Neurourology and Urodynamics 34:92–97 (2015)

Differentiation and Interaction of Tibial Versus Spinal Nerve Stimulation for Micturition Control in the Rat

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Aims: To determine time course of the bladder inhibitory response to unilateral or bilateral stimulation of the tibial nerve (TN) and spinal nerve (SN) as well as the interaction of stimulation at these two sites. **Methods:** In anesthetized female rats, a wire electrode was placed under either one or both of the TN or L6 SN. A cannula was placed into the bladder via the urethra. Saline infusion induced bladder rhythmic contraction (BRC). **Results:** Compared to SN neuromodulation, TN neuromodulation is less efficacious. The first 5-min stimulation at three times motor threshold on the SN and TN decreased the BRC frequency to 9% and 69% of controls, respectively. In contrast to SN stimulation, bilateral TN neuromodulation is not more effective than unilateral and sustained TN stimulation results in an apparent desensitization of the bladder response. If a 15-min TN stimulation was applied, BRCs were shutdown only during the first 5 min of stimulation. If a 5-min stimulation, using sufficient current to abolish BRC, is repeated, at least 20 min between stimulations was required in order for the responses to the first and second stimulations to be equivalent. Finally, stimulation of the TN combined with SN never produced a significantly greater effect than TN or SN stimulation alone. **Conclusions:** Based on the current experiments, it would appear that SN neuromodulation of bladder activity is preferable to TN stimulation and there is no evidence to suggest that stimulation at both sites would offer a therapeutic advantage over spinal stimulation alone. *Neurourol. Urodynam. 34:92–97, 2015.* © 2013 The Authors. *Neurourology & Urodynamics* published by Wiley Periodicals, Inc.

Key words: bladder; micturition; neuromodulation; spinal nerve; tibial nerve

INTRODUCTION

Neuromodulation has been established as an effective treatment for patients with overactive bladder (OAB) where first-line therapies, such as the muscarinic antagonists, do not provide sufficient efficacy.[1] Currently, the two most common approaches to neuromodulation are sacral spinal nerve (SN) stimulation using the InterStim® device and percutaneous tibial nerve (TN) stimulation using the Urgent PC®. Sacral neuromodulation is included in the OAB treatment guidelines of American Urological Association (AUA), European Association of Urology, and International Continence Society.[2,3] Percutaneous TN stimulation is listed as optional therapy in the AUA treatment guidelines.[2]

Using a rat model in which reflex bladder rhythmic contraction (BRC) is induced by filling with saline, we have characterized the relationship between inhibition of reflex bladder contraction and the frequency and intensity of SN stimulation.[4] We have also compared the effects of stimulation of different bladder nerves, including spinal, tibial, and genital.[5] Dorsal genital nerve stimulation has not been approved clinically for OAB treatment and is not included in this study. We now report on a further characterization of the response of the isovolumetric rat bladder to TN and SN stimulation, including a comparison of bilateral and unilateral stimulation, evaluation of desensitization to continuous and repeated stimulation, and the effects of simultaneous stimulation of spinal and TNs.

Results in the rat BRC model appear to correlate with data from other animal models and with clinical observations. The characterization of the bladder effects of TN stimulation, and comparison of these effects to those of SN stimulation, as well as the interaction of stimulation at these two sites, could identify mechanistic differences and provide information useful in the use of nerve stimulation for the treatment of OAB.

MATERIALS AND METHODS

Female Sprague–Dawley rats (200–300 g) were anesthetized with urethane (i.p., 1.2 g/kg, 200 mg/ml in saline, Sigma–Aldrich, St. Louis, MO). Anesthetized rats were maintained at 37°C with a heating pad and were euthanized by CO_2 asphyxia upon completion of experiments. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Medtronic and Non-clinical Research Board of Medtronic (Minneapolis, MN).

To deliver unipolar TN stimulation, a bared portion of a Teflon-coated, 40-guage, stainless steel wire (Cooner Wire Co., Chatsworth, CA) was placed bilaterally under each TN, which was exposed on the medial side of both hindlimbs above the ankle (72 rats, Fig. 1A). For SN stimulation, the wire was placed bilaterally under each L6 SN (44 rats, Fig. 1B). Electrodes were also placed under both left TN and left L6 SN ipsilaterally (21 rats), and right TN and left SN contralaterally (26 rats). The wire electrode(s) were positioned, secured with silicone adhesive, and connected to a Grass S88 stimulator (Grass Medical Instruments, Warwick, RI), through stimulus isolation unit(s) (SIU-BI, Grass Medical Instruments). A needle electrode under the skin of the tail served as the ground.

