot). The response to 10 Hz was measured at Tmot, 2 × Tmot, 3 × Tmot, and 0.6 mA (∼6 × Tmot). When the stimulation frequency range was 1–20 Hz, the response to stimulation was proportional to the current intensity.
optimal stimulation parameters had been identified, mechanistic studies were performed using capsaicin to evaluate the role of primary C-fiber afferents (see above) and pancuronium, a neuromuscular blocker, to evaluate a potential role of efferent nerve activation (31, 32).

MATERIALS AND METHODS

Female Sprague-Dawley rats, weighing 200–300 g ($n = 164$), were anesthetized with urethane (2 ip injections, 4 min apart, total 1.2 g/kg). Anesthetized rats were maintained at 37°C with a heating pad during the studies and were euthanized by CO$_2$ asphyxia upon completion of experimental procedures. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Medtronic and the Non-clinical Research Board of Medtronic (Minneapolis, MN).

To record bladder contractions, a cannula (PE-50) was placed into the bladder via the urethra and secured with a suture tie. The urethral cannula was connected via a T-type connector to a pressure transducer (ADInstruments MLT0380D, Colorado Springs, CO) of the data-acquisition system (ADInstruments, ML880/P), and the signal of intravesical pressure was put through a DC amplifier (ADInstruments, ML228). The other end of the T connector was attached to a syringe pump.

To deliver electrical stimulation, a wire electrode was placed bilaterally under the L6 SN (Fig. 1). The skin around the dorsal sacral and thoracic area was shaved, and a dorsal midline incision was made from approximately SN L3 to S2; the L6/S1 posterior processes were exposed. The S1 processes were removed, and the L6 nerve trunks were localized caudal and medial to the sacroiliac junction. After the wire electrode was placed with two bared portions of teflon-coated, 40-gauge, stainless steel wire (Cooner Wire, Chatsworth, CA) under each nerve, silicone adhesive (Kwik-Cast, World Precision Instruments) was applied to cover the wire around the nerve, and the skin incision was sutured shut. The electrode was connected to a Grass S88 stimulator, through a stimulus isolation unit (SIU-BI, Grass Medical Instruments). A needle electrode under the skin of the tail served as the ground. The stimulator generated pulses to both nerves serially.

Electrical stimulation of the SN evoked hind-toe twitches and/or pelvic floor muscle contraction. In each rat, the threshold current ($T_{\text{mot}}$) was defined as the lowest intensity to evoke the first, barely discernable skeletal muscle contraction. Biphasic pulses (pulse width 0.1 ms) of different intensities ($T_{\text{mot}} \times 6$, $T_{\text{mot}}$) were used to stimulate the SN at frequencies ranging from 0.01 to 100 Hz.

To induce BRC, saline was infused into the bladder via the syringe pump at a rate of 50 l/min to induce a micturition reflex (here defined as bladder contraction of a magnitude $>10$ mmHg). The infusion rate was then lowered to 10 l/min and continued until three to five consecutive contractions were established. At this time, BRC will continue when saline infusion is terminated. After a 15-min control period, nerve stimulation was applied for 10 min and the BRC was recorded for 20 min poststimulation. Two parameters of BRC were evaluated: frequency/interval and amplitude. Data were calculated in 5-min bins, having three control periods, two periods during stimulation, and four periods after stimulation. In the case of the “shutdown” of BRC induced by high-intensity SN stimulation, the amplitude of the bladder contractions was “0” and data were excluded. All data were compared with the mean response during the last 5 min before stimulation. In an additional 26 rats, SN stimulation to different durations (0, 2, 5, 10, and 20 min) was evaluated. Data during stimulation were the means of each 5-min bin except that 2-min stimulation was calculated in a 2-min bin.

