Effects of pudendal neuromodulation on bladder function in chronic spinal cord-injured rats

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Background/Purpose: Few studies have investigated the feasibility of using pudendal neuromodulation to regulate bladder function in spinal cord-injured (SCI) animals. The present study aimed to determine the effects of electrical activation of the pudendal sensory branch on improving voiding functions in rats 6 weeks after a spinal cord injury and to explore the underlying neuromodulatory mechanisms.

Methods: Two urodynamic measurements were used to assess the effects of electrical stimulation (ES) on bladder and urethral functions: simultaneous recordings of the intravesical pressure (IVP)
during continuous isotonic transvesical infusion (i.e., isotonic IVP) and external urethral sphincter (EUS) electromyography (EUS-EMG), and simultaneous recordings of transvesical pressure under isovolumetric conditions (i.e., isovolumetric IVP) and urethral perfusion pressure (UPP).

Results: Six weeks after the SCI, the rats showed voiding dysfunction, as indicated by abnormal cystometric measurements (e.g., increased volume threshold, increased contraction amplitude, and increased residual volume, and decreased voided volume). The voiding efficiency (VE) decreased to 13% after the SCI, but increased to 22–34% after applying pudendal afferent stimulation. In addition, pudendal stimulation significantly increased the EUS burst period and increased the difference between the UPP and the high-frequency oscillation (HFO) baselines, and changed the time offset between bladder and EUS activities. These findings suggest that pudendal afferent stimulation improved the VE by prolonging the micturition interval, decreased the urethral resistance, and recovered detrusor-sphincter dysynergia during the voiding phase.

Conclusion: This study demonstrates the feasibility of using pudendal neuromodulation in chronic SCI rats. These results could aid in developing an advanced neural prosthesis to restore bladder function in clinical settings.

Materials and methods

The experiment protocols involving the use of animals in this study were approved by the Institutional Animal Care and Use Committee of Taipei Medical University and Hospital (Taipei, Taiwan). Female Sprague–Dawley rats (n = 60) that weighed 250–300 g were used in the study. The rats were divided into two equal groups: the normal control (NC) group and the SCI group. The NC group rats received a sham operation with no damage to the spinal cord, whereas the SCI group rats received spinal cord transection. The spinal cord injury surgical procedures were performed, based on a previous report.11 Spinal cord transections were performed under 2–2.5% isoflurane anesthesia using aseptic surgical techniques. After a T8–T9 laminectomy, the dura matter, spinal cord, and spinal roots were cut with fine scissors. The severed ends of the spinal cord typically retracted 1–2 mm. They were inspected under a surgical microscope to ensure complete transection. The overlying muscle and skin were sutured. Animals were treated with an antibiotic (ampicillin, 200 mg/kg, intramuscular) for 7–10 days. To prevent overdistension of the bladder, urine was expressed manually every 6–8 hours until automatic micturition developed at approximately 10–14 days postsurgery. The bladder was then expressed two to three times daily.

Urodynamic and electromyographic recordings

After a 6-week recovery period, all rats were anesthetized with subcutaneous urethane (1.2 g/kg). The sensory branch of the unilateral pudendal nerve was exposed through a posterior approach.7,8 A bipolar cuff electrode was

Introduction

The lower urinary tract (LUT) system comprises two components: the reservoir (i.e., urinary bladder) and the outlet [i.e., bladder neck, urethra, and external urethral sphincter (EUS)], which are regulated by a complex neural control system. The two components typically exhibit reciprocal activities that are coordinated by a descending projection from the pontine center.1 A spinal cord injury above the lumbosacral level causes detrusor-sphincter dyssynergia (DSD) and induces simultaneous contractions of the bladder and the EUS during voiding, which obstructs evacuation of the urine from the bladder.2–4 Chronic DSD may increase bladder pressure and may result in vesicourethral reflux and renal failure.5 Daily urethral catheterization can aid patients in voiding urine from the bladder; however, catheterization can cause serious problems such as frequent urinary tract infections and a reduced quality of life.

