Comparison of neural targets for neuromodulation of bladder micturition reflex in the rat

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Submitted 18 June 2012; accepted in final form 7 August 2012

Su X, Nickles A, Nelson DE. Comparison of neural targets for neuromodulation of bladder micturition reflex in the rat. Am J Physiol Renal Physiol 303: F1196–F1206, 2012. First published August 8, 2012; doi:10.1152/ajprenal.00343.2012.—Spinal nerve (SN) stimulation inhibits the bladder rhythmic contraction (BRC) in anesthetized rats. This preparation was used to study the effects of electrical stimulation of the tibial nerve (TN) and the dorsal nerve of the clitoris (DNC) on BRC. Stimulation of the TN and DNC for 10 min produced a frequency- and intensity-dependent attenuation of the frequency of bladder contractions. As observed with the SN, 10-Hz stimulation of either TN or DNC produced the greatest degree of inhibition, with lower or higher frequencies being either less efficacious or inactive. In contrast to the prolonged inhibition produced by SN stimulation, both TN and DNC stimulation produced “short” lasting inhibition of bladder contractions and the maximal inhibition occurred during stimulation. TN stimulation was effective over only a narrow range of current intensities [3–4 × motor threshold current for inducing a toe twitch (T_mot)] and only at a frequency of 10 Hz. Stimulation of TN at 10 Hz, 3 × T_mot inhibited BRC to 23% of control. Ten-hertz DNC stimulation at 2 × T_EAS, the threshold current for evoking a reflex anal sphincter contraction, decreased the frequency of contractions to 4% of control. Although compared with the respective threshold current the BRC response was more sensitive to DNC compared with TN stimulation, the absolute current required to reduce BRC using DNC stimulation appeared to be higher. Comparing the effects of TN and DNC stimulation to our previous results with SN stimulation, SN stimulation produces the largest duration and efficacy of bladder inhibition.

INTERSTIM therapy (Medtronic), utilizing electrical stimulation of the sacral spinal nerve (SN, S3), is an established treatment modality for patients with urge incontinence, urinary frequency, and urinary retention. Neuromodulation applied to the SN is thought to modulate the micturition reflex by stimulating the fast conducting afferent fibers of the SN to alter the transmission of sensory input from the bladder to the pontine micturition center (28). The SN is composed of nerve fibers emerging from the pelvic nerve, the pudendal nerve, and the somatic nerve bundles that derive from the sciatic nerve, including the tibial nerve (TN) and others (20). It is possible that stimulation of different nerve roots may produce bladder inhibitory effects with a different time course and different sensitivity to stimulation frequency and/or intensity.

The pelvic nerve, a component of the sacral SN, consists of bladder afferents and parasympathetic efferent fibers of the bladder, urethra, and pelvic floor (1, 20). Direct pelvic nerve stimulation evokes pelvic floor muscle contraction and a synergistic response of bladder detrusor contraction and urethral relaxation (20). It is not known if adjusting the stimulation parameters could allow pelvic nerve stimulation to inhibit the micturition reflex. Furthermore, the difficulty of electrode implantation limits the clinical utility of this nerve root as a site for neuromodulation. In any case, pelvic nerve activation is not required for a bladder inhibitory effect since direct neuromodulation of the pudendal nerve is efficacious (21, 25).

As observed with stimulation of sacral roots, neural stimulation of the pudendal nerve has been postulated to tighten the urethral sphincter and inhibit detrusor hyperreflexia (33). Interestingly, electrical stimulation of the afferent axons of pudendal nerve branches can coordinate urethra and bladder function in the rat (4) and restore the synergistic activity of the bladder and urethra in the cat (29, 35). Furthermore, clinical evidence suggests that electrical stimulation of the penile or clitoral nerve, the major sensory elements in the pudendal nerves of males and females, respectively, reduces detrusor overactivity in patients with multiple sclerosis (8), spinal cord injury (5, 10, 12), and combined neurogenic detrusor overactivity and detrusor-sphincter dyssynergia (19).

Indeed, the therapeutic rationale for neuromodulation of bladder activity by stimulating SN, pudendal, or dorsal genital nerves may not depend solely on contraction of the pelvic floor and sphincter. Stimulation of TN evokes muscle contraction of the hind legs but not the pelvic floor. There seems little anatomic and physiological correspondence between nerve-mediated contraction of leg skeletal muscle and the bladder micturition reflex. However, stimulation of fibers innervating the leg can modulate the bladder micturition reflex and produce an inhibitory action on bladder function in the cat (6, 18, 23, 30, 31), nonhuman primate (15), and humans (7, 11, 14, 21, 34).