Fig. 1. Diagram representing spinal nerve stimulation system. A: experimental setup. B: schematic drawing of the stimulation waveform. C: bilateral electrode implantation. Two bared portions of a single wire were placed under each of the spinal nerves serially, and bilateral stimulation was achieved by passing current in a parallel circuit. D: histogram of motor threshold ($T_{\text{mot}}$) to spinal nerve stimulation.
In five rats, an attempt was made to see whether additional saline infusion could overcome SN stimulation-evoked BRC inhibition. After a 5-min period of bladder contractions, 10-Hz SN stimulation at a maximal intensity (>3 * T_{mot}), which completely abolished bladder contractions, was used to produce a shutdown of BRC. Then saline (60–130 μl) was further infused into the bladder to raise the bladder volume (and therefore, pressure) until periodic BRC was reestablished. Nerve stimulation was maintained during additional saline infusion. All data were calculated in 5-min bins for control, during reestablished BRC, and poststimulation.

In eight rats, one jugular vein and one carotid artery were cannulated with polyethylene tubing for intravenous administration of pancuronium and arterial pressure measurement during neuromuscular blockade, respectively. Pancuronium (1 mg/kg iv) was administered at the start of an experiment, and the effect of stimulation was compared with control rats. The effectiveness of pancuronium was demonstrated by the failure of SN stimulation to produce skeletal muscle twitches even at high stimulation intensity.

**Desensitization of primary afferent C fibers.** Thirty-two rats were treated with capsaicin (20 mg/ml, in 10% ethanol, 10% Tween 80, and 80% physiological saline) or vehicle. Capsaicin (125 mg/kg) or vehicle was given subcutaneously (sc) in the hindlimb in divided doses on 2 consecutive days: 25 and 50 mg/kg at a 12-h interval on day 1 and 50 mg/kg on day 2. Injections were performed under isoflurane anesthesia. Four days after the last injection, an eye-wipe test was performed on unanesthetized animals immediately before the bladder experiments. A drop of 100 μg/ml capsaicin solution was instilled into the eye, and the number of defensive wiping movements was counted (9, 13). After the test, the eye was irrigated with physiological saline and then the animals were anesthetized to measure the effect of SN stimulation on BRC as described above.

**Compounds.** Urethane (MW: 89.09, dissolved in saline), capsaicin (MW: 305.41, dissolved in 10% ethanol and 10% Tween 80), and pancuronium (MW: 732.67, dissolved in water) were purchased from Sigma-Aldrich (St. Louis, MO).

**Data analysis.** All data are expressed as means ± SE. Results were analyzed with Student’s t-test or ANOVA with repeated measures by Prism 5 (GraphPad Software, San Diego, CA). A value of P < 0.05 was considered statistically significant.

**RESULTS**

The threshold current (T_{mot}) at which first visible motor contraction occurred was 0.18 ± 0.01 mA (n = 107; range: 0.01–0.45 mA; 95% confidential interval: 0.16–0.20 mA, Fig. 1D). The muscle contraction became stronger, and additional muscle groups were involved as the stimulation current was increased. The experiments employed multiple intensities using multiples (1–4) of T_{mot} as well as 0.6 mA, which averaged 5.6 ± 2.0 * T_{mot}.

**Effect of high-intensity SN stimulation on bladder contractions.** There was no significant change in BRC during a 45-min recording if electrical stimulation was not applied (Fig. 2A). Depending on the stimulation frequency and current intensity, electrical stimulation of the SN attenuated the frequency of bladder contractions, either eliminating bladder contractions (Fig. 2B) or reducing the contraction frequency during electrical stimulation.

Figure 3 summarizes intensity- and frequency-dependent effects of high-intensity SN stimulation on BRC. Maximal inhibition appeared during stimulation, while after termination of the stimulus, bladder contractions returned to control levels. However, inhibition of BRC at the highest intensities [4 * T_{mot} or 5.6 * T_{mot} (0.6 mA) at 10 Hz] of SN stimulation was sustained for 10 min poststimulation (prolonged inhibition). At 10-Hz stimulation, inhibition of the contraction frequency was stronger as the current intensity increased (Fig. 3A). Two * T_{mot}, 3 * T_{mot}, 4 * T_{mot}, and 0.6 mA (5.6 * T_{mot}) significantly (P < 0.05 vs. control, n = 13) decreased the frequency of contractions during stimulation to 65.56 ± 17 (n = 9), 10.64 ± 8 (n = 8), 6.25 ± 6 (n = 7), and 0% of controls (n = 7), respectively.