Electrical neuromodulation such as sacral anterior root neuromodulation was successfully introduced to treat patients with a spinal cord injury and voiding dysfunction.6 However, despite its clinical efficacy, sacral neuromodulation is not widely accepted by these patients because of the need for a dorsal rhizotomy, which can inhibit residual sensations and reflexes such as defecation, erection, ejaculation, and lubrication. Therefore, a more effective neuroprosthesis is required to restore bladder function in patients with a spinal cord injury and voiding dysfunction. Recent animal and human studies indicate that electrical stimulation (ES) of the pudendal nerve encourages the augmenting reflex, and thereby improves bladder voiding in patients with voiding dysfunction.7–10 Thus, pudendal nerve modulation by ES could be an effective approach to treat voiding dysfunction.

Rats have gained great popularity as the primary species to investigate LUT functions. Therefore, SCI rats have been extensively used to investigate physiological changes in the regulation of urine storage and micturition reflexes.11–13 However, to our knowledge, few studies have investigated the feasibility of using pudendal neuromodulation to regulate bladder function in a rat model of chronic spinal cord injury. Thus, this study primarily aimed to investigate the feasibility of electrically activating the pudendal sensory branch to improve bladder dysfunction in rats 6 weeks after a spinal cord injury and to explore the underlying neuromodulatory mechanisms.
mounted on the isolated pudendal sensory branch for ES. Regulated current, cathodic, monophasic pulses were applied for 0.1 ms by a stimulator with an amplitude of 0.01–0.2 mA and a frequency of 2 Hz. The stimulators were controlled by using the LabView program (National Instruments, Austin, TX, USA), which sampled and analyzed the intravesical pressure (IVP) signals and generated a voltage signal to each stimulator through a digital-to-analog converter.7,8 The stimulator was triggered when the cystometric pressure reached a preset threshold.

Two preparations were used to quantify the effect of ES on the pudendal nerve on LUT functions. The first experimental series (n = 12 for each group) involved simultaneous IVP recordings during isotonic transvesical infusion with an open urethra (i.e., isotonic IVP) and EUS electromyography (EUS-EMG). Surgical procedures for the simultaneous recordings of isotonic IVP and EUS-EMG were performed as described in previous reports.7,14,15 The tail vein was catheterized for fluid and drug administration, and the body temperature was maintained at 36–38°C with a recirculating water blanket. The urinary bladder was exposed via a midline abdominal incision. A polyethylene (PE)-50 tube was inserted into the bladder lumen to measure the isotonic IVP. Before the insertion, the bladder end of the PE tube was heated to form a collar and then passed through a small incision at the apex of the bladder dome. The tube was secured with a purse-string suture and the abdominal wall was closed with a nylon suture. By using a three-way stopcock, the PE tube was connected to an infusion pump for filling the bladder and to a pressure transducer (Deltran DPT-100; Utah Medical Products, Midvale, UT, USA) for monitoring the IVP. Two insulated silver wire electrodes (0.05 mm in diameter) with exposed tips were inserted bilaterally into the lateral aspect of the midurethra behind the pubic symphysis for the EUS-EMG recordings. After manually emptying the bladder, transvesical cystometry was performed at a fixed infusion rate of 0.12 mL/min with saline in the NC group rats and 0.4 mL/min for SCI rats. Electrical stimulation was applied during voiding contractions that were triggered when the isotonic IVP exceeded 20 cmH2O (or 25 cmH2O for the SCI rats), and ES was ceased when the pressure dropped below this threshold.

