Neural pathways involved in sacral neuromodulation of reflex bladder activity in cats

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1Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania; 2Department of Urology, China Rehabilitation Research Center, School of Rehabilitation Medicine, Capital Medical University, Beijing, China; 3Department of Biomedical Engineering, Beijing Jiaotong University, Beijing, China; 4Medtronic Neuromodulation, Minneapolis, Minnesota; and 5Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

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Zhang F, Zhao S, Shen B, Wang J, Nelson DE, Roppolo JR, de Groat WC, Tai C. Neural pathways involved in sacral neuromodulation of reflex bladder activity in cats. Am J Physiol Renal Physiol 304: F710–F717, 2013. First published January 2, 2013; doi:10.1152/ajprenal.00334.2012.—This study examined the mechanisms underlying the effects of sacral neuromodulation on reflex bladder activity in chloralose-anesthetized cats. Bladder activity was recorded during cystometrograms (CMGs) or under isovolumetric conditions. An S1–S3 dorsal (DRT) or ventral root (VRT) was electrically stimulated at a range of frequencies (1–30 Hz) and at intensities relative to threshold (0.25–2T) for evoking anal/toe twitches. Stimulation of DRTs but not VRTs at 1T intensity and frequencies of 1–30 Hz inhibited isovolumetric rhythmic bladder contractions. A 5-Hz DRT stimulation during CMGs was optimal for increasing (P < 0.05) bladder capacity (BC), but stimulation at 15 and 30 Hz was ineffective. Stimulation of the S1 DRT was more effective (increases BC to 144% and 164% of control at 1T and 2T, respectively) than S2 DRT stimulation (increases BC to 132% and 150% of control). Bilateral transection of the hypogastric or pudendal nerves did not change the inhibitory effect induced by S1 DRT stimulation. Repeated stimulation of S1 and S2 DRTs during multiple CMGs elicited a significant (P < 0.05) increase in BC (to 155 ± 11% of control) that persisted after termination of the stimulation. These results in cats suggest that the inhibition of reflex bladder activity by sacral neuromodulation occurs primarily in the central nervous system by inhibiting the ascending or descending pathways of the spinobulbospinal micturition reflex.

SACRAL NEUROMODULATION, which is an FDA-approved therapy for the treatment of idiopathic overactive bladder or nonobstructive urinary retention (12, 20), requires surgery to implant a stimulator (InterStim, Medtronic, Minneapolis, MN) and insert an electrode into a sacral foramen to stimulate a sacral spinal root. Although sacral neuromodulation has been used for more than a decade to treat urinary incontinence as well as urinary retention (3, 32), the mechanisms underlying this therapy are still uncertain (1, 10).

Several mechanisms are possible to account for the effects of sacral neuromodulation on bladder function, including: 1) direct activation of motor axons innervating the external urethral sphincter (EUS), which increases urethral outlet resistance or increases EUS afferent firing that in turn modulates bladder sensory or motor pathways in the central nervous system (6, 14); 2) direct activation of afferent projections to the spinal cord that in turn modulate central bladder sensory or motor pathways (4, 5, 13, 15); and 3) direct activation of afferent projections to the spinal cord that evoke sympathetic or EUS reflexes that inhibit the bladder or close the urethral outlet, respectively (11, 16, 30, 31).

This study was undertaken to establish a convenient animal model for analyzing the effects of sacral neuromodulation on reflex bladder activity and to determine if afferent or efferent axons in the sacral spinal roots are the target of electrical stimulation during neuromodulation and if central and/or peripheral mechanisms are responsible for the modulation of bladder function. We used a chloralose-anesthetized cat model with a closed urethral outlet so that changes in reflex bladder activity are not influenced indirectly by fluid flowing in the urethra. Because this model has been used recently to study the effects of pudendal and tibial neuromodulation (15, 26), the experiments will facilitate comparisons between the three types of neuromodulation and may provide information that could be used to improve therapy by optimizing stimulation location or stimulation parameters.

MATERIALS AND METHODS

All protocols involving the use of animals in this study were approved by the Animals Care and Use Committee at the University of Pittsburgh.