The inhibitory effect of SN stimulation was also frequency-dependent, with a U-shaped curve where 10-Hz stimulation produced maximal inhibition of BRC. Figure 3B shows the time course of the mean responses of BRC frequency with different SN stimulation frequencies. Two-way ANOVA analysis demonstrates a significant inhibition of SN stimulation on BRC frequency with stimulation frequencies of 0.5–50 Hz SN stimulation, while 0.01, 0.1, and 100 Hz failed to attenuate bladder contractions. SN stimulation did not reduce the amplitude of bladder contractions (Fig. 3, C and D), except 0.6 mA (5.6 * T_{mot}), 50 Hz, which produced a mild but significant inhibition of the contraction amplitude to 76.24 ± 6% of control (n = 6, P < 0.05, Student’s t-test).

**Fig. 2.** Typical experimental records showing bladder reflex contraction (mmHg) during 45-min recording. A: no significant change in isovolumetric bladder contraction during 45-min recording without electrical stimulation. B: high intensity of spinal nerve stimulation (0.6 mA, 10 Hz, pulse width 0.1 ms) abolished bladder contractions. Black bars indicate 10-min duration of spinal nerve stimulation.
Figure 4A shows that high-intensity SN stimulation abolished the BRC. Further saline infusion increased the bladder threshold pressure (basal pressure). BRC was reestablished with a similar frequency but lower amplitude of bladder contractions, despite the presence of electrical stimulation. Figure 4, B and C, summarizes effects of high-intensity SN stimulation on amplitude and frequency of the reestablished BRC. SN stimulation produced a significant inhibition of the amplitude of bladder contractions but not frequency of bladder contractions (n=13, P<0.05, Student’s t-test) during this period.

SN stimulation at current intensities above T_{mot} triggers a visible skeletal muscle contraction (toes, pelvic floor, tail, or legs) which occurs during the inhibition of BRC. To eliminate the possibility that the inhibition of BRC is an indirect consequence of skeletal muscle contraction, a subset of animals (n=8) was pretreated with pancuronium (1 mg/kg), which produces paralysis of striated muscle. Stimulation that produced 39% inhibition of BRC in untreated rats (5.6 * T_{mot} at 0.5 Hz) showed an equal degree of bladder inhibition in paralyzed rats (61 ± 14%, P = 0.34), even though skeletal muscle contractions were eliminated.

Effect of SN stimulation at T_{mot} intensity on bladder contractions. Figures 5 and 6, A and B, show the effect of SN stimulation at T_{mot} on BRC at different stimulation frequencies. Figure 5, A and C, shows no changes in bladder contractions in response to SN stimulation at 1 and 20 Hz, respectively. Stimulation at 10 Hz also did not produce an obvious inhibition of bladder contractions during stimulation but decreased the frequency of BRC after termination of the electrical stimulation (poststimulation inhibition).

SN stimulation at T_{mot} (0.10 ± 0.02 mA) produced a much narrower range of inhibition with only 10-Hz, but not 1- or 20-Hz, attenuating frequency of bladder contractions (Fig. 6A). Stimulation at 10 Hz decreased BRC to 34 ± 11% of control (n=10, vs. control, n=13, P < 0.05), with maximal inhibition appearing at 10 min poststimulation. SN stimulation did not reduce the amplitude of bladder contractions (Fig. 6B).

The poststimulation inhibitory effect on bladder contractions for different durations of SN stimulation was evaluated. Figure 6C shows that 0 (n = 8)-, 2 (n = 7)- and 5-min (n = 7) stimulation periods (10 Hz) failed to attenuate bladder contraction frequency. Ten (n = 11)- and 20-min
(n = 9) stimulation significantly decreased the frequency of bladder contractions to 44 ± 14% of control (n = 11) and 56 ± 15% of control (n = 9), 10 min poststimulation, respectively, vs. control, n = 7, P < 0.05, 2-way ANOVA. There was no significant difference in responses to neurostimulation during the 10- or 20-min stimulation periods.

**Effect of capsaicin on neuromodulation of bladder contractions.** To examine the involvement of C-fiber sensory afferents in

![Fig. 4. Reestablishment of bladder reflex contractions following “shutdown” evoked by high-intensity spinal nerve stimulation. A: raw traces of isovolumetric bladder reflex contraction (mmHg) to high intensity of spinal nerve stimulation (pulse width 0.1 ms) at 10 Hz. Further saline infusion reestablished the bladder contractions. Black bars indicate spinal nerve stimulation period. B and C: effects of high-intensity spinal nerve stimulation on intensity and frequency of bladder contractions.](image)

![Fig. 5. Raw traces of isovolumetric bladder reflex contraction (mmHg) following threshold intensity of spinal nerve stimulation (pulse width 0.1 ms) at 1 (A), 10 (B), and 20 Hz (C). Note a strong inhibition of bladder contractions post-spinal nerve stimulation at 10 Hz. The black bars indicate 10-min duration of spinal nerve stimulation.](image)
SN stimulation, rats were treated with capsaicin to eliminate capsaicin sensitive C fibers. The effectiveness of this capsaicin treatment was examined using the eye wipe test, where a drop of 100 μg/ml capsaicin solution was applied to the eye and the number of eye wipes in 20 s was counted. Vehicle-treated rats had vigorous wiping in response to the drop of capsaicin (11.3 ± 1.06 wipes, n = 16). In contrast, rats receiving three injections (sc) of capsaicin demonstrated no responsiveness to ocular capsaicin (n = 16), demonstrating a strong loss of capsaicin-sensitive C-fiber afferents. The ability of this treatment to induce desensitization of pain responsiveness was evident, since the rats showed signs of pain behaviors (involuntary twitching, hyperactivity, or immobility) after the first injection of capsaicin. No aversive behaviors were observed with the two subsequent injections.

In another case (Fig. 7B), SN stimulation at T_{mot} (10 Hz) attenuated bladder contractions, especially after termination of electrical stimulation.

As seen above, high-intensity stimulation at 0.6 mA (~6 * T_{mot}) produced acute inhibition of bladder contraction frequencies. This was true in both vehicle- and capsaicin treated rats (P < 0.05) during current stimulation. However, high-intensity stimulation produced a stronger inhibition of BRC in capsaicin-treated rats (to 18 ± 14% of control, n = 6, vs. 63 ± 17% of control in vehicle-treated rats, n = 7, P < 0.05). T_{mot} intensity stimulation in both vehicle- and capsaicin-treated rats produced a poststimulation inhibition of bladder contractions, with a trend toward stronger inhibition in capsaicin-treated rats (P = 0.49).