It is possible that electrical stimulation of large afferent fibers of the TN from the somatic tissue projecting to the same spinal segment as those innervating the bladder and urethra inhibit the intraspinal transmission of bladder sensory signaling. In this case, stimulation of the medial nerve (MN), which innervates the front limb and projects into the cervical spinal cord, a different spinal segmental distribution from TN (and also L6 SN), would not be expected to influence the micturition reflex.

The comparison of effects of neuromodulation for bladder control between the dorsal nerve of the penis, the compound pudendal nerve trunk, and the S1 sacral nerve, has been evaluated in the cat (25). Stimulation at each of these sites was effective for inhibition of bladder activity. The maximum inhibition was equivalent, but stimulation of the dorsal nerve of the penis produced inhibition over a wider range of frequencies/intensities. Snellings and Grill (25) did not evaluate the effects of TN stimulation. Quantitative comparison of the
relative efficacy of the optimal stimulation parameters on TN, dorsal genital nerve, and SN should help to explain the beneficial effects of neuromodulation and may also influence the selection of anatomical targets for clinical neuromodulation.

Using an in vivo rat model of the bladder rhythmic contraction (BRC), we (28) recently reported optimal stimulation parameters for SN stimulation for attenuation of bladder contraction frequency. In the rat, the SN from L6 to S1 contributes fibers to the innervation of the lower urinary tract and pelvic floor [including the dorsal nerve of the clitoris (DNC)]. The lumbosacral trunk that originates from L3-L5 mainly gives rise to the TN (16). Our goal was to utilize the rat BRC model to optimize the stimulation parameters (current intensity and frequency) for TN and DNC stimulation and to compare the effects with SN stimulation-induced inhibition of bladder contraction. To confirm the segmental selectivity of inhibition of bladder function, stimulation of the MN was also evaluated.

MATERIALS AND METHODS

Female Sprague-Dawley rats ($n = 135$) weighing 200–300 g 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 and were euthanized by CO$_2$ asphyxia after completion of the experiment. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Medtronic and Nonclinical Research Board of Medtronic (Minneapolis, MN).

To record bladder contractions, a cannula (PE50) was placed into the bladder via the urethra and secured with a suture, while a suture loop was placed and tightened around the entire external urethral meatus. The urethral cannula was connected via a T-connector to a pressure transducer (MLT0380D; ADInstruments, Colorado Springs, CO) and data acquisition system (ML880/P; ADInstruments). The intravesical pressure signal was amplified for recording (ML228; ADInstruments). The other end of the T-connector was attached to a syringe pump.

To deliver unipolar TN stimulation, two bared portions of a teflon-coated, 40-guage, stainless steel wire (Cooner Wire, Chatsworth, CA) were placed bilaterally under each TN, which was exposed on the medial side of both hindlimbs above the ankle (Fig. 1A). A needle electrode under the skin at base of the tail served as the ground. For DNC stimulation, the wire was placed bilaterally under each DNC, which was exposed by a 1-cm incision on the top of the urethra (see Fig. 5A). A needle electrode under the skin of the abdominal midline, 1 cm above pubic symphysis, served as the ground. For MN stimulation, a wire electrode was placed under each MN on the medial side of both forelimbs. A needle electrode under the skin of the forelimbs, 1 cm peripheral

Fig. 1. Diagram representing tibial nerve stimulation system. A: experimental setup. Two bared portions of a single wire placed under each of the tibial nerve (TN) serially and bilateral stimulation was achieved by passing current in a parallel circuit. B: histogram of motor threshold ($T_{mot}$) to TN stimulation. TN stimulation evokes toe twitches, and spinal nerve (SN) stimulation evoked tow twitches and pelvic floor muscle contraction. Broken-lined square and circle indicate the visually observed area of muscle contraction. C: electromyography (EMG) activities of the external urethral sphincter (EUS) and external anal sphincter (EAS) to electrical stimulation (arrow, artifact in shaded area) of the SN at 0.1 mA ($T_{mot}$) and 0.4 mA. No EMG activity was observed following TN stimulation at either 0.01 mA ($T_{mot}$) or 0.9 mA. D: summary data of stimulus-response functions of increased EMG activities from the EAS and EUS to graded intensity of the SN stimulation to 1 mA. EMG response was modified to the percentile of the response at 1 mA (% control).
to the cathode, served as the ground. After the wire electrodes were placed, 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 (Grass Medical Instruments) through a stimulus isolation unit (SIU-BI; Grass Medical Instruments).

In each rat, the threshold current for biphasic pulse (pulse width = 0.1 ms, 10 Hz for 2–6 s) stimulation was defined as motor threshold ($T_{\text{mot}}$), which was different depending on the nerve root studied. The $T_{\text{mot}}$ to TN or MN stimulation was defined by the lowest intensity to evoke any of skeletal muscle contractions, similar to SN stimulation (28). Current intensity was adjusted until the muscle contraction was just discernible in each animal. Stimulation of TN and MN evoked muscle contractions from hind-toe/leg and paw/forelimb, respectively.