Multiple isotonic IVP and EUS-EMG parameters were measured to quantify the effects of ES on voiding7,8: volume threshold, contraction amplitude, contraction duration, residual volume, voided volume, and voiding efficiency (VE; the ratio of the voided volume to the volume threshold). Furthermore, the duration of the EUS burst period (BP) was calculated (Figure 1).8,15,19

Several parameters were measured for the isovolumetric IVP and UPP recordings14,18: the maximal amplitude of the reflex bladder contractions; the baseline UPP (i.e., the average UPP baseline between reflex bladder contractions); the baseline high-frequency oscillation (HFO), which is the average HFO baseline in the UPP during reflex bladder contractions; the HFO amplitude (i.e., the peak-to-peak HFO amplitude in the UPP during a reflex bladder contraction); the duration of the HFOs; and the difference between the UPP and HFO baselines (Figure 2).

Coordination between the bladder and EUS activities

To determine whether pudendal afferent stimulation affected the coordination between the bladder and EUS activities during voiding, the isotonic IVP and EUS-EMG data obtained from the first experimental series were further analyzed using a custom-designed computational software program. The EUS-EMG data were first rectified, and then smoothed by resampling to achieve an effective sampling rate of 10 Hz. The first derivative of the isotonic IVP and the resampled EUS-EMG data were used to determine the rate of change in the IVP and EUS-EMG recordings during a voiding cycle. The rates of change in the IVP and EUS-EMG recordings were subsequently normalized and plotted as a time-domain function (Figure 3). The time offset between the peaks of the IVP and EUS-EMG derivatives for each rat was averaged from six voiding cycles. The statistical data were obtained from six animals. The time offset calculation was performed as previously described.20

Histological examination

Midurethral histological examinations were performed in six NC rats and six SCI rats. The animals were euthanized by a urethane overdose. Their urethras were removed, fixed in 10% buffered formalin, and embedded in paraffin. The midurethral section was then cut into 5-mm sections and stained with hematoxylin and eosin.19
Data analysis

All data are presented as the mean ± standard deviation. A two-way analysis of variance (ANOVA) was used to compare the parameters obtained from all examinations. The ANOVA was followed by the Student–Newman–Keuls post-hoc test using SigmaStat software (Systat Software, Inc., San Jose, CA, USA). A value of $p < 0.05$ was considered significant for all analyses.

Figure 1  The typical patterns of isotonic transvesical pressure under continuous transvesical infusion (i.e., isotonic IVP; top tracing) and external urethral sphincter electromyography (EUS-EMG; bottom tracing) recorded in the normal control (NC) rats and spinal cord-injured (SCI) rats. (A) In a NC rat, bladder micturition contractions [indicated by an asterisk (*)] are induced by a constant-rate (0.12 mL/min) intravesical saline infusion, which was accompanied by large amplitude EUS-EMG activity. (B) Multiple small nonvoiding contractions precede the first bladder micturition contraction (*) during continuous intravesical saline infusion (0.2 mL/min). Compared to the NC rats, cystometric parameters in the SCI rats are significantly increased in the volume threshold and contraction amplitude and duration. (C) The recording-period micturition (denoted in 1A) at a faster time scale. A prolonged EUS-EMG burst period is apparent during a voiding contraction in the NC rats. (D) The recording-period micturition (denoted in 1B) at a faster time scale. In the SCI rats, EUS-EMG did not show burst activity during a nonvoiding contraction. (E) The recording-period micturition (denoted in 1B) at a faster time scale. Several fragments of short bursts usually appear during a voiding contraction in the SCI rats. BP = burst period; EMG = electromyography; EUS = external urethral sphincter; IVP = intravesical pressure; NC = normal control; SCI = spinal cord-injured.
Results

The effect of ES on the isotonic IVP and EUS-EMG

Figure 1 illustrates the simultaneous recordings of the isotonic IVP and EUS-EMG in NC rats and SCI rats without ES. Compared to the NC rats, the chronic SCI rats exhibited significant differences in cystometric parameters such as increased volume threshold, increased contraction amplitude, increased contraction duration, and increased residual volume (Table 1). The SCI rats also exhibited a significant decrease in the VE, compared to the NC rats (13.0% vs. 83.5%, respectively). We further evaluated the effects of ES of the pudendal nerve on isotonic IVP parameters in the NC rats and SCI rats. No tested ES parameter conferred a significant effect on the isotonic IVP variables in the NC rats (Table 1). However, pudendal neuromodulation significantly altered the VE in the SCI rats: ES at lower intensities (0.01 to 0.05 mA) significantly increased the VE from 13% to approximately 22 to 40%, but there were no significant effects at higher stimulation amplitudes (0.1 to 0.2 mA).

A long BP in the EUS-EMG was apparent during voiding contractions in the NC rats (Figure 1C). However, in the SCI rats, EUS-EMG revealed no BP during small nonvoiding bladder contractions that preceded the first bladder micturition contraction (Figures 1B and 1D); however, several short BP fragments usually appeared during voiding contractions (Figure 1E). Compared to the NC rats, the SCI rats also exhibited significantly shorter BPs, which were significantly increased at lower stimulation intensities (0.01 to 0.05 mA; Table 1).

The effect of ES on the isovolumetric IVP and UPP

Figure 2 shows the simultaneous recordings of the isovolumetric IVP and UPP in the NC rats and SCI rats without ES. The isovolumetric bladder contractions were associated with urethral relaxation and high-frequency oscillations (HFOs) in the UPP that appear during urethral relaxation in the NC rats and SCI rats. The recordings are quantified by using the baseline UPP, baseline HFOs, differences between the UPP and HFO baselines, duration of HFOs, HFO amplitude, and maximal amplitude of reflex bladder contractions. HFO = high-frequency oscillation; IVP = isotonic intravesical pressure; NC = normal control; SCI = spinal cord-injured; UPP = urethral perfusion pressure.
differences between the UPP and HFO baselines were comparable to those detected in the NC rats.

Differences between the UPP and HFO baselines in the SCI rats significantly increased at the lower stimulation intensity of 0.05 mA; the ES did not alter the UPP baseline but did decrease significantly the HFO baseline. By contrast, baseline differences were insignificantly altered at the higher stimulation intensity of 0.2 mA in the SCI rats.

Figure 3 illustrates the effects of pudendal afferent stimulation (ES) of 0.05 mA on temporal relationships between the bladder and external urethral sphincter (EUS) activities during a voiding cycle in (A) the normal control (NC) rats and (B) the spinal cord-injured (SCI) rats. The first derivative of the isotonic intravesical pressure (IVP) and resampled EUS electromyography (EUS-EMG) data are normalized and plotted as a function of time. The time offset between the peaks (i.e., maximal rate change) of the isotonic IVP (red solid line) and EUS-EMG (black dashed line) activities was determined in rats without and with ES. The ES (indicated by the horizontal blue bar) is delivered when the bladder pressure exceeds a preset threshold and is ceased when the pressure returns to below this threshold. ES = electrical stimulation; EUS-EMG = external urethral sphincter electromyography; IVP = intravesical pressure; NC = normal control; SCI = spinal cord-injured.

Temporal relationships between the isotonic IVP and EUS-EMG activities

Figure 3 illustrates the effects of pudendal afferent stimulation on the coordination between the bladder and EUS activities. The temporal relationships between the bladder and EUS activities during a voiding cycle were determined by calculating the time offset between the detection of the
peak (i.e., maximal rate change) of the IVP and EUS-EMG derivatives. Table 3 summarizes the results of the time offset obtained from the NC rats and SCI rats without and with ES. In the NC rats, the IVP derivative peak was detected earlier than the EUS-EMG derivative peak, and the time offset was approximately 0.62 seconds (Table 3 and Figure 3A). The NC rats with concomitant pudendal nerve ES subsequently showed no significant differences in the time offset. By contrast, the EUS-EMG derivative peak was detected earlier than the IVP derivative peak in the SCI rats without concomitant ES (Figure 3B), which resulted in a negative time offset (~3.25 s). A lower stimulation intensity of 0.05 mA interestingly produced a positive time offset of approximately 0.61 seconds, which was comparable to the time offset detected in the NC rats. This finding indicated that low stimulation amplitudes enhanced the coordination between the bladder and EUS activities in the SCI rats. However, the time offset was not affected by the stimulation intensity of 0.2 mA.