DISCUSSION

In contrast to other rat models which tested the effect of S1 SN stimulation on the micturition reflex (11, 23, 25, 39, 40, 43), we delivered electrical stimulation at the L6 level through which most mechanosensitive afferent fibers innervating the urinary bladder pass in the rat. SN stimulation inhibited the frequency of volume-induced BRC, with the magnitude of the inhibition directly proportional to the applied current (stimulus intensity). Stimulation produced a delayed inhibition at the threshold current and prolonged inhibition at high intensity. Although the reason for this difference in time course is unknown, it is possible that the different stimulation parameters activate different micturition reflex circuits or act via different mechanisms. The inhibitory effect of SN stimulation is likely to be mediated by both afferent and efferent mechanisms. Lower intensities of stimulation may act through the afferent limb of the reflex arc and increase bladder capacity. Higher intensities may additionally act through efferent mechanisms. Lower intensities of stimulation may act through the efferent limbs of the reflex arc and attenuate amplitude of detrusor contractions as well as by increasing bladder capacity. The inhibitory effects of SN stimulation on bladder contractions were also frequency dependent. For inhibition of bladder contractions by both low- and high-intensity stimulation, 10-Hz stimulation was optimal. Finally, current stimulation produced a greater inhibition of BRC frequency in capsaicin-pretreated vis-à-vis vehicle-treated rats.
suggesting that an activation of fast-conducting fibers might be associated with neuromodulation of the bladder micturition reflex.

SN stimulation inhibited BRC frequency and often temporarily eliminated voiding contractions. This effect has been observed in many studies with compounds targeting the bladder afferent pathway, by several different molecular mechanisms (12, 16, 18, 19, 30, 37). Shutdown of BRC is a consequence of an increased pressure threshold for induction of contractions since additional saline can reestablish BRC even in the presence of electrical stimulation. Since the urethra is ligated in this model, voiding cannot occur; however, efficacy in the anesthetized BRC model is predictive of an increased bladder capacity and voided volume, as measured by cystometry in conscious animals (19). The ureters were not ligated in this study, and bladder overactivity could result from enhancement of urine production.

The shutdown is not due to a reduction of endogenous urine production. The basal urine production rate for a 250-g female SD rat is very low, only 4 $\mu$L/min based on 25 ml·kg$^{-1}·24$ h$^{-1}$ (24); it has been well validated that BRC is maintained even when the ureters are ligated to eliminate any urine outflow from the kidney.

Over a wide frequency range (0.1–100 Hz), SN stimulation failed to change the amplitude of BRC, except 0.6 mA, 50 Hz, which produced a mild inhibition of bladder amplitude. This suggests the SN-mediated neuromodulation may not directly depress the contractility of detrusor smooth muscle when BRC was not abolished. However, the SN stimulation-mediated shutdown at high-intensity current may be mediated via both afferent and efferent actions, since SN stimulation suppresses the amplitude of bladder contractions during the reestablishment of BRC.

Fig. 7. Effect of capsaicin on neuromodulation of the micturition reflex. A and B: typical experimental records showing effects of spinal nerve stimulation by high current intensity (0.6 mA, $6 * T_{mot}$, 0.5 Hz; A) and threshold current intensity ($T_{mot}$, 10 Hz, pulse width 0.1 ms; B) on bladder reflex contraction (mmHg) in rats with capsaicin (125 mg/kg sc) pretreatment 4 days before the bladder reflex contraction study. The black bars indicate 10-min duration of spinal nerve stimulation. C and D: time course for the effect of spinal nerve stimulation at high intensity (0.6 mA, $6 * T_{mot}$, 0.5 Hz; C) and threshold intensity (10 Hz; D) on frequency of bladder reflex contraction in rats with vehicle and capsaicin (125 mg/kg sc) pretreatment 4 days before the bladder reflex contraction study. The responses are represented as a percentage of control (% control), where the baseline response before stimulation is defined as 100%. Significant differences between the tests and control values were demonstrated by ANOVA followed by Bonferroni posttest. Shaded areas are responses during electrical stimulation. *$P < 0.05$ Bonferroni posttest. In capsaicin-pretreated rats, high intensity of current stimulation produced a stronger inhibition of frequency of bladder contractions ($P < 0.05$ vs. vehicle treated, 2-way ANOVA); $T_{mot}$ intensity stimulation produced a trend of stronger inhibitory effects but was not statistically significant ($P = 0.03$ vs. vehicle-treated, 2-way ANOVA).
Our finding that 10-Hz stimulation produced the strongest inhibition of bladder contractions is consistent with clinical applications of SN or pudendal nerve stimulation in patients (36). For sacral nerve stimulation, neuromodulation frequency is typically adjusted to 10–14 Hz (Medtronic InterStim Therapy, Implant Manual). The frequency dependence for bladder inhibition by pudendal nerve and perigenital nerve stimulation in normal and spinal cord-injured cats has also been studied. Maximal inhibition was obtained when the nerve was stimulated at 3–10 Hz (4, 5, 33, 35, 41). Boggs et al. (5) found that, in spinally transected cats, pudendal nerve stimulation at 10 Hz was more effective in inhibiting bladder activity than either 33 or 100 Hz and that stimulation at 100 Hz had no activity, either inhibitory or excitatory, in at least 50% of the animals studied. Considering the differences in experimental design (species, stimulation site, stimulation duration), the frequency-inhibition relationships are very consistent between the current rat study and the previously reported cat studies.