Pelvic floor muscle contraction and sphincter twitches to SN stimulation (28) were not observed with either MN or TN stimulation. To better characterize the difference, the electromyography (EMG) of the external urethral sphincter (EUS) and external anal sphincter (EAS) was measured in response to TN or MN stimulation or L6 SN stimulation at 10 Hz with variable intensities from 0.01 to 1 mA for 1 s. The L6 SN stimulation method has been described previously (28).

The EAS and EUS EMG activities were obtained by bipolar intramuscular needle electrodes inserted in parallel through the skin just outside the anocutaneous junction into the EAS or EUS. The wire was secured to the tail with tape and connected to preamplifiers. Action potentials of EMG were initially amplified through a low-noise AC differential amplifier (model 1700; A-M Systems) and processed using the AD data acquisition program (ML880/P; ADInstruments).

Since the major component of DNIC fibers are sensory, the threshold current was determined based on a reflex contraction from the EAS with a delayed EMG signal (>6–8 ms) following the electrical stimulation of the DNIC (25). In each rat, the threshold ($T_{\text{EAS}}$) was defined by the lowest intensity to evoke the reflex EAS EMG.

To induce BRC, saline was infused into the bladder via the syringe pump at a rate of 50 μl/min to induce a micturition reflex. The infusion rate was then lowered to 10 μl/min and continued until three to five consecutive contractions were established. At this time, saline infusion was terminated and BRC was continued at regular intervals. After a 15-min control period, nerve stimulation was applied for 10 min and the BRCs were recorded for 20-min poststimulation.

Data analysis. The EMG activities from the EAS and EUS were integrated and calculated as the area under the curve for 20 ms with a 2-ms delay (eliminating the electrical artifact). The mean EMG area under the curve of a train of 10 stimulation pulses was calculated following electrical stimulation. Responses were normalized by comparison with the response to stimulation at 1 mA (% control) and plotted against the current intensity on a semilogarithmic scale. The $E_{50}$ (intensity to produce 50% response) and the 95% confidence interval (CI) were calculated by Prism (from the 20 to 80% component of nonlinear regression fitting curves, GraphPad Software, San Diego, CA) according to the equation $Y = 100/[1 + 10^{(\text{Log}E_{50} - X)} * \text{HillSlope}]$, where $Y$ is normalized EMG response and $X$ is log of current intensity. The slope factor (HillSlope) is not constrained.

Two parameters of BRC were evaluated: frequency/interval and amplitude. Data were calculated in 5-min bins, with three during control, two during stimulation, and four following stimulation. When the BRC was completely suppressed by high intensity stimulation, the frequency of the bladder contraction was designated “0” and amplitude data for that interval was excluded. All data were compared with the mean response during the 5 min before stimulation. All data are expressed as means ± SE. Results were analyzed with Student t-test or ANOVA with repeated measures by Prism 5 (GraphPad Software). Bonferroni posttest was used to determine the statistical significance between individual points. A value of $P < 0.05$ was considered statistically significant.

Compounds. Urethane (molecular weight: 89.09, dissolved in saline) was purchased from Sigma-Aldrich (St. Louis, MO).

RESULTS

Effect of TN stimulation on BRC. The $T_{\text{mot}}$ at which first visible motor contraction occurred to TN stimulation was 0.16 ± 0.02 mA ($n = 43$; range: 0.01–0.48 mA; 95% CI: 0.13–0.20 mA; Fig. 1B). The muscle contraction was first observed at the toe or paw (area indicated in Fig. 1B, inset with broken-lined square), and additional muscle groups were involved as the stimulation current was increased. In contrast to L6 SN stimulation, there were no pelvic floor contractions or EAS and EUS twitches with TN stimulation. Figure 1C demonstrates that the EAS EMG and EUS EMG were not associated with TN stimulation at either $T_{\text{mot}}$ intensity (0.01 mA) or the maximum intensity tested (0.9 mA). In comparison, electrical stimulation of L6 SN ($T_{\text{mot}}$ distribution histogram was reported previously; Ref. 28) evoked muscle contractions at toe or anal sphincter or pelvic floor (area indicated in Fig. 1B, inset with broken-lined circle). Action potentials from the EAS and EUS were recorded to have latencies of 2–20 ms with a very constant latency and pattern within each rat. Figure 1C shows the traces of EAS EMG and EUS EMG to electrical stimulations of L6 SN at 0.1 mA ($T_{\text{mot}}$) and 0.4 mA. The EAS EMG and EUS EMG increased in a graded fashion to increased electrical stimulation. The stimulus-response functions are presented in Fig. 1D. The $E_{50}$ (mA) determined from nonlinear regression fitting curves for the EAS EMG and EUS EMG were 0.34 ± 0.03 mA (95% CI: 0.29–0.40) and 0.22 ± 0.04 mA (CI: 0.13–0.30), respectively.