### Histology of the urethra of the NC rats and SCI rats

Histological examination of midurethral transverse sections revealed numerous circular bands of muscle fibers in the striated muscle layer. The width of the four striated muscle regions close to the two diagonal lines was quantified in the NC rats and SCI rats (Figure 4). All four striated muscle regions were significantly thicker in the SCI rats than in the NC rats (Figure 5).

### Discussion

This study aimed to investigate the feasibility of applying electrical activation to the pudendal sensory branch to

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**Table 1** The effects of electrical stimulation of pudendal afferents on isotonic intravesical pressure and external urethral sphincter electromyography parameters.

<table>
<thead>
<tr>
<th></th>
<th>Volume threshold (mL)</th>
<th>Contraction amplitude (cmH2O)</th>
<th>Contraction duration (s)</th>
<th>Residual volume (mL)</th>
<th>Voided volume (mL)</th>
<th>Voiding efficiency (%)</th>
<th>Burst period (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC rats without ES</td>
<td>0.8 ± 0.1</td>
<td>30.2 ± 4.2</td>
<td>23.9 ± 2.9</td>
<td>0.14 ± 0.06</td>
<td>0.70 ± 0.11</td>
<td>83.5 ± 5.6</td>
<td>4.82 ± 0.32</td>
</tr>
<tr>
<td>2 Hz, 0.01 mA</td>
<td>0.8 ± 0.1</td>
<td>33.1 ± 4.4</td>
<td>23.3 ± 3.2</td>
<td>0.17 ± 0.1</td>
<td>0.66 ± 0.1</td>
<td>79.7 ± 5.6</td>
<td>4.75 ± 0.38</td>
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<tr>
<td>2 Hz, 0.03 mA</td>
<td>0.9 ± 0.1</td>
<td>33.2 ± 2.8</td>
<td>22.5 ± 3.3</td>
<td>0.20 ± 0.04</td>
<td>0.70 ± 0.1</td>
<td>77.8 ± 4.7</td>
<td>4.89 ± 0.44</td>
</tr>
<tr>
<td>2 Hz, 0.05 mA</td>
<td>0.9 ± 0.1</td>
<td>28.8 ± 3.0</td>
<td>22.2 ± 2.8</td>
<td>0.19 ± 0.08</td>
<td>0.67 ± 0.1</td>
<td>80.7 ± 7.1</td>
<td>4.85 ± 0.43</td>
</tr>
<tr>
<td>2 Hz, 0.1 mA</td>
<td>0.8 ± 0.1</td>
<td>30.4 ± 3.9</td>
<td>22.9 ± 2.5</td>
<td>0.15 ± 0.04</td>
<td>0.63 ± 0.1</td>
<td>81.1 ± 4.6</td>
<td>4.67 ± 0.50</td>
</tr>
<tr>
<td>2 Hz, 0.2 mA</td>
<td>0.9 ± 0.1</td>
<td>29.4 ± 3.0</td>
<td>23.1 ± 2.3</td>
<td>0.18 ± 0.07</td>
<td>0.67 ± 0.1</td>
<td>79.2 ± 5.9</td>
<td>4.86 ± 0.27</td>
</tr>
<tr>
<td>SCI rats without ES</td>
<td>1.7 ± 0.3**</td>
<td>45.0 ± 2.5**</td>
<td>40.4 ± 4.6**</td>
<td>1.45 ± 0.25**</td>
<td>0.21 ± 0.05**</td>
<td>13.0 ± 3.2**</td>
<td>2.72 ± 0.25</td>
</tr>
<tr>
<td>2 Hz, 0.01 mA</td>
<td>1.6 ± 0.3</td>
<td>42.5 ± 4.1</td>
<td>37.6 ± 3.9</td>
<td>1.24 ± 0.24</td>
<td>0.35 ± 0.04*</td>
<td>22.2 ± 4.0*</td>
<td>4.13 ± 0.33*</td>
</tr>
<tr>
<td>2 Hz, 0.03 mA</td>
<td>1.8 ± 0.3</td>
<td>40.3 ± 2.9</td>
<td>44.5 ± 3.5</td>
<td>1.27 ± 0.22</td>
<td>0.56 ± 0.05*</td>
<td>30.9 ± 3.4*</td>
<td>4.86 ± 0.34*</td>
</tr>
<tr>
<td>2 Hz, 0.05 mA</td>
<td>1.7 ± 0.2</td>
<td>43.6 ± 5.9</td>
<td>41.3 ± 3.8</td>
<td>1.13 ± 0.19*</td>
<td>0.57 ± 0.04*</td>
<td>34.0 ± 3.1*</td>
<td>4.43 ± 0.62*</td>
</tr>
<tr>
<td>2 Hz, 0.1 mA</td>
<td>1.7 ± 0.2</td>
<td>40.3 ± 3.4</td>
<td>31.0 ± 3.6*</td>
<td>1.42 ± 0.21</td>
<td>0.23 ± 0.03</td>
<td>14.3 ± 2.8</td>
<td>3.04 ± 0.26</td>
</tr>
<tr>
<td>2 Hz, 0.2 mA</td>
<td>1.8 ± 0.2</td>
<td>44.3 ± 4.1</td>
<td>30.9 ± 2.8*</td>
<td>1.65 ± 0.17</td>
<td>0.16 ± 0.02</td>
<td>8.8 ± 1.5</td>
<td>2.93 ± 0.21</td>
</tr>
</tbody>
</table>

The values are presented as the mean ± the standard deviation (n = 12).

* Indicates a significant difference between the NC rats and SCI rats without ES (p < 0.05).

** Indicates a significant difference between the NC rats and SCI rats without ES (p < 0.05).

ES = electrical stimulation; NC = normal control; SCI = spinal cord-injured; UPP = urethral perfusion pressure.

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**Table 2** The effects of electrical stimulation of pudendal afferents on isovolumetric intravesical pressure and urethral perfusion pressure parameters.

<table>
<thead>
<tr>
<th></th>
<th>Baseline UPP (cmH2O)</th>
<th>Baseline HFOs (cmH2O)</th>
<th>HFOs amplitude (cmH2O)</th>
<th>Maximum amplitude of the bladder contraction (cmH2O)</th>
<th>HFOs duration (s)</th>
<th>Difference between UPP and HFOs baselines (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC rats without ES</td>
<td>22.9 ± 1.3</td>
<td>13.3 ± 1.1</td>
<td>3.4 ± 0.6</td>
<td>51.9 ± 2.9</td>
<td>15.2 ± 2.3</td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>2 Hz, 0.05 mA</td>
<td>23.1 ± 0.8</td>
<td>13.3 ± 0.8</td>
<td>3.4 ± 0.4</td>
<td>54.5 ± 2.2</td>
<td>15.6 ± 1.7</td>
<td>9.8 ± 0.9</td>
</tr>
<tr>
<td>2 Hz, 0.2 mA</td>
<td>22.9 ± 1.6</td>
<td>12.8 ± 1.1</td>
<td>3.5 ± 0.4</td>
<td>54.7 ± 3.0</td>
<td>16.1 ± 1.5</td>
<td>10.1 ± 1.2</td>
</tr>
<tr>
<td>SCI rats without ES</td>
<td>22.2 ± 1.2</td>
<td>16.9 ± 1.2**</td>
<td>1.7 ± 0.2**</td>
<td>36.8 ± 2.2**</td>
<td>10.9 ± 1.5**</td>
<td>5.3 ± 0.5**</td>
</tr>
<tr>
<td>2 Hz, 0.05 mA</td>
<td>22.1 ± 0.8</td>
<td>14.2 ± 1.3*</td>
<td>1.6 ± 0.1</td>
<td>38.4 ± 2.6</td>
<td>9.2 ± 1.0</td>
<td>7.9 ± 0.7*</td>
</tr>
<tr>
<td>2 Hz, 0.2 mA</td>
<td>21.1 ± 1.0</td>
<td>15.6 ± 1.0*</td>
<td>1.8 ± 0.2</td>
<td>37.2 ± 2.1</td>
<td>6.5 ± 1.0*</td>
<td>5.5 ± 0.7</td>
</tr>
</tbody>
</table>

The values are presented as the mean ± the standard deviation (n = 12).