Over a wide range of stimulation frequencies, we did not observe an excitatory effect which might be clinically relevant for the treatment of urinary retention. This is different from other reports using stimulation of the pudendal or the deep perianal nerves (20–40 Hz) (35, 41). Pudendal nerve stimulation induced bladder contractions in normal cats (33 Hz) (6) and in acute (33 Hz) (5), 20 Hz (27), or chronic spinal cord-injured cats (at 20 Hz) (33, 34). Such bladder excitatory effects were observed when the bladder was filled with saline to 66–80% volume threshold (2, 3, 5, 8). The current study, not using an animal disease model of retention, was not designed to study augmentation of bladder contraction by neuromodulation as an approach to the treatment of urinary retention in humans.

The inhibitory effects of SN stimulation on bladder contractions were intensity dependent as well; attenuation was stronger with increases in the stimulation current. The marked inhibition of BRC frequency by high-intensity SN stimulation (above T_{mot}) occurs in either normal rats or rats pretreated with pancuronium to block skeletal muscle contractions, showing that the inhibitory effect of SN stimulation is unlikely to be mediated through skeletal muscle contractions. A similar observation has been demonstrated for bladder inhibition in response to vaginal nerve stimulation in the rat (17). Poststimulation inhibitions appeared as a prolonged and delayed inhibition following high-intensity (above T_{mot}) and low-intensity (T_{mot}) nerve stimulation, respectively. The poststimulation inhibition is quite reproducible and sustained. Bycroft et al. (7) occasionally observed a delayed “rebound” detrusor contraction following cessation of stimulation of sacral nerve roots in spinal cord-injured patients. We observed no evidence of a rebound in bladder excitation. The poststimulation inhibitory effects are not present when the stimulation durations are short (e.g., seconds long) (35, 41) and were only evoked if stimulation was applied for a sufficient duration, 10 min here in this study and 5 min in that of Jiang and Lindstrom (17). Twenty-minute stimulation produced an equal degree of poststimulation inhibition to 10-min stimulation. The prolonged or delayed inhibitory effects have been observed in InterStim Therapy in humans, where neuromodulation leads to lasting continence, e.g., the patients remain continent even when stimulation is off (clinical observation).

It seems that mechanisms of the inhibitory effects during electrical stimulation with high-intensity currents differ from poststimulation inhibition. The former is an “on-and-off” switch and needs only a few seconds to a few minutes to have a full effect while poststimulation inhibition by SN stimulation appears to be another mechanism of neuromodulation, which needs a longer time to develop. Prolonged inhibition may resemble the long-term depression of the central micturition reflex (17). Delayed inhibition by low-stimulation currents suggests that the effect requires some time to develop or that, during SN stimulation, both excitation and inhibition occur and that the excitation, but not inhibition is lost immediately after termination of nerve stimulation.

The inhibitory effects have been reported not only in spinal cord-intact animals [e.g., in cats to pudendal nerve stimulation (5), in rats to SN stimulation (23)] but also in spinal cord-injured animals where the spinobulbospinal micturition reflex is not reserved (5, 33, 39, 43). Thus, although it is difficult to attribute the inhibitory effect on neuromodulation solely to a spinal reflex, at least a spinobulbospinal pathway is not the only mechanism.