The effect of TN stimulation on BRC was determined using multiples (1–4) of $T_{\text{mot}}$ as well as 0.6 mA, which averaged 4.69 ± 1.17 $T_{\text{mot}}$. There was no significant change in BRC during a 45-min recording if electrical stimulation was not applied to any nerve roots (Fig. 2A). Depending on the stimulation frequency and current intensity, electrical stimulation of the TN 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 TN stimulation on BRC, with a U-shaped curve where $3 \times T_{\text{mot}}$, 10-Hz stimulation produced maximal inhibition of BRC. Maximal inhibition appeared during stimulation; after termination of the stimulus, bladder contractions returned to control levels. Regardless of stimulus parameter, TN stimulation did not produce poststimulation inhibition of BRC.

Significant inhibition ($P < 0.05$, vs. control, two-way ANOVA; $n = 10$) of the frequency of bladder contractions by TN stimulation (10 Hz) was produced only at current intensities of $3 \times T_{\text{mot}}$ (total current: 0.40 ± 0.08 mA) or $4 \times T_{\text{mot}}$ (0.59 ± 0.10 mA) of TN stimulation (Fig. 3, A and C) to 23.08 ± 14% (n = 6) and 55.08 ± 14% (n = 7) of controls (n = 10), respectively. Lower or higher intensities failed to attenuate bladder contraction frequency.

The inhibitory effect of TN stimulation was also frequency dependent. Ten-hertz stimulation produced the strongest inhibition. Figure 3B shows no changes in bladder contractions in response to TN stimulation at 1 and 50 Hz. Two-way ANOVA analysis demonstrated a significant inhibitory effect of TN stimulation on bladder contraction frequency when stimulation frequency was 10 and 20 Hz (Fig. 3B), the frequency of BRC was reduced to 23 ± 13% (means ± SE; n = 6) and 76 ± 13% (n = 7) of controls at 10 and 20 Hz, respectively. TN stimu-
tion did not reduce the amplitude of bladder contractions (Fig. 3, C–E).

**Effect of MN stimulation on BRC.** Electrical stimulation of the MN evoked front-toe twitches; $T_{\text{mot}}$ was 0.26 ± 0.03 mA (95% CI: 0.20–0.32 mA; $n$ = 17). Stimulation of the MN at 10 Hz, with stimulus intensities of $1 \times T_{\text{mot}}$ (total current: 0.36 ± 0.04 mA), $2 \times T_{\text{mot}}$ (0.67 ± 0.06 mA), $3 \times T_{\text{mot}}$ (0.88 ± 0.15 mA), $4 \times T_{\text{mot}}$ (0.67 ± 0.09 mA), and $5 \times T_{\text{mot}}$ (0.84 ± 0.12 mA) had no effect on the frequency or amplitude of BRC (Fig. 4).

**Effect of DNC stimulation on BRC.** Determination of the current required to activate the reflex arc between the DNC and

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**Fig. 3.** Parameter effects of the tibial nerve stimulation on isovolumetric bladder contractions. Responses are represented as a percentage of control, where the baseline response before stimulation is defined as 100%. A: time-course response of frequency of the bladder reflex contraction to tibial nerve stimulation (10 Hz, pulse width 0.1 ms) at different current intensities. Significance of differences between the tests to $3 \times T_{\text{mot}}$ and $4 \times T_{\text{mot}}$ stimulation and control values was demonstrated by ANOVA test. *$P$ < 0.05, Bonferroni posttest. B: time-course response of frequency of the bladder reflex contraction to tibial nerve stimulation ($3 \times T_{\text{mot}}$, pulse width 0.1 ms) at different current frequencies. Significance of differences between the tests to 10 and 20 Hz stimulation and control values was demonstrated by ANOVA test. *$P$ < 0.05, Bonferroni posttest. C–E illustrate intensity ($C$)- and frequency ($D$ and $E$)-dependent effects of tibial nerve stimulation on the frequency and amplitude of bladder contractions. The response of contraction is the mean value as a percentage of control (% control) during 10-min stimulation (the shaded areas in $A$ and $B$). Significance of differences between the tests and control values was demonstrated by Student’s $t$-test. *$P$ < 0.05. Animal numbers are indicated either under or over each symbol.