In each rat, the threshold current (T_{mot}) for biphasic pulses (pulse width = 0.1 msec, 10 Hz for 2–6 sec) stimulation was

Dirk De Ridder led the peer-review process as the Associate Editor responsible for the paper.
Contract grant sponsor: Medtronic, Inc.
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E-mail: xin.su@medtronic.com
Received 10 June 2013; Accepted 4 September 2013
Published online 22 October 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/nau.22506

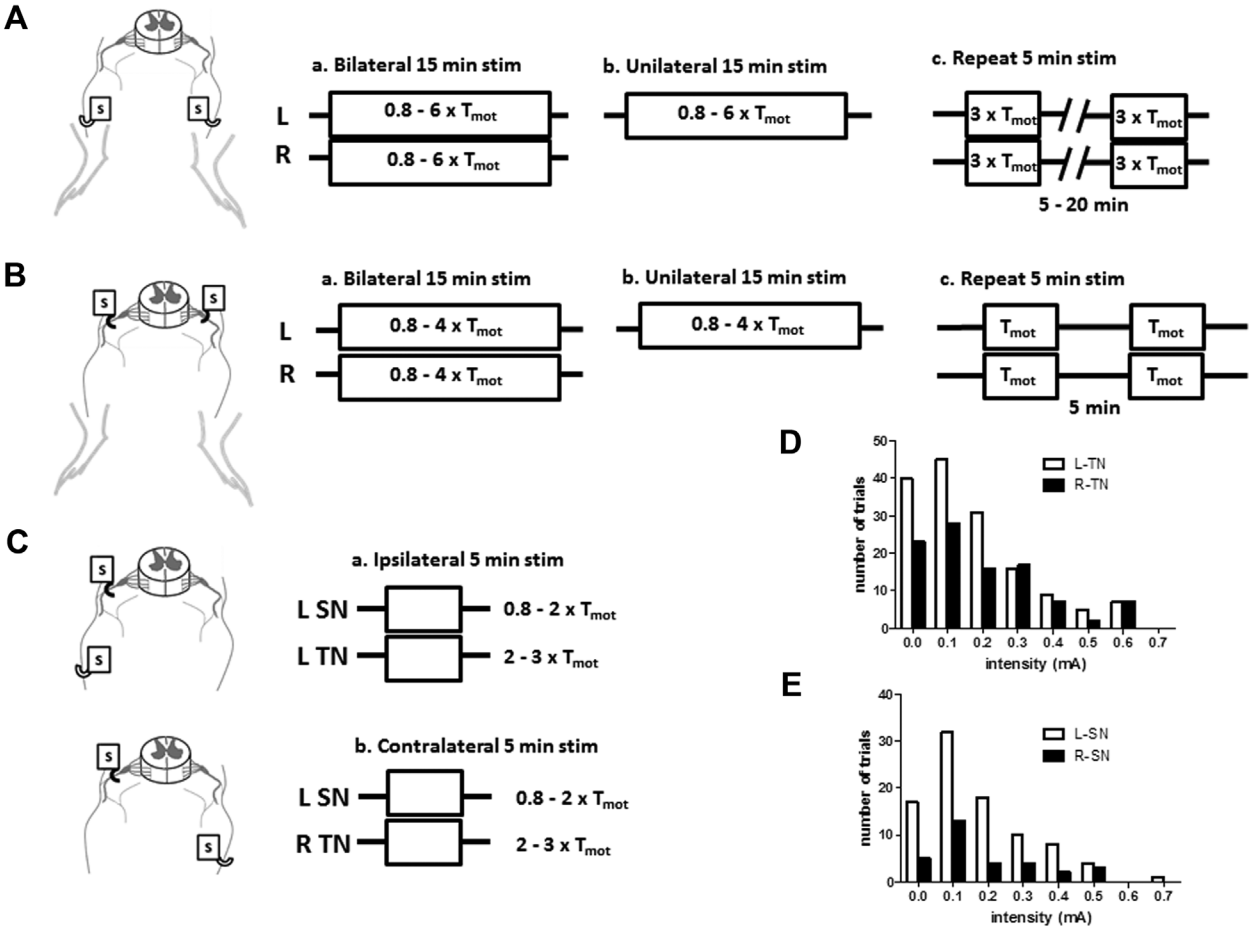

Fig. 1. Experimental model for tibial (TN) and spinal nerve (SN) stimulation (S, Stim). **A**, **B**: Experimental setup and location of TN and SN stimulation. Unilateral stimulation was delivered on the left (L) side of the TN or SN and bilateral stimulation was applied simultaneously on both left and right (R) side of the nerve. Stimulation (10 Hz, pulse width 0.1 msec) intensities varied from 0.8 times motor threshold intensity (T_{mot}) up to six times T_{mot}. **C**: Combination of unilateral stimulation of SN and TN. **D**, **E**: Histogram of motor threshold (T_{mot}) distribution to TN and SN stimulation.

defined as the lowest current required to evoke the first, barely observable, muscle contraction (hind-toe twitches and/or pelvic floor muscle contraction, 4, 5). For bilateral stimulation, the T_{mot} was measured on each side separately, to allow differentiation of muscle responses to left or right nerve stimulation.

A cannula (PE50) was inserted into the bladder via the urethra, and secured with a suture tie for intravesical pressure recording and saline infusion. The urethral cannula was connected via a T-type connector to a pressure transducer of the data acquisition system (AD Instruments MLT0380D, Colorado Springs, CO) and the signal of intravesical pressure was put through a DC amplifier (AD Instruments, ML119). The other end of the T connector was attached to a syringe pump. 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 (here defined as bladder contraction of a magnitude >10 mmHg). The infusion rate was then lowered to 10 μl/min and continued until three to five consecutive contractions were established. At this time, BRC will continue when saline infusion is terminated. Each trial of recording lasted for up to 50 min including 15 min control, 5–15 min nerve stimulation, and 20 min post-stimulation. Two trials of the testing were performed with a random stimulation parameter in some rats. The bladder was emptied after finishing the first trial and BRC was re-established by saline infusion. The second stimulation was applied at least 40 min after the first stimulation. A total of 295 trials were studied in 163 rats.

Electrical stimulation at a fixed frequency of 10 Hz, which has been shown to be optimal for inhibition of bladder contractions by both low and high intensity stimulation,[4,5] was tested unilaterally or bilaterally for 15 min (Fig. 1A-a,b and B-a,b). The repeated 5 min stimulations were also assessed from variable length of 5–20 min between stimulations to allow recovery of the bladder inhibitory responses to TN stimulation from desensitization (Fig. 1A-c).

Finally, the combination of unilateral stimulation of SN and TN was examined using a 5-min stimulation protocol. The stimulation period of 5 min was chosen based on the results of a sustained 15-min TN stimulation which showed that bladder contractions were shut down only during the first 5 min of stimulation (see Results section). With unilateral SN stimulation on the left side (0.8–2 times T_{mot}), the stimulation of either the ipsilateral and contralateral TN (2–3 times T_{mot}) was tested for a total of 10 possible combinations (Fig. 1C). The stimulation intensity ranges were chosen based on the intensity dependent bladder inhibitory response curve to stimulations on either SN or TN alone. Either lower current intensities below which no response occurs or can be measured, or higher intensities above those producing a maximum response, were not tested.

Data Analysis

SN or TN stimulation did not reduce the amplitude of bladder contractions,[4,5] therefore only effects on frequency/interval of BRC were studied. Data were calculated in 5 min bins, each having three control periods, two periods during stimulation, and four periods after stimulation. All data were normalized to the mean response during the 5 min immediately prior to stimulation. All data are expressed as mean ± SEM. Time course for the BRC response to stimulation was analyzed using repeated measures ANOVA (Prism 5, GraphPadSoftware, Inc., San Diego, CA). Bonferroni post-test was used to determine the statistical significance between individual time points. Student's *t*-test was utilized to compare mean responses during stimulation. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The threshold current (T_{mot}) at which first visible motor contraction occurred to TN stimulation was 0.17 ± 0.01 mA (n = 253; range: 0.01–0.6 mA; 95% confidence interval [CI]: 0.15–0.19 mA, Fig. 1D). T_{mot} for SN stimulation was 0.17 ± 0.01 mA (n = 121; range: 0.01–0.7 mA; 95% CI: 0.14–0.20 mA, Fig. 1E). The T_{mot} is distributed approximately normally, irrespective of TN and SN or left side and right side of the nerve roots.