A centrally mediated long-term depression in the micturition reflex pathway may be more important than a stimulation-induced modification of peripheral afferent nerve activity, since neither tonic after-discharge nor mechanical sensitivity of afferent nerves is altered by electrical stimulation (17). Even though the relative contribution of peripheral and central actions are not yet known, acute peripheral nerve activation evoked by SN stimulation is the trigger pointing toward neuromodulation of the bladder micturition reflex.

Administration of capsaicin 4 days before the experiments in a dose that is known to desensitize bladder C-fiber afferents blocked the eye-wipe response but did not significantly alter BRC, showing that bladder sensory C fibers are not essential for micturition in the rat (9, 22). In these rats, the inhibitory effects of SN stimulation on bladder contractions remain, supporting the primary involvement of fast-conducting fibers.

Pretreatment of rats with capsaicin to desensitize the primary afferent C fibers appears to potentiate the inhibitory effect of SN stimulation on BRC frequency. This potentiation is clear in the case of high-current SN stimulation (Fig. 7C). In rats stimulated at the threshold current, no difference is observed during stimulation, but the magnitude of the delayed inhibitory effect tended to be greater following C-fiber desensitization (Fig. 7D).

It is possible that activation of Aβ fibers in the sacral nerve root by low-current stimulation excites central inhibitory pathways. The SN is composed of a wide range of fiber types, including myelinated Aβ and Aδ fibers, as well as unmyelinated C fibers (28, 30, 42). The large myelinated Aδ or Aβ fibers should be stimulated by low-current intensity while the small unmyelinated C fibers are activated by high current intensity (20). T_{mot} values of the SN stimulation did not differ between capsaicin- or vehicle-treated rats. Accordingly, the delayed bladder inhibition by T_{mot} current stimulation may result from an action only at the large myelinated fibers.

Activation of C-fiber afferents is not necessary for SN stimulation action; however, high-current stimulation may activate the unmyelinated C fibers (unwanted) in addition to myelinated Aδ or Aβ fibers (desired) in vehicle-treated rats. Many bladder afferent C fibers in the anesthetized rat are mechanosensitive afferent fibers responding to bladder distension (28). Activation of these C fibers would stimulate BRC, and consequently weaken the inhibitory effect of bladder contractions associated with SN stimulation. This could ex-
plain the potentiation of high-current SN-mediated inhibition by capsaicin-induced C-fiber desensitization.

In summary, we have optimized the parameters of SN stimulation in a preclinical model and demonstrated time course responses of bladder inhibition to different intensities of SN stimulation. Current explanations of peripheral or central neuromodulation are based on the longer stimulation periods regularly used in patients or on repeated stimulation over several days in experimental animals (see Ref. 14 for a review). The present study, using only 10-min stimulation, cannot directly address the sustained bladder inhibition seen in overactive bladder patients after InterStim therapy. In addition, isovolumetric bladder contraction is a nonpathological micturition reflex and the study was performed in urethane-anesthetized rats although urethane has minimal influence on cardiovascular function in the rat compared with other general anesthetics (1, 21). Further experiments using conscious cystometry in chronic models of bladder overactivity will target the mechanisms by which neuromodulation acts to relieve the symptoms of overactive bladder.

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DISCLOSURES

The authors are Medtronic employees.

AUTHOR CONTRIBUTIONS

Author contributions: X.S. and D.E.N. provided conception and design of research; X.S. and A.N. performed experiments; X.S. and D.E.N. analyzed data; X.S., A.N., and D.E.N. interpreted results of experiments; X.S. and D.E.N. prepared figures; X.S. drafted manuscript; X.S. and D.E.N. edited and revised manuscript; X.S., A.N., and D.E.N. approved final version of manuscript.

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