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**Fig. 2.** Typical experimental records showing the bladder reflex contraction (mmHg) during a 45-min recording. A: typical experimental record showing that if stimulation was not applied there was no change in bladder contractions. B: tibial nerve stimulation ($3 \times T_{\text{mot}}$, 10 Hz, pulse width 0.1 ms) abolished the bladder contraction. Black bars indicate 10-min duration of tibial nerve stimulation.
the anal sphincter (long latency) was performed. Figure 5B shows the traces of EAS EMG to electrical stimulations of DNC at 0.1 mA and 1.2 mA. Low-intensity stimulation of 0.1 mA evoked a fast action potential from the EAS with a latency of 2 ms, and high-intensity stimulation (1.2 mA) evoked a slow action potential with 17 ms latency. The action potentials had a very constant latency, and the electrical pattern was uniform in each rat. The mean threshold current to DNC stimulation in all animals used was $0.98 \pm 0.06$ mA ($n = 42$; range: 0.28–2.0 mA; 95% CI: 0.85–1.1 mA; Fig. 5C). Stimulation evoked a reflex EAS EMG signal with a delay of $16.47 \pm 0.69$ ms ($n = 42$; range: 7.0–30 ms; 95% CI: 15.08–18.87 ms; Fig. 5D).
Because the mean threshold current to DNC stimulation was relatively high and the safe limit for electrical stimulation from this preparation was not studied, an arbitrary level of $2 \times T_{EAS}$ was used as the upper limit for this test (24). The experiments employed multiple intensities using multiples ($0.2–2$) of $T_{EAS}$.

Depending on the stimulation frequency and current intensity, electrical stimulation of the DNC attenuated the frequency of bladder contractions. Figure 6 shows typical results of electrical stimulation of the DNC on BRC in three rats. In these experiments, the DNC was stimulated with a current of 1.2 mA, which was slightly below the threshold value ($0.8 \times T_{EAS}$) at 0.01 Hz (1 pulse every 100 s), bladder contraction frequency was not inhibited (Fig. 6A). The same current intensity of $0.8 \times T_{EAS}$ (0.56 mA) at 10-Hz stimulation in another rat slightly attenuated bladder contractions (Fig. 6B).

An intense current of 1.4 mA, $\sim 2 \times T_{EAS}$ of DNC stimulation of 10 Hz, completely abolished the BRC (Fig. 6C). There was no functional delay between application of the stimulus and effect on the bladder, i.e., the next contraction expected to occur was abolished when the current was applied. The contractions reappeared $\sim 3$ min after the 10-min stimulation was terminated; within 10 min after termination of stimulus the contraction frequency had returned to the prestimulation value. DNC stimulation had no effect on the amplitude of bladder contraction except under conditions that produced complete abolition of these contractions (Fig. 7, C and D).

Figure 7 summarizes intensity- and frequency-dependent effects of DNC stimulation on BRC. There appeared to be a linear relationship between stimulation intensity and inhibition of contraction frequency. At 10-Hz stimulation, inhibition of the contraction frequency was stronger as the current intensity increased (Fig. 7, A and C). Statistically significant inhibition ($P < 0.05$, vs. control, Student’s t-test; $n = 9$) was attained at 0.8, 1, and $2 \times T_{EAS}$ to $57.53 \pm 13\%$ ($n = 9$), $56.30 \pm 17\%$ ($n = 8$), and $3.71 \pm 3\%$ of controls ($n = 5$), respectively. Maximal inhibition appeared during stimulation, while after termination of the stimulus, bladder contractions returned to control levels. At the two lower stimulation intensities, there appeared to be some accommodation, since maximal inhibition was not maintained through the entire 10-min stimulation (Fig. 7A). At $2 \times T_{EAS}$, inhibition was maintained throughout the stimulation.

The inhibitory effect of DNC stimulation was also frequency-dependent with a U-shaped curve where a 10-Hz stimulation produced maximal inhibition of BRC. If the DNC was stimulated with a current slightly below the threshold value ($0.8 \times T_{EAS}$), using a broad frequency range, from 0.01 Hz (1 pulse every 100 s) to 500 Hz, bladder contraction frequency was inhibited with 1- to 100-Hz stimulation (Fig. 7D; $P < 0.05$, Student’s t-test).

**DISCUSSION**

Comparing the effects of TN and DNC stimulation to our previous results (28) with SN stimulation, shows a similar U-shaped frequency dependence, with each showing maximum...
inhibition at 10 Hz (Fig. 8A). All stimulations failed to alter the BRC amplitude, suggesting that subsaturating neuromodulation of these three nerve roots may share the same mechanism and are not directly depressing the contractility of detrusor smooth muscle. Stimulation of the MN, which innervates the front foot and projects into the cervical spinal cord, a different spinal segment than TN (and also L6 SN), does not modulate bladder function. This is consistent with inhibition of intraspinal transmission of visceral input from the bladder and urethra by the somatic nerve, although activation of supraspinal circuits may also be involved. The BRC had a different sensitivity to stimulation of the three nerve roots (Fig. 8, B and C) and a different time-course response (Fig. 9). Both TN and DNC stimulations produced “short” lasting inhibition of bladder contractions. TN stimulation is effective only at a frequency of 10 Hz and over a narrow range of current intensities (3–4 times $T_{\text{mod}}$). Absolute current required to reduce BRC using DNC stimulation appears to be higher. These differences may be a consequence of the specific characteristics of the nerve, its tissue environment, and the composition of different fiber types. Overall, SN stimulation produces the strongest bladder quieting response; delayed and prolonged inhibitory effects are only observed with this stimulation site.

**Neuromodulation of bladder function is nerve target specific.**

The SN, DNC, TN, but not MN are effective nerve targets for modulation of bladder function. Although there are differences in the profiles, stimulation at the SN, DNC, and TN inhibits the frequency of spontaneous bladder contraction. Stimulation at any one of these sites will send afferent impulses to the spinal cord at the lumbarosacral level, through which pass most mechano-sensitive afferent fibers innervating the rat urinary bladder. In contrast, stimulation of the MN, which would send impulses to the cervical spinal level, has no effect on bladder contraction. Therefore, a spinal reflex links the afferent nerve stimulation to inhibition of bladder contraction. Previous studies in both cat (23) and monkey (15) have shown that electrical stimulation of the TN and peroneal nerves in the hindlimb inhibits reflex bladder activity. Stimulation of hind foot but not the front foot is effective in suppression of bladder activity (30). Taken together, the spinal segmental distribution of the stimulated nerve pathways is a critical factor in the efficacy of neuromodulation on bladder control.

Different nerve distribution may contribute to the different functional response to nerve stimulation. TN stimulation triggers only toe twitches but not pelvic floor contraction and sphincter contractions from the EAS and EUS. DNC stimula-

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**Fig. 7. Effects of clitoral nerve stimulation on isovolumetric bladder contractions.** Responses are represented as a percentage of control (% control), where the baseline response before stimulation is defined as 100%. A: time-course response of frequency of the bladder contraction to DNC stimulation (10 Hz, pulse width 0.1 ms) at different current intensities. Significance of differences between the tests to 0.8 $\times$ T$_{\text{EAS}}$, 1 $\times$ T$_{\text{EAS}}$, and 2 $\times$ T$_{\text{EAS}}$ stimulation and control values was demonstrated by ANOVA test. * $P < 0.05$, Bonferroni posttest. B: time-course response of frequency of the bladder reflex contraction to DNC stimulation (0.8 $\times$ T$_{\text{EAS}}$, pulse width 0.1 ms) at different current frequencies. Significance of differences between the test to 10-Hz stimulation and control values was demonstrated by ANOVA test. * $P < 0.05$, Bonferroni posttest. C and D: intensity- and frequency-dependent effects of DNC stimulation on the frequency and amplitude of bladder contractions. Response of contraction is the mean value as a percentage of control (% control) during 10-min stimulation (the shaded areas in A and B). Significance of differences between the tests and control values was demonstrated by Student’s t-test. * $P < 0.05$. Animal numbers are indicated either under or over each symbol.
which a maximal poststimulation inhibition was observed and analyzed.

somatic muscles and sphincter muscles, or to a reflex evoked
stimulation of the efferent nerves, as motor neurons innervate
motor function (28) or EMG of the EAS or EUS (current
(0.1–0.2 mA) when detected either by visual observation of
response to SN stimulation shows a similar response threshold
projects to the pelvic floor. Sphincter contraction in re-
tional motor effects of SN stimulation confirm that the SN has
triggers pelvic floor contraction, sphincter contraction, and toe
twitches.

Fig. 8. Comparison of neuromodulation on bladder contraction frequency to
SN, TN, and DNC stimulation. A: U-shaped frequency dependent bladder
inhibitory effect. Response of contraction is the mean value as a percentage of
control (% control) during 10-min stimulation except SN stimulation at T_{mot} of
which a maximal poststimulation inhibition was observed and analyzed. T_{EAS}
is threshold evoking a reflex contraction from the external anal sphincter. B and
C: intensity-dependent effects. Response of contraction is the mean value as a
percentage of control (% control) during 10-min stimulation. Current intensity
is expressed as either proportional to threshold or absolute current value.

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C: intensity-dependent effects. Response of contraction is the mean value as a
percentage of control (% control) during 10-min stimulation. Current intensity
is expressed as either proportional to threshold or absolute current value.