Figure 2 shows typical results of bilateral, electrical stimulation of the TN or SN on BRC (3 times T_{mot}, 10 Hz, 15 min). Stimulation attenuated bladder contractions. There was no functional delay between application of the stimulus and effect on the bladder, that is, the next contraction expected to occur was abolished when the current was applied. However, the contractions re-appeared before TN stimulation was terminated (desensitization). In contrast, inhibition of BRC using the same parameters (3 times T_{mot} at 10 Hz) of SN stimulation was sustained for ~2 min post-stimulation.

Figure 3 shows that nerve stimulation inhibited the frequency of spontaneous bladder contractions. If stimulation on the TN was applied continuously for 15 min, the frequency of spontaneous bladder contractions returned to pre-stimulation values before the end of the stimulation period (Fig. 3A). Significant inhibition to TN stimulation was produced by the first 5 min stimulation at intensities equal or greater than three times T_{mot} (vs. without stimulation, n = 29, $P < 0.05$, unpaired Student's *t*-test), and by the second or third 5 min stimulation at intensities five and six times T_{mot} (vs. same time control without stimulation, n = 29, $P < 0.05$, unpaired Student's *t*-test, Fig. 3B). The first 5-min bilateral TN stimulation at 10 Hz, three times motor threshold decreased the frequency of contractions to 69.49 ± 8% of control (n = 6).

The decrease in bladder contraction frequency produced by the first 5-min stimulation at three and four times T_{mot} was significantly greater than that produced by the second or third 5 min stimulation ($P < 0.05$, paired Student's *t*-test). These data again show decreased response to sustained TN stimulation. The inhibitory response was equal, regardless of whether unilateral or bilateral stimulation was applied (Fig. 3B).

Figure 3C and D shows the effect of SN stimulation at 10 Hz on BRC at different stimulation intensities. The inhibition of the contraction frequency to SN stimulation was greater than that of TN stimulation. The lowest intensity of SN stimulation that produced a statistically significant bladder inhibition ($P < 0.05$, vs. without stimulation, n = 29, unpaired Student's *t*-test) was 0.8 times T_{mot} for bilateral stimulation and two times T_{mot} for unilateral stimulation, to 67.46 ± 15% (n = 9), and 32.52 ± 16% (n = 6) of controls, respectively. The first 5-min bilateral SN stimulation at three times motor threshold decreased the frequency of contractions to 9.42 ± 9% of control (n = 7). The inhibitory effect to bilateral stimulation at T_{mot} is significantly greater than that produced by unilateral SN stimulation ($P < 0.05$, unpaired Student's *t*-test, Fig. 3D).

A comparison of the bladder inhibitory response to TN and SN stimulation shows that with either of continuous (15 min) or repeated 5 min stimulation, there is a clear difference between the responses to stimulation at the two sites. With only a 5-min interval between two 5 min stimulations, the second SN stimulation (T_{mot} intensity, bilateral) produced a response (38.33 ± 22% control) equal to the first (46.56 ± 27% control, n = 4, $P > 0.05$, paired Student's *t*-test). In contrast, the second tibial stimulation (three times T_{mot} intensity) produced no inhibition (105.98 ± 12% control, n = 16, combined data from 9 bilateral with 7 unilateral stimulations) while the first