* Indicates a significant difference between the NC rats and SCI rats without and with ES (p < 0.05).

** Indicates a significant difference between the NC rats and the SCI rats without ES (p < 0.05).

ES = electrical stimulation; HFO = high-frequency oscillation; UPP = isovolumetric intravesical pressure; NC = normal control; SCI = spinal cord-injured; UPP = urethral perfusion pressure.
improve bladder voiding functions in chronic SCI rats. The results demonstrated that low-intensity ES (0.01–0.05 mA) significantly increased the VE from 13% to approximately 22–34% in rats with a chronic spinal cord injury (Table 1). Improvements in bladder voiding indicated ES significantly altered the activity of pudendal afferents during voiding such as urethral resistance, EUS-BP, and coordination between bladder and EUS activities.

Urethral relaxation in the voiding phase as enhanced by ES is one explanation for the improved VE. In rats with a chronic spinal cord injury, urethral resistance during the storing phase (i.e., baseline UPP) revealed no significant changes; however, urethral resistance during the voiding phase (i.e., baseline HFO) was significantly higher, and consequently decreased the difference between the UPP and HFO baselines (i.e., increased urethral resistance during the voiding phase), which would impede bladder voiding. According to Poiseuille’s law, the resistance at which a fluid flows through a pipe is inversely proportional to the pipe diameter raised to the fourth power.21 The urethra is more complex because it is not a rigid pipe and has elastic components; however, the urethral cross-sectional diameter or area remains a critical factor that dominates urethral resistance. The present study demonstrated that pudendal afferent stimulation increased urethral relaxation (i.e., increased urethral diameter) by significantly decreasing the baseline HFOs and increasing the difference between the UPP and HFO baselines (Table 2), which would accelerate voiding of urine from the bladder.

Previous studies indicate that pudendal sensory feedback is required to determine EUS burst activity during voiding.22 The EUS burst activity represents the phasic relaxation and opening of the outlet, which are essential for efficient voiding.23,24 Our results demonstrated that ES elongated the EUS-BP in the SCI rats (Table 1), which indicated that the sensory feedback augmented by pudendal afferent stimulation enhanced the patterning of the EUS burst activity. In addition, the prolonged EUS-BP implied that the urethral outlet was open for a longer duration for urine voiding, and this could have enhanced the VE induced by pudendal afferent stimulation.

Table 3 The effects of electrical stimulation of pudendal afferents on the time offset between the bladder and external urethral sphincter activities.

<table>
<thead>
<tr>
<th>Time offset (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC rats without ES</td>
</tr>
<tr>
<td>2 Hz, 0.05 mA</td>
</tr>
<tr>
<td>2 Hz, 0.2 mA</td>
</tr>
<tr>
<td>SCI rats without ES</td>
</tr>
<tr>
<td>2 Hz, 0.05 mA</td>
</tr>
<tr>
<td>2 Hz, 0.2 mA</td>
</tr>
</tbody>
</table>

The values are presented as the mean ± the standard deviation (n = 6).

* Indicates a significant difference between the NC rats and SCI rats without ES (p < 0.05).

** Indicates a significant difference between the NC rats and SCI rats without ES (p < 0.05).

ES = electrical stimulation; NC = normal control; SCI = spinal cord-injured.

Coordination between the urinary bladder and EUS is necessary for normal voiding, which is modulated by a spinal neural pathway that links the bladder to EUS activity during the micturition reflex. Such a pathway can be modulated by pudendal afferent stimulation via different transmission routes. First, pudendal afferent stimulation may activate the micturition switch in the pons and simultaneously deliver excitatory output to the sacral
The present study further demonstrated that abnormal temporal relationships between the bladder and EUS activities in the SCI rats could be reverted by pudendal afferent stimulation to nearly a normal time course (0.05 mA; Table 3). Therefore, the increased VE in the SCI rats may be partially attributable to the effects of pudendal stimulation on alleviating DSD. To our knowledge, this is the first study to investigate the effects of pudendal stimulation on the coordination between the bladder and EUS activities. The effects of pudendal neuromodulation on DSD and VE nevertheless warrant further exploration.

In this study, simultaneous recordings of the isovolumetric IVP and UPP in rats with a chronic spinal cord injury revealed abnormal IVP and UPP parameters such as increased baseline HFOs and decreased amplitude in bladder contractions and decreased duration of HFOs (Table 2). These abnormalities may be attributable to the reorganization of the micturition reflex pathway and histological alterations induced in the LUT by a chronic spinal cord injury.

A normal micturition reflex controlling the bladder and EUS depends on spinal pathways passing through the pontine micturition center, which is modulated by A-delta afferent nerves. However, in chronic SCI rats, the centrally mediated control of this reflex was eliminated. Thus, this reflex is dependent only on spinal pathways modulated by C-fiber afferent nerves, which are involved in the initiation of DSD and/or detrusor hyperreflexia. This neural reorganization contributes to abnormal bladder and urethral activities. Furthermore, morphological changes in the bladder and EUS caused by a spinal cord injury are other possible factors that induced abnormal IVP and UPP activities. This possibility was supported by the significant increase in the EUS thickness after the spinal cord injury in our study (Figure 5), and by previous reports demonstrating that a spinal cord injury induces bladder-wall tissue remodeling such as hypertrophy and fibrosis. To our knowledge, this is the first study to report quantification of morphological changes in the EUS in rats with a spinal cord injury.

In the present study, the bladder voiding dysfunction in SCI rats was significantly enhanced by ES at lower intensities of 0.01–0.05 mA, as evidenced by improvements in isovolumetric IVP, isovolumetric IVP, and UPP (Tables 1 and 2). However, higher amplitudes of 0.1–0.2 mA, exerted inhibitory effects on voiding functions, as indicated by certain cytometric parameters such as decreased duration of bladder contractions and HFOs. The varying responses evoked at low and high stimulation intensities in rats were consistent with those reported in previous animal studies. High-amplitude stimulation of pudendal afferents reportedly induce bladder inhibition in cats and in humans. However, the frequency and stimulus train duration also have crucial roles in determining the response polarity in cats. The voiding efficiency (VE) in SCI rats without concomitant ES has been reported at only approximately 83%, which is lower than the approximately 98–99% VE measured in conscious animals. This limitation presumably results from the anesthesia, which inhibits bladder emptying in all rats. Similar low voiding efficiencies have been reported in other studies and presumably resulted from urethane anesthesia inhibiting reflex bladder contractions and reducing the contraction pressure during micturition. The physiological functions of the bladder and EUS appear to be preserved under urethane, and urethane is, as Matsuura and Downie state, "the most suitable anesthetic for physiological experiments that require demonstration of reflex micturition." Anesthetic effects are a limitation of the present study; however, the facilitative effects of electrical pudendal neuromodulation on bladder emptying were readily detected in the SCI rats.
This study showed that electrical activation of pudendal afferents improved the VE in rats with a spinal cord injury by decreasing the urethral outlet resistance, prolonging the micturition interval, and improving the synergy of the bladder and EUS activities during voiding. The ability to translate the present results to humans remains unclear. However, the feasibility of using pudendal nerve stimulation to improve bladder voiding functions in models of chronic spinal cord injury warrants further exploration.

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References

Pudendal neuromodulation in chronic SCI rats