Although the effects of either SN or TN stimulation on
afferent function and sensation could not be evaluated, addi-
tional motor effects of SN stimulation confirm that the SN has
projections to the pelvic floor. Sphincter contraction in re-
sponse to SN stimulation shows a similar response threshold
(0.1–0.2 mA) when detected either by visual observation of
motor function (28) or EMG of the EAS or EUS (current
study). Sphincter EMG activity might be related to a direct
stimulation of the eff erent nerves, as motor neurons innervate
somatic muscles and sphincter muscles, or to a reflex evoked
by activation of afferent nerves that coexist in the axons of the
stimulated neuron. Sphincter contractions following TN elec-
trical stimulation were not observed.

EUS contraction to SN stimulation may produce a reflex
detrusor inhibition. The urethral-detrusor inhibitory reflex may
be eliminated in the current experimental preparation, since the
urethra was expanded with a catheter. Indeed, the marked
inhibition of BRC frequency by SN stimulation occurs in rats
pretreated with pancuronium to block skeletal muscle contrac-
tions indicates that the inhibitory effect of SN stimulation is
unlikely to be mediated through skeletal muscle contractions
(28). Therefore, it seems that sphincter contractions or pelvic
muscle contraction are not required for the neuromodulation on
bladder functions; however, they may contribute to different
efficacy and duration of bladder inhibition observed in re-
sponse to SN or DNC stimulation.

Frequency-dependent effect of neuromodulation is similar
across all three nerve roots. If frequency-response curves are
compared, SN, TN, and clitoral nerve stimulation each show a
maximal inhibitory effect at 10 Hz. (Fig. 8A). TN stimulation
appears to have the narrowest effective range with only 10 Hz
producing significant inhibition. If spinal and clitoral prepara-
tions are stimulated at low intensity, the magnitude of inhibi-
tion at 10 Hz is similar. Data with SN stimulation at high
intensity (6 x T_{mot}) show that 10 Hz still produces the
maximum effect, but complete abolition of the response is now
produced, and significant inhibition is observed from 0.5 to 50
Hz (28). Our finding that a 10-Hz stimulation produced the
strongest inhibition on bladder contractions is consistent with
clinical applications of SN or pudendal nerve stimulation in
patients (33). Using InterStim Therapy on the SN, neuromodu-
lation frequency is typically adjusted to 10–14 Hz (Medtronic
InterStim Therapy, Implant Manual). The frequency depen-
dence 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 (2, 3, 29, 35). Boggs et al. (3)
found that, in spinaly transacted cats, pudendal nerve stimu-
tion at 10 Hz was more effective in inhibiting bladder activity
than either 33 or 100 Hz and that stimulation at 100 Hz (28).

Intensity-dependent effects of neuromodulation differ among
SN, DNC, and TN stimulation. The maximum efficacy of DNC
stimulation, TN stimulation (current study) and SN stimulation
(28) differ. The minimal effective current intensity in this study
to TN stimulation was 0.40 ± 0.09 mA (3 x T_{mot}), and DNC
stimulation was 0.90 ± 0.11 mA (0.8 x T_{EAS}), comparing with
0.10 mA ± 0.02 mA (T_{mot} delayed inhibition at Fig. 9A; Ref.
28) to SN stimulation. If stimulus intensity is expressed as a
multiple of the threshold current, the clitoral nerve is more
sensitive than the SN and TN (Fig. 8B). However, since the
threshold for the SN (0.1 mA) is 10 times lower than that of

AJP-Renal Physiol • doi:10.1152/ajprenal.00343.2012 • www.ajprenal.org
the clitoral nerve (1.0 mA), if current intensity is expressed as the actual current used to excite the nerves, the potency order is reversed, and the SN and TN are activated by lower current (Fig. 8C). The maximum inhibition to electrical stimulation of the DNC (2 × T_EAS) and TN (3 × T_mot) reduces the frequency of the bladder contractions to 4 ± 3% (n = 5) and 23 ± 14% of controls (n = 6), respectively. Electrical stimulation of the SN (6 × T_mot) can completely abolish bladder contractions (Fig. 9B1). Therefore, in our studies in the rat, in terms of maximal bladder responses to optimal stimulation parameters and the current efficacy thresholds, the SN is the most efficacious nerve target for neuromodulation.

In the nonhuman primate, the maximum efficacy of neuromodulation, using the optimum stimulation parameters, has been reported to be greater when administered to the SN compared with TN and perineal nerve (15).