Animal preparation. The experiments were conducted in 19 cats (8 male and 11 female, 2.6–4.0 kg) under α-chloralose anesthesia (60–70 mg/kg iv, supplemented as needed) and with initial isoflurane anesthesia (2–4% in oxygen) for surgery. Heart rate and blood oxygen level were monitored by a pulse oximeter (9847V, NONIN Medical, Plymouth, MN) with the sensor attached to the tongue. Systemic blood pressure was monitored via a catheter inserted in the right carotid artery. The mean blood pressure (129 ± 5 mmHg) and heart rate (128 ± 6 beats/min) were not changed significantly before, during, and after sacral DRT stimulation. Anesthetics and fluids were administered via the right cephalic vein, and airway access was secured with a tracheotomy tube. The ureter on the right side was cut and tied. The left ureter was drained externally. A double-lumen catheter (5F) was inserted through the urethra into the bladder and secured by a ligature around the urethra. One lumen of the catheter was connected to a pump to infuse the bladder with saline (1–3 ml/min), and the other lumen was attached to a pressure transducer to monitor the bladder activity. In some experiments, sutures were placed bilaterally around the hypogastric or pudendal nerves for cutting these nerves at the end of the experiments by withdrawing the sutures.

The spinal cord and cauda equina were exposed between the L4 and Cx1 vertebrae via a dorsal laminectomy. The spinal dura was cut...
and the dorsal and ventral roots were separated for electrical stimulation. The animal was mounted in a modified Narishige “Eccles” spinal cord frame in which the hip was supported by metal pins, and the spinous process at the rostral end of the laminectomy was secured with a clamp. The skin, cut midsagittally from L4 to S3, was tied along each margin to form a pool that was filled with warmed (35–37°C) mineral oil. The temperature of the animal was maintained at 36–38°C using a heating pad during the experiments.

**Stimulation protocol.** In the first group of experiments ($N = 3$ cats), the bladder was infused with saline to a volume slightly above the micturition threshold [100–110% of bladder capacity (BC)] to induce isovolumetric rhythmic bladder contractions. Monophasic pulses (0.2-ms pulse width) generated by an electrical stimulator (S88, Grass Medical Instruments, Quincy, MA) were delivered in each cat via a pair of hook electrodes first to the sacral DRTs and then to the sacral VRTs in the order of S1, S2, and S3. Eight different frequencies (1, 3, 5, 7, 10, 15, 20, 30 Hz) were tested on every root in a random order to determine the effect on isovolumetric bladder contractions. Stimulation intensity was at the threshold (T) for inducing either anal sphincter or toe twitching, which was determined at the beginning of each experiment.

In the second group of experiments ($N = 16$ cats), the effect of stimulating S1 or S2 DRT was further tested during CMGs, which consisted of a slow infusion of saline (1–3 ml/min) starting with an empty bladder until the first micturition contraction occurred. BC is defined as the bladder volume threshold to induce the first micturition contraction during a CMG. Initially, two or three control CMGs were performed without stimulation to obtain the control BC and evaluate reproducibility. Then, S1 DRT stimulation at 5 Hz and at different intensities (1/4, 1/2, 1, and 2T) was applied during repeated CMGs. This was followed by two control CMGs without stimulation and a series of repeated CMGs with S1 DRT stimulations at 1T intensity and at several frequencies (5, 15, 30 Hz). After testing S1 DRT stimulation, the same stimulation parameters were tested again on the S2 DRT. At least two control CMGs without stimulation were performed after testing the different intensities or the different frequencies to detect any poststimulation effect induced by repeated stimulation during multiple CMGs. The acute inhibitory effect was

![Fig. 1. Effect of S1 dorsal root (DRT)/ventral root (VRT) stimulation on isovolumetric bladder contractions. A: S1 DRT stimulation; intensity = 0.3 V (threshold for inducing anal twitching) and pulse width = 0.2 ms. B: S1 VRT stimulation; 0.07 V is the threshold intensity for inducing leg movement, and pulse width = 0.2 ms. C: after the pattern of isovolumetric contraction changed with time, S1 DRT stimulation inhibited bladder activity at the threshold intensity (0.2 V) but S1 VRT stimulation did not inhibit the bladder at threshold intensity (0.15 V). The black bars under the pressure trace indicate the stimulation duration. The recordings in A and B are from the same cat, but recordings in C are from another cat.](http://ajprenal.physiology.org/.../by/10.220.32.247/on June 10, 2017)
RESULTS

Effect of stimulating afferent or efferent pathways in the sacral spinal roots on reflex bladder activity. At threshold intensity for inducing anal sphincter or toe twitching, electrical stimulation of the S1 or S2 DRT at frequencies of 1–30 Hz in three animals inhibited the large-amplitude (40–80 cmH2O) isovolumetric rhythmic bladder contractions (Fig. 1A, and Table 1). The bladder inhibition induced by S3 DRT stimulation was observed in two of three animals (Table 1). However, sacral VRT stimulation was not effective in inhibiting the bladder contractions (Fig. 1, B and C) except in one cat during S1 VRT stimulation at 20–30 Hz (Table 1). It is noteworthy that the pattern of isovolumetric bladder contractions changed with time, but this change did not influence the result of S1 DRT/VRT stimulation (Fig. 1C).

Effect of S1/S2 DRT stimulation on bladder activity. The slow infusion of saline during CMGs induced large-amplitude (average 78.6 ± 4.8 cmH2O; range 40–120 cmH2O) bladder contractions when the bladder volume reached the BC (average 11.6 ± 1.2 ml; range 4.1–20.5 ml). The amplitude of bladder contractions was not significantly (P > 0.05) influenced by DRT stimulation (76.0 ± 4.3 cmH2O after 5 Hz S1 DRT stimulation at 1T intensity), but the BC was significantly increased by the stimulation (Fig. 2). The inhibitory effect of S1 or S2 DRT stimulation on reflex bladder activity was dependent on both stimulation intensity and frequency (Figs. 2 and 3). When applied during a CMG, S1 DRT stimulation at 5 Hz significantly (P < 0.05) increased BC to 144 ± 12% and 164 ± 12% of control at 1T and 2T intensity, respectively (Fig. 2 and Fig. 3A), while S2 DRT stimulation significantly (P < 0.05) increased BC to 132 ± 12% and 150 ± 12% of control, respectively (Fig. 3C). Increasing intensity from 1T to 2T did not significantly increase the inhibitory effect (Fig. 3, A and C). S1 or S2 DRT stimulation at ¼ or ½T was ineffective in increasing bladder capacity. There was no significant difference between the threshold intensities for stimulation of S1 DRT (0.21 ± 0.03 V) and S2 DRT (0.15 ± 0.01 V).

Analysis of the effect of different frequencies revealed that S1 DRT stimulation at 1T intensity significantly (P < 0.05) increased BC to 147 ± 14% of control at 5 Hz, but was ineffective at 15 and 30 Hz (Figs. 2 and 3B). Similarly S2 DRT stimulation at 1T intensity was only effective at 5 Hz in increasing BC (Fig. 3D).

Bladder compliance was also measured at the bladder volumes equal to the control bladder capacity. DRT stimulation (5 Hz, 1T) did not significantly (P > 0.05) increase bladder compliance during CMGs (control: 1.2 ± 0.2 ml/cmH2O; S1 and 3). When applied during a CMG, S1 DRT stimulation at 5 Hz significantly (P < 0.05) increased BC to 144 ± 12% and 164 ± 12% of control at 1T and 2T intensity, respectively (Fig. 2 and Fig. 3A), while S2 DRT stimulation significantly (P < 0.05) increased BC to 132 ± 12% and 150 ± 12% of control, respectively (Fig. 3C). Increasing intensity from 1T to 2T did not significantly increase the inhibitory effect (Fig. 3, A and C). S1 or S2 DRT stimulation at ¼ or ½T was ineffective in increasing bladder capacity. There was no significant difference between the threshold intensities for stimulation of S1 DRT (0.21 ± 0.03 V) and S2 DRT (0.15 ± 0.01 V).

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Table 1. Stimulation frequencies and sacral spinal roots effective in inhibiting isovolumetric bladder contractions at threshold intensity for inducing anal/toe twitching

<table>
<thead>
<tr>
<th>Dorsal root</th>
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<tr>
<td>Cat 1</td>
<td>3–30 Hz</td>
<td>1–5 Hz</td>
<td>NE</td>
</tr>
<tr>
<td>Cat 2</td>
<td>3–30 Hz</td>
<td>1–30 Hz</td>
<td>3–30 Hz</td>
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<td>Cat 3</td>
<td>1–30 Hz</td>
<td>1–30 Hz</td>
<td>1–5 Hz</td>
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<thead>
<tr>
<th>Ventral root</th>
<th>S1</th>
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<tbody>
<tr>
<td>Cat 1</td>
<td>20–30 Hz</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Cat 2</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Cat 3</td>
<td>NE</td>
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NE, not effective.
DRT stimulation: $1.5 \pm 0.3 \text{ ml/cmH}_2\text{O}$; S2 DRT stimulation: $2.1 \pm 0.6 \text{ ml/cmH}_2\text{O}; N = 16 \text{ cats}$.

Poststimulation inhibition induced by repeated S1/S2 DRT stimulation. Repeated stimulation of S1 or S2 DRT during multiple CMGs (as shown in Fig. 2) induced a poststimulation inhibitory effect that was evident as an increase in BC even after termination of the stimulation (Fig. 4A). The BC was significantly ($P < 0.05$) increased to $126 \pm 6\%$ and $155 \pm 11\%$ of control after repeated S1 or S2 DRT stimulation, respectively (Fig. 4B). The increase in BC was significantly ($P < 0.05$) larger after repeated S2 DRT stimulation compared with repeated S1 DRT stimulation. The first control CMG in Fig. 4A was performed before any stimulation was applied. The second control CMG in Fig. 4A was performed after repeated CMG tests of S1 DRT stimulation, similar to the protocol used in the experiment shown in Fig. 2. The third control CMG in Fig. 4A was performed after repeated CMGs during which S1 DRT stimulation and then S2 DRT stimulation were tested. The results from 16 experiments like the one shown in Fig. 4A are summarized Fig. 4B.

Effect of hypogastric or pudendal nerve transection on bladder inhibition induced by S1 DRT stimulation. At the end of some experiments, bilateral transection of the hypogastric ($N = 5$ cats) or pudendal ($N = 5$ cats) nerves did not change the ability of S1 DRT stimulation to inhibit reflex bladder activity and increase BC (Figs. 5 and 6). An inhibitory effect was elicited by S1 DRT stimulation within 5–10 min after the nerve transection during isovolumetric bladder contractions (Figs. 5B and 6B) or during repeated CMGs after the nerve transections (Figs. 5, C and D, and 6, C and D). The basal bladder capacities in the absence of S1 DRT stimulation were not significantly ($P > 0.05$) changed after either hypogastric nerve transection ($97.5 \pm 19.4\%$ of pretransection value, $N = 5$ cats) or pudendal nerve transection ($107.9 \pm 8.2\%$ of the pretransection value, $N = 5$ cats). The amplitude of bladder contractions was also not significantly ($P > 0.05$) changed after either hypogastric nerve transection (before $75.1 \pm 9.6 \text{ cmH}_2\text{O}$ vs. after $81.8 \pm 11.4 \text{ cmH}_2\text{O}; N = 5$ cats) or pudendal nerve transection (before $77.2 \pm 9.9 \text{ cmH}_2\text{O}$ vs. after $71.1 \pm 9.3 \text{ cmH}_2\text{O}; N = 5$ cats).

**DISCUSSION**

This study in cats revealed that stimulation of afferent pathways rather than efferent pathways in sacral spinal roots is effective in inhibiting reflex bladder activity (Fig. 1 and Table 1), suggesting sacral neuromodulation’s effects are centrally mediated. This inhibitory effect, which is stimulation intensity and frequency dependent (Figs. 2 and 3), consists not only of an acute effect that occurs during stimulation and then rapidly dissipates after the end of stimulation but also of a poststimulation inhibition that maintains an increase in BC even after termination of the stimulation (Fig. 4). Bilateral transection of the hypogastric (Fig. 5) or pudendal nerves (Fig. 6) did not eliminate the acute inhibitory effects of S1 DRT stimulation indicating that afferent and efferent pathways in these nerves are not essential for the effects of sacral neuromodulation in the cat. These results suggest that the inhibition of reflex bladder activity by sacral neuromodulation occurs primarily in the central nervous system by inhibiting the ascending or descending pathways of the spinobulbospinal micturition reflex.

The possible contribution of peripheral mechanisms to sacral neuromodulation was important to evaluate because stimulation of a sacral spinal nerve can directly activate motor axons to the EUS, or reflexively activate these axons or lumbar sympathetic efferent axons that can modulate reflex bladder activity (6, 15, 16). When sympathetic efferent pathways in the hypogastric nerves are activated, they directly inhibit bladder smooth muscle and suppress synaptic transmission in bladder parasympathetic ganglia (7–9). These changes could increase...
bladder compliance as well as increase BC. Although previous studies (16, 29) indicate that the sympathetic reflexes may play a role in bladder inhibition during pudendal nerve stimulation, an increase in bladder compliance was not observed in this study during sacral DRT stimulation. In addition the increase in BC elicited by S1 DRT stimulation was not eliminated after hypogastric nerve transection (Fig. 5). Therefore, it seems unlikely that the sympathetic reflexes play an important role in inhibition of the micturition reflex by sacral neuromodulation in the cat.

Electrical stimulation of perigenital skin area (27), vagina (16), or EUS (6) can also suppress bladder reflexes in animals presumably by inducing afferent firing in the pudendal nerve that in turn suppresses central micturition reflex pathways. Therefore, it is possible that sacral DRT stimulation could reflexively activate pudendal efferent nerves and induce contractions of EUS or vaginal muscles, which could in turn elicit pudendal afferent firing (14) and inhibit bladder activity. However, it is unlikely that this mechanism played an important role in the sacral neuromodulation because bilateral pudendal nerve transection did not alter the inhibition in the present experiments.

Clinical application of sacral (S3) neuromodulation utilizes a stimulation intensity slightly below the motor threshold for inducing toe or anal twitching but above sensory threshold (1, 17, 18). Although bladder inhibition has been shown in rats by chronically (8 h/day) stimulating the S1 spinal root at an intensity slightly below the motor threshold (33, 34), acute inhibition of the bladder during S1 spinal root stimulation could only be observed at an intensity greater than 3 times motor threshold (19). A recent study in rats (23) also indicated that L6 spinal root stimulation at motor threshold intensity and 0.1-ms pulse width only induced a transient poststimulation inhibition, but not an acute inhibition during the stimulation. However, our current results in cats show that S1 or S2 DRT stimulation is effective in inducing both acute inhibition (Figs. 1–3) and poststimulation inhibition (Fig. 4) of reflex bladder activity. Previous studies also showed in cats that electrical stimulation of the S1 spinal root (22) or S2 spinal root (21) acutely inhibited reflex bladder activity. These results suggest that in cats the S1 or S2 spinal root might be equivalent to the S3 spinal root in humans. In addition, S1 or S2 root stimulation in cats can induce acute inhibition of bladder reflex activity at motor threshold intensity (Figs. 2 and 3) (22), which is similar to the stimulation intensity used in S3 neuromodulation in humans. Therefore, electrical stimulation of S1 or S2 spinal roots during acute experiments in cats might be an appropriate animal model to investigate the possible neural mechanisms underlying sacral neuromodulation.

Sacral neuromodulation in humans often uses stimulation frequencies of 10 or 15 Hz (1, 17). Our study in cats shows that 15-Hz stimulation of S1 or S2 DRT is effective in inhibiting isovolumetric rhythmic bladder contractions (Fig. 1 and Table 1) but does not significantly increase BC during repeated CMG tests (Figs. 2 and 3). Only low frequency (5 Hz) was effective in increasing BC when the stimulation was applied during a
Previous studies in cats (21, 22) also showed that 7–10 Hz was the optimal frequency range for S1 or S2 spinal root stimulation to inhibit reflex bladder activity. If the results of these cat studies are applicable to humans, then lower stimulation frequencies (5–7 Hz) might be more effective than the commonly used 10–15 Hz in clinical application of sacral neuromodulation. However, a recent clinical study (17) indicated that sacral neuromodulation at 5 Hz had a similar efficacy to 10- to 40-Hz stimulation in the treatment of overactive bladder. This discrepancy between the animal and clinical data might be due to the fact that clinical studies (17) very likely include a poststimulation inhibitory effect elicited by prolonged stimulation, while the animal studies (Fig. 3) (21, 22) measure an acute, rapidly reversible inhibition during a CMG that only lasts for 10–20 min (see further discussion below).

It is noteworthy that a significant poststimulation inhibition was induced by repeated stimulation of the S1 or S2 DRT in cats (Fig. 4). This poststimulation effect influenced the evaluation of stimulation intensity and frequency (Figs. 2 and 3), because the control BC in each testing group (see Fig. 3) to which the results were normalized was significantly increased following each group of tests (see Fig. 4). This increase in control BC might have occluded the acute increase in BC during a subsequent test of DRT stimulation. For example, the increase in BC by S2 DRT stimulation is smaller than S1 DRT stimulation (comparing Fig. 3, A and C). Similarly, the poststimulation effect might have caused the apparent ineffectiveness of 15-Hz stimulation in increasing BC (Fig. 3, B and D).

Although a wide range of stimulation frequencies (1–30 Hz) was effective in inhibiting isovolumetric rhythmic bladder contractions (Fig. 1 and Table 1), this was not evident when measuring the stimulation-induced increase in BC during repeated CMG tests (Fig. 3). This discrepancy further suggests that the poststimulation effect occurring within each testing group was relatively small, the acute inhibitory effects induced by stimulation of different intensities/frequencies could still be compared within an individual testing group (Figs. 2 and 3). Thus our conclusion that 5-Hz stimulation is better than other frequencies (15 or 30 Hz) in inducing the acute inhibition during a CMG (Fig. 3, B or D) seems justified.

Fig. 5. Role of hypogastric nerves in bladder inhibition induced by S1 DRT stimulation. A: S1 DRT stimulation increased bladder capacity before bilateral transection of hypogastric nerves. B: S1 DRT stimulation inhibited isovolumetric bladder contractions before and after bilateral transection of hypogastric nerves. C: S1 DRT stimulation also increased bladder capacity after bilateral transection of hypogastric nerves. D: summarized results from 5 cats showing that the increases of bladder capacity by S1 DRT stimulation before and after bilateral hypogastric nerve transection are not statistically different (Student’s t-test). The bladder pressure traces from top to bottom were measured from the same animal in sequence. The black bars under bladder pressure trace indicate stimulation duration. Intensity threshold: T = 0.2 V; pulse width: 0.2 ms; infusion rate: 2 ml/min in A and C.
and the effective stimulation duration, intensity, and frequency to induce the poststimulation effect are warranted.

Our previous studies in cats (25, 27, 29) have shown that pudendal nerve stimulation is frequency dependent, i.e., low frequency (3–10 Hz) is inhibitory but high frequency (20–30 Hz) is excitatory. Meanwhile tibial nerve stimulation in cats (26) can inhibit bladder activity in a wide range of stimulation frequencies (5–30 Hz), but low frequency (5 Hz) is better than high frequency (30 Hz) in inducing acute inhibition during the stimulation. S1 DRT stimulation in this study exhibited a frequency response similar to tibial nerve stimulation but different from pudendal nerve stimulation. In addition, post-stimulation inhibition was not observed in cats during pudendal nerve stimulation (24, 28), but did occur during tibial nerve stimulation (26) as well as S1 or S2 DRT stimulation in this study. These results suggest that activation of limb afferents in the dorsal roots during sacral neuromodulation might play a dominant role in reducing bladder dysfunction.

Although the small number of animals used in the VRT stimulation experiments (Table 1) limits a statistical analysis, it provided sufficient information to eliminate efferent mechanisms as the major contributors to the bladder inhibition induced by sacral neuromodulation. The small number of cats (N = 3, Table 1) used for VRT stimulation allowed us to identify the DRT as the major contributor and focus our investigation on DRT-induced inhibition (N = 16, Figs. 3 and 4). For the same reason, S3 DRT was not systematically investigated due to its inconsistent response (see Table 1). Our study clearly identified central mechanisms in the spinal cord and/or brain as the major contributors to sacral neuromodulation, and therefore future research should focus on central rather than peripheral mechanisms of sacral neuromodulation.

This study in cats, which examined the neural pathways involved in sacral neuromodulation of reflex bladder activity, has raised the possibility that electrical stimulation of sacral afferent nerves elicits prolonged changes in bladder activity that are maintained following stimulation, as well as acute, rapidly reversible inhibitory effects that only occur during stimulation. This information may improve sacral neuromodulation therapy by optimizing the stimulation locations or parameters. The animal model established in this study could also be used in future studies to investigate the possible neurotransmitter mechanisms underlying sacral neuromodulation therapy (15, 25).

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**DISCLOSURES**

D. E. Nelson is a Medtronic employee.

**AUTHOR CONTRIBUTIONS**

NEURAL PATHWAYS INVOLVED IN SACRAL NEUROMODULATION


REFERENCES