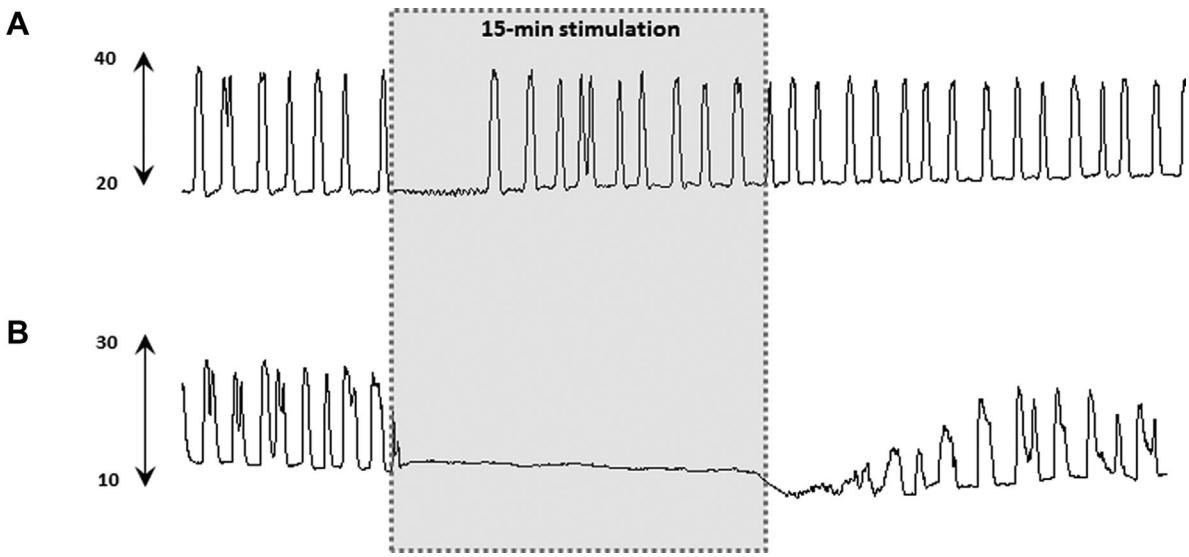

Fig. 2. Typical experimental records showing the bladder rhythmic contraction (mmHg) to sustained 15-min bilateral tibial nerve stimulation (**A**) and spinal nerve (**B**) at three times motor threshold intensity (10 Hz, pulse width 0.1 msec). Grey area indicates duration of nerve stimulation.

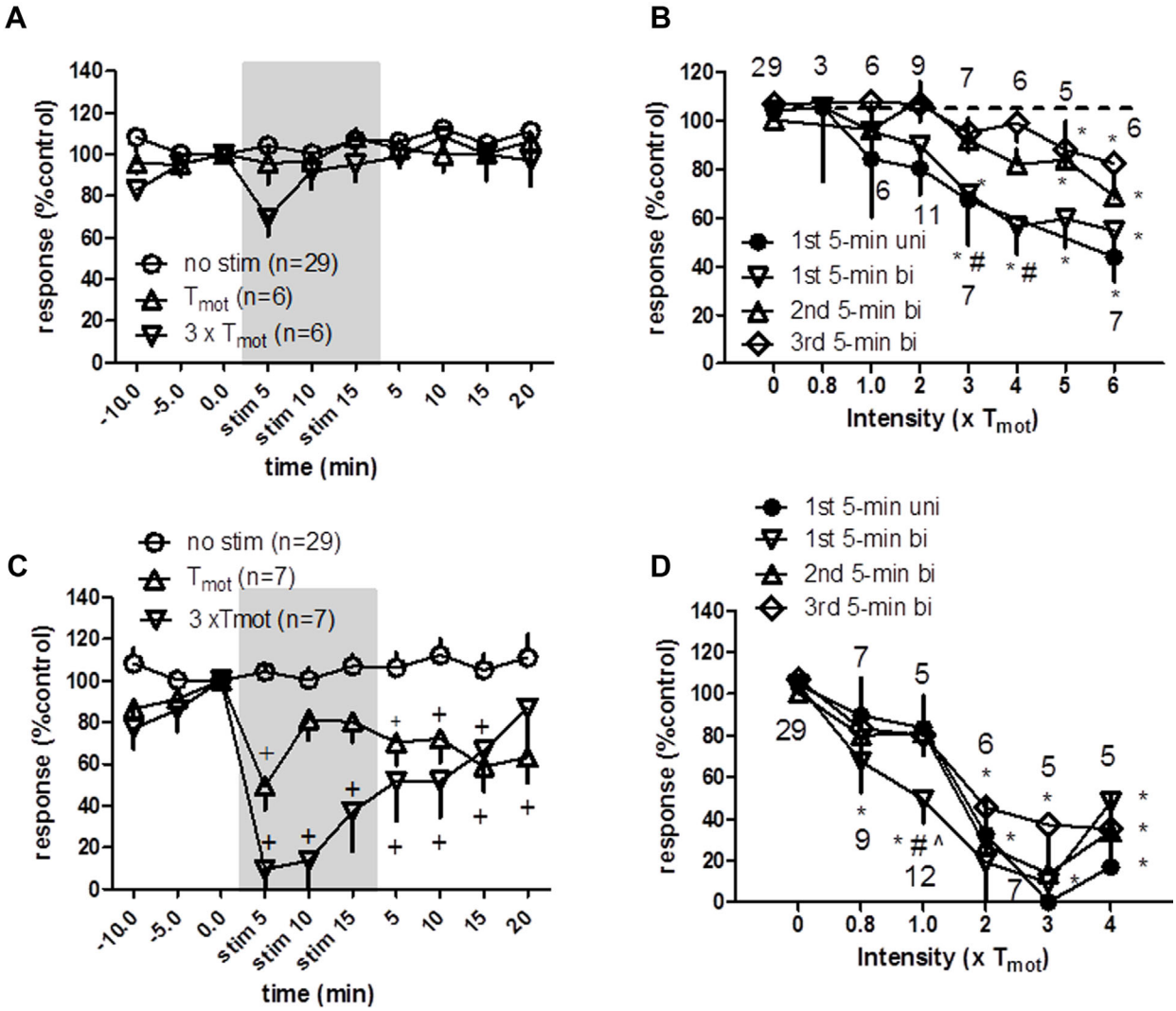

Fig. 3. Effects of tibial nerve (**A**, **B**) and spinal nerve stimulation (**C**, **D**: 10 Hz, pulse width 0.1 msec) on the frequency of the bladder rhythmic contraction. A, C: Time course response of frequency of the bladder rhythmic contraction to bilateral tibial nerve (A) and spinal nerve (C) stimulation at motor threshold (T_{mot}) and threefold of T_{mot} (3 times T_{mot}). Shaded areas are responses during electrical stimulation. +, $P < 0.05$, versus control without stimulation; repeated measures ANOVA, Bonferroni post-test. B, D: Intensity dependent effects of unilateral (uni) and bilateral (bi) tibial nerve (B) and spinal nerve (D) stimulations on frequency of bladder contractions during electrical stimulation. *X*-axis denoted increasing current intensity relative to multiples of motor threshold (T_{mot}) stimulation. The mean contraction frequency during stimulation is expressed as a percentage of the control response prior to stimulation (% control). *, $P < 0.05$, versus values without stimulation, unpaired Student's *t*-test; #, $P < 0.05$, first 5-min stimulation versus second and third 5-min stimulation, *, $P < 0.05$, unilateral versus bilateral, unpaired Student's *t*-test. The number of animals is indicated in each symbol.

stimulation reduced bladder contraction to 65.44 ± 11% control ($P < 0.05$, paired Student's *t*-test, Fig. 4C).

The effect of combined stimulation of spinal and TNs is shown in Figure 5. With unilateral SN stimulation, the stimulation of either the ipsilateral or contralateral TN was tested. With 10 possible combinations tested, there was no significant additive effect of stimulation at the two sites. Only in the case of low intensity spinal (T_{mot}) and high intensity tibial (3 times T_{mot}) stimulation was there any additive effect, but the difference between spinal + tibial stimulation and tibial stimulation alone was not statistically significant.

DISCUSSION

The rat BRC model has been used for prediction of the effect of nerve stimulation on bladder function.[4,5] Since the urethra is ligated, actual voiding is not measured. Furthermore normal rats are used, so bladder muscle and its innervations are normal. Nevertheless, considering the limitation of this animal model, the ability of nerve stimulation to inhibit the frequency of reflex bladder contractions is in good agreement with the ability to normalize micturition parameters in other animal models, such as cystometry in bladder irritated rats[6] and rhythmic contraction and cystometry in anesthetized cats,[7] as well as with clinical observations in OAB patients.[8]

We have reported that both TN and SN stimulation was effective for inhibition of bladder reflex contractions.[4,5] In those experiments, a single wire electrode was positioned under both sides of the nerve and current intensities were not controlled separately, and would depend on the functional impedance at each implantation site. In the present study, we specifically tested and controlled the delivery to both sides independently allowing a more complete characterization of stimulation thresholds for unilateral and bilateral stimulation of the SN and

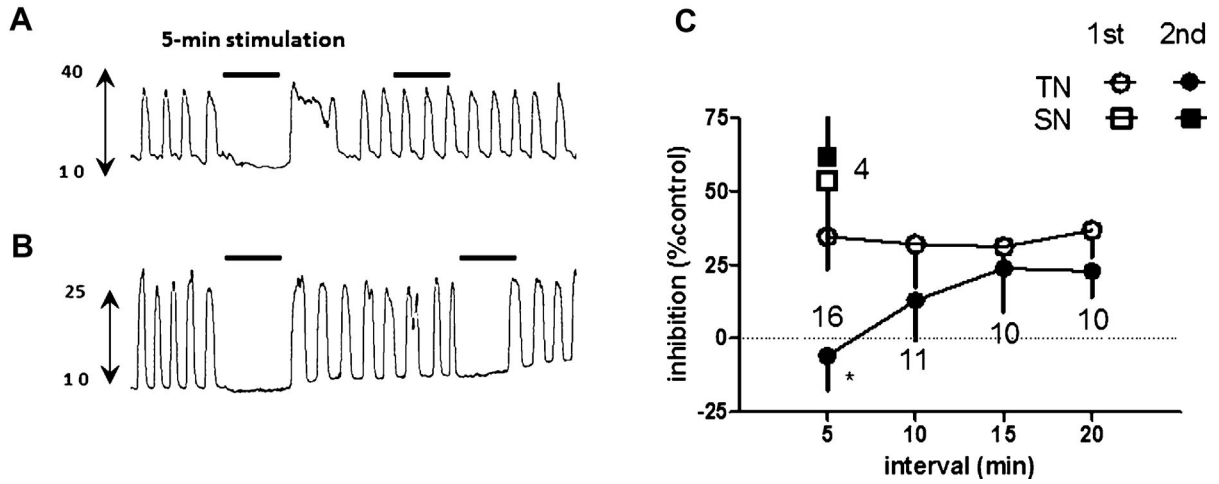

Fig. 4. Effects of bladder inhibitory response to repeated 5-min stimulations (10 Hz, pulse width 0.1 msec). **A**, **B**: Typical experimental records showing the bladder rhythmic contraction (mmHg) to two 5 min tibial nerve (TN) stimulations at three times motor threshold intensity (10 Hz, pulse width 0.1 msec). Horizontal bars indicate 5 min duration of nerve stimulation. The contraction traces in (A) illustrate that with an interval of 10 min or less between TN stimulations, the response to a second stimulation was completely blunted; with 15 min between stimulations, the first and second stimulations were equieffective (B). **C**: Inhibition of the bladder rhythmic contraction to first and second TN stimulation at three times motor threshold intensity and spinal nerve (SN) at motor threshold intensity with increasing interval between stimulations (*X*-axis). The number of animals is indicated in each symbol. *, $P < 0.05$, first 5-min stimulation versus second 5-min stimulation, paired Student's *t*-test.

TN. Even though stimulation pulses were delivered differently in these three studies, it was consistently observed that the BRC had a different sensitivity to stimulation of the TN and SN and that the response had a different time course. SN stimulation produces bladder inhibition with a longer duration and greater efficacy than TN stimulation.

The rat SN is composed of nerve fibers emerging from the TN and along pelvic nerve, and other somatic nerve bundles; the TN originates from the SNs L4–L6.[9,10] It is possible that TN neuromodulation activates some, but not all of the fibers involved in SN neuromodulation. TN stimulation triggers only toe twitches but SN stimulation evokes additional pelvic floor contraction and urethral sphincter contractions, which may produce further reflex detrusor inhibition, although effects on the urethra are unlikely since the urethra was expanded with a catheter in this preparation.[5] In addition, SN has higher fiber density than TN,[10] which may be responsible for the lower stimulation currents required for activation of a sufficient number of fibers for bladder control and may also contribute to the higher effectiveness of SN mediated bladder neuromodulation.

High intensity stimulation of the TN or SN induces a strong skeletal muscle contraction. Previously we found that the marked inhibition of BRC frequency by SN stimulation occurs in rats pretreated with pancuronium to block skeletal muscle contractions.[4] Therefore, such "unwanted" contraction does not seem to contribute to the bladder inhibition observed in response to SN or TN stimulation.

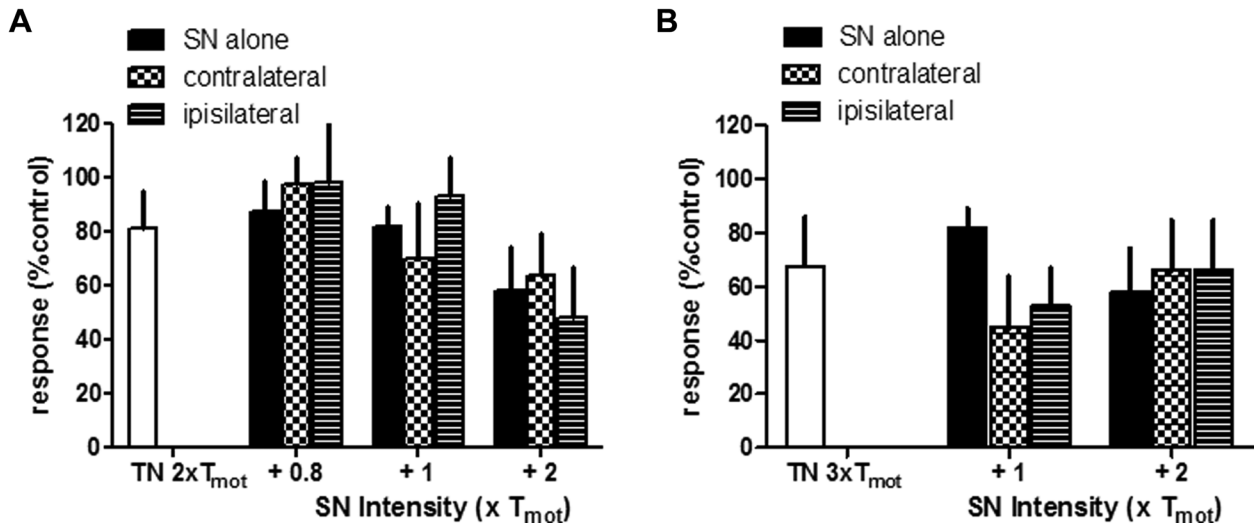

Fig. 5. Intensity-dependent effects of combination of spinal nerve (SN) and tibial nerve (TN) stimulation on the frequency of bladder contractions. Unilateral tibial nerve stimulation was tested at intensity of two times motor threshold (T_{mot}, **A**) and three times T_{mot} (**B**). The responses are represented as a percentage of pretreatment values (% control), where the baseline response before stimulation is defined as 100%.

There were some differences in the relationship between stimulation intensity and bladder inhibition; in particular, TN stimulation was effective over a narrow range (3–4 times T_{mot}) and the duration of the inhibition was less than that from SN stimulation. These results may be a consequence of the more rapid diminution of the response to TN vis-Á-vis SN stimulation which we observed in the current study (see Fig. 3). Compared with stronger bladder inhibition to bilateral vis-Á-vis unilateral SN stimulation, bilateral stimulation of the TN failed to produce more effective attenuation of bladder contractions. Further experimentation will be required to explain this difference. Bilateral symmetry of nerve responsiveness to electrical stimulation was demonstrated by equal motor threshold distribution between the left and right nerve roots.

Though there is a difference in the results of bilateral stimulation on the SN and TN, the lack of a clear additive effect between spinal and TN stimulation would suggest that stimulation at either of these two sites inhibits the bladder via a similar effect on the micturition reflex arc. However, it is still possible that TN and SN stimulation target different points on the reflex arc and that the impulses from the different stimulation sites may travel through different neuronal pathways before reaching the brainstem. Both TN stimulation and low intensity SN stimulations do not alter the BRC amplitude, suggesting their common mechanism of action through afferent limb of the micturition reflex arc.[5,11] Higher intensities of SN stimulation may directly depress the contractility of detrusor smooth muscle through the efferent limb.[4]

Both tibial and spinal neuromodulation have been found to be effective in relieving symptoms in patients with OAB. No side-by-side comparison of the two therapies in patients has been reported. It is difficult to compare reported results from individual patients since spinal stimulation is applied continuously[12] and tibial stimulation is used intermittently (one 30-min stimulation once per week[13]). Tibial stimulation is applied intermittently since it is done trans-cutaneously as an office procedure rather than by a permanently implanted device. It is possible however, that stimulation at this site would not be effective if applied continuously, due to the potential for desensitization.

A significant additive effect between spinal and TN stimulation was not observed (Fig. 4). However, there was a suggestion that the higher intensity tibial stimulation (3 times T_{mot}) could be additive with a low intensity spinal stimulation (Fig. 5B). Further experiments could determine whether this interaction is a consistent effect and if it could be enhanced by using different stimulation parameters.

CONCLUSION

Based on this rat model, it would appear that neuromodulation of bladder activity by SN stimulation is preferable to TN stimulation. The data generated in this study does not suggest that stimulation at both sites would offer a therapeutic advantage over spinal stimulation alone.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Greg Molnar for helpful comments and Dr. Matt Kelly for study coordination. The manuscript was edited by J. Paul Hieble Scientific Writing. The research was funded by Medtronic, Inc.

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