Different nerve fiber composition may contribute to the different efficacy. It has been reported that effective inhibition of bladder contractions was produced by stimulation of fast conducting nerve fibers (e.g., conduction velocity > above 50 m/s; Ref. 17). When rats were chronically treated with capsaicin to desensitize primary C fibers, the inhibitory effects of SN stimulation on bladder contractions are not significantly reduced (28). Those studies suggest an important involvement of fast-conducting fibers in the action of neuromodulation. The SN has better defined separation between fast-conducting and slow-conducting fiber populations (22); therefore, the nerve fibers of the SN will be excited with better selectivity. In addition, in nerves having higher densities, lower stimulation currents are needed to activate a sufficient number of fibers. The higher fiber density of the SN, estimated from the interspace parameters (22), may also contribute to its higher sensitivity as a target for bladder neuromodulation.

Comparing the relationship between stimulus intensity and effect on the bladder shows that the TN stimulation differs from the SN and DNC stimulation. SN and DNC stimulations produce an inhibitory effect that is directly proportional to current intensity until the maximal response is attained. The effect is then maintained at higher stimulus intensities. However, the TN is stimulated, 10-Hz and 3 × T_mot intensity produce the strongest inhibition on bladder contractions. Increasing or decreasing of either the frequency or intensity reduces the magnitude of the inhibition. Increasing the stimu-
lus intensity beyond $3 \times T_{\text{max}}$ results in a loss of the inhibitory effect.

Such a unique intensity-dependent effect is supported by a study from Morrison et al. (18). They recorded firing of pelvic parasympathetic nerves innervating the rat bladder in response to electrical stimulation of the TN when the bladder was expanded. They observed that stimulation of myelinated A α-fibers of the TN elicited a strong reflex inhibition of efferent discharges followed by a subsequent slight increase in parasympathetic activity. Additional stimulation of unmyelinated C-fibers of the TN (20 V) produced a very late strong reflex inhibition and excitation of pelvic efferent discharge. Thus the net result on bladder activity might depend on the balance of excitatory and inhibitory actions to stimulation of different fiber types within the TN; stimulation of these different fiber types could be dependent on current intensity. The large myelinated A6- or Aα-fibers should be stimulated by low current intensity while the small unmyelinated C fibers are activated by high current intensity (13). The maximal inhibitory effect of bladder contraction to $3 \times T_{\text{max}}$ intensity of TN stimulation in this study might be due to the optimal stimulation of inhibitory parasympathetic nerves at this intensity level.

Effect durations. Although the frequency-response and intensity-response relationships between genital and SN stimulation are similar, there is an important difference. Figure 9A compares the effect of DNC and spinal stimulation on the frequency of spontaneous bladder contraction. In each case, the nerve was stimulated at its respective threshold intensity at a frequency of 10 Hz for 10 min. The magnitude of the maximum inhibition obtained is approximately equal. However, stimulation of spinal afferents has little effect during stimulation, instead producing its maximal effect after termination of the stimulus (28). In contrast, DNC stimulation produces an immediate effect, with the maximum inhibition being observed within a few minutes, and the contraction frequency nearly returning to control values by the end of the 10-min stimulation.

TN and DNC stimulation does not totally abolish bladder contraction at the optimal intensity during stimulation (Fig. 9B1), and the effect persists after termination of the stimulation but not for longer than 5 min (Fig. 9, B2 and B3). SN stimulation abolishes bladder contraction during stimulation, and the effect lasts for ≥10 min after termination of stimulation (Fig. 9B3).

In the cat, TN stimulation elicited prolonged poststimulation inhibition of bladder activity; the duration of the poststimulation inhibition is proportional to the length of stimulation; inhibition can persist for ≥1.5–2 h following a 30-min stimulation (32). Clinically, percutaneous stimulation of the TN uses a 30-min stimulation once per week for 12 consecutive wk for office-based treatment of overactive bladder symptoms (34) and SN stimulation uses continuous stimulation with an implanted stimulator/electrode (9). Since SN and TN stimulation have not been administered clinically using a similar treatment protocol, it is not known whether the two stimulation sites will produce a similar degree of poststimulation inhibition of bladder activity in patients with hyperactive bladder.

The use of a 10-min stimulation suggests that SN stimulation produced the longest duration of bladder inhibition. Since we found some differences in the bladder inhibitory action of SN, TN, and clitoral nerve stimulation in the rat, it is possible that the clinical profile of these procedures could differ as well. Further clinical studies using similar treatment protocols will allow an accurate comparison of different targets for neuromodulation to relieve the symptoms of overactive bladder.

ACKNOWLEDGMENTS

We are grateful to Dr. Greg Molnar for valuable discussions, Matthew Kelly for study coordination, and J. Paul Hieble for valuable input on the manuscript.

 Portions of this work were presented in abstract form (26, 27).

DISCLOSURES

Authors are employees of Medtronic.

AUTHOR CONTRIBUTIONS

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

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