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To cite this article: Tsung-Hsun Hsieh et al 2016 J. Neural Eng. 13 046001

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Chronic pudendal neuromodulation using an implantable microstimulator improves voiding function in diabetic rats

Tsung-Hsun Hsieh¹,²,³, Yin-Tsong Lin⁴,⁵, Shih-Ching Chen⁶,⁷ and Chih-Wei Peng⁴,⁶,⁷,⁸

¹ Graduate Institute of Neural Regenerative Medicine, Taipei Medical University, Taipei, Taiwan
² Department of Physical Therapy and Graduate Institute of Rehabilitation Science, College of Medicine and Healthy Aging Research Center, Chang Gung University, Taoyuan, Taiwan
³ Neuroscience Research Center, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan, Taiwan
⁴ School of Biomedical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan
⁵ Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan
⁶ Department of Physical Medicine and Rehabilitation, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
⁷ Department of Physical Medicine and Rehabilitation, Taipei Medical University Hospital, Taipei, Taiwan
⁸ Graduate Institute of Biomedical Materials and Tissue Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan

E-mail: cwpeng@tmu.edu.tw

Received 14 October 2015, revised 13 April 2016
Accepted for publication 22 April 2016
Published 17 May 2016

Abstract

Objective. Few studies have investigated the feasibility of using chronic pudendal neuromodulation for improving voiding function in patients with diabetes who are also experiencing urinary retention. The present study investigated the effects of chronic electrical stimulation (ES) of the sensory branch of the pudendal nerve on voiding function in diabetic rats.

Approach. A custom-made implantable microstimulation system was designed and manufactured for chronic implantation in normal control (NC) and diabetic rats. After three or six weeks of pudendal neuromodulation, the intravesical pressure, external urethral sphincter electromyograms (EUS-EMGs), and urine flow rate (UFR) of all rats were simultaneously recorded to assess the effects of chronic pudendal ES on voiding function. Morphological changes in pudendal axons were assessed through hematoxylin and eosin (H&E) staining.

Results. Abnormal cystometric measurements, including an increased volume threshold, contraction amplitude, and residual volume (RV) as well as a decreased burst period (BP) indicated voiding dysfunction in the diabetic rats with sham ES for three or six weeks. However, real ES for three or six weeks reversed these abnormal results; these parameters became similar to those of the NC rats and, consequently, voiding efficiency (VE) increased significantly from 17%–45% to 51%–57%. The cross-sectional area and axonal density of the pudendal nerve decreased in all the diabetic rats with sham ES for six weeks; however, these values increased significantly after real ES for six weeks. Significance. This study demonstrated the feasibility of
using chronic pudendal neuromodulation for improving voiding function in diabetic rats. These results may facilitate the development of an advanced neural prosthesis for restoring bladder function in clinical settings.

Keywords: neuromodulation, electrical stimulation, voiding, intravesical pressure

(Some figures may appear in colour only in the online journal)

Introduction

Sacral neuromodulation (SNM) was approved by the US Food and Drug Administration in 1997 for treating urinary urge incontinence, urgency, frequency, and nonobstructive urinary retention resulting from detrusor underactivity [1]. More than 100,000 people worldwide have received SNM with an InterStim device (Medtronic, Minneapolis, MN, USA) [2]. Although the precise mechanism underlying SNM remains unclear, previous studies have reported that in patients with urinary retention, SNM modulates the urethral afferent activity, restores the sensation of bladder fullness, and reduces the inhibition of detrusor muscle contraction [3, 4].

Diabetic cystopathy is characterized by typical symptoms including an impaired sensation of bladder fullness, increased bladder capacity, decreased bladder contractility, detrusor sphincter dyssynergia (DSD), and elevated postvoid residual urine volumes [5–7]. Clinically, chronic diabetes-induced urinary retention may increase bladder pressure and vesicourethral reflux, and even cause renal failure. Standard therapy, including pharmacological, behavioral, and surgical approaches, was initially used for treating patients with cystopathy [8]. However, cystopathy is often refractory to the aforementioned interventions, and many patients must resort to intermittent self-catheterization. Therefore, patients frequently experience urinary tract infections and typically have a low quality of life.

Although SNM is approved and successfully used for the long-term treatment of various urological diseases, to our knowledge, few studies have explored the feasibility of using electrical neuromodulatory approaches for improving bladder function in patients with diabetes who are also experiencing urinary retention [9]. In addition, the pudendal nerve is currently used as the optimal neuromodulation site for SNM because of the lack of therapeutic effects of S3 stimulation in some patients [3, 10]. By contrast, pudendal nerve stimulation can provide optimal neuromodulatory effects through the simultaneous activation of S2–S4 afferents [11].

In this study, we investigated whether chronic electrical stimulation (ES) of pudendal afferents for three or six weeks improves bladder function in a diabetic animal model of urinary retention. Most neuromodulatory studies have been limited to acute animal experiments because of the lack of suitable implantable microstimulation devices. To determine the effects of chronic pudendal neuromodulation, we developed a custom-made implantable microstimulation system and implanted it in rats for neuromodulatory experiments. Rats were evaluated after three or six weeks of pudendal neuromodulation, and their intravesical pressure (IVP), external urethral sphincter electromyograms (EUS-EMGs), and urine flow rate (UFR) were simultaneously recorded during continuous cystometrographic monitoring in order to assess the effects of ES on voiding function. The pudendal nerve was histologically assessed through hematoxylin and eosin (H&E) staining to quantify the morphological changes in pudendal axons.

Methods

Implantable microstimulation system

The implantable microstimulation system developed for this study was comprised of a microstimulator and biocompatible cuff electrode for chronic implantation. The microstimulator module was mostly fabricated with surface mount device (SMD) components and a 3 V coin battery (CD2032, Panasonic, Osaka, Japan) mounted on a double-layer printed circuit board. The battery was utilized for powering the electronic circuit of the microstimulator and for outputting a 20 Hz monophasic rectangular stimulation current with a stable, low-stimulation intensity (fixed pulse duration, 0.1 ms; amplitude, 10 μA). The microstimulator module was further hermetically sealed using medical grade silicone (Dow Corning® 184 Silicone Elastomer, USA). The hermetrical sealing of the implant is essential for preventing body fluids from leaking into the implanted device, causing it to malfunction. For chronic implantation, a biocompatible cuff electrode was fabricated using a platinum wire and medical grade silicone sheet. The electrode was connected to the stimulator module as an interface for delivering the electrical stimuli to the unilateral pudendal sensory nerve.

Animal preparation

All animal experiments were approved by the Institutional Animal Care and Use Committee of Taipei Medical University and Hospital. Female Sprague–Dawley rats (n = 96) weighing 250–300 g were used in the experiment of chronic pudendal neuromodulation. The rats were randomly assigned to two groups: diabetic and normal control (NC) groups (n = 48 each). Each group was subdivided into four groups for three or six weeks of sham and real ES (figure 1). After a 24 h fast, diabetes was induced using a single tail vein injection of 65 mg kg⁻¹ streptozotocin (STZ; Sigma, St. Louis, MO, USA) freshly dissolved in a 0.1 M citrate buffer solution at pH 4.5 [12]. Three days after the STZ treatment, the blood glucose level was estimated from a blood sample obtained by cutting the tail. Rats with a blood glucose level of ≥300 mg/day were deemed diabetic and therefore underwent
implantation with a customized microstimulation system. Similarly, the NC rats were treated with a vehicle (i.e. normal saline), and the microstimulation system was implanted three days later.

Fully functional microstimulation systems were implanted in all these rats by using isoflurane (2.0%–2.5%) through aseptic surgical techniques including hermetic packaging and implanting the system within the subcutaneous tissue over the lower back. In addition, a bipolar cuff electrode for chronic pudendal neuromodulation was applied to the sensory branch of the unilateral pudendal nerve through a posterior approach [12, 13]. After the implantation, the rats were administered an antibiotic (ampicillin 200 mg kg\(^{-1}\), intramuscular) for seven days.

**Current threshold for the activation of pudendal axons**

To examine the minimum current intensity for the activation of pudendal axons, additional rats (\(n = 6\) normal rats) were used only to quantify the current threshold required to evoke the pudendal–pudendal reflex (i.e. an EUS-EMG response evoked with the stimulation of the pudendal sensory branch). The stimulus parameters were fixed at 1 Hz, with a 0.1 ms pulse width.

**Protocols for chronic pudendal neuromodulation**

Beginning on the day following implantation, 30 min of pudendal neuromodulation was provided daily to the rats in the real ES subgroups for three or six weeks. Regulated current cathodic, monophasic pulses (pulse width, 0.1 ms) with a fixed stimulation frequency (10 \(\mu\)A) and intensity (20 Hz) were used to stimulate the sensory branch of the pudendal nerve. During neuromodulation, the rats were kept awake and allowed to move freely in their cages because the stimulation intensity was lower than the motor threshold and did not induce any stress responses.

**Simultaneous recording of IVP, EUS-EMG, and UFR**

After three or six weeks of pudendal neuromodulation, all the rats were anesthetized using urethane (1.2 g kg\(^{-1}\), subcutaneous), and their IVP, EUS-EMG, and UFR were simultaneously recorded to assess the effects of chronic pudendal microstimulation on lower urinary tract (LUT) function. The surgical procedure for the recordings was previously reported [12, 14]. The femoral vein was catheterized for fluid infusion, and the body temperature was maintained at 36–38 °C by using a recirculating water blanket. A midline abdominal incision was made to expose the bladder, and a polyethylene (PE) tube (PE-50) was inserted into the bladder lumen for IVP measurements. Furthermore, the bladder end of the PE tube was heated to form a collar, passed through a small incision at the apex of the bladder dome, and secured using a purse-string suture. A three-way stopcock was used to connect the PE tube to an infusion pump for filling the bladder with physiological saline and to a pressure transducer (P23XL-1, Becton Dickinson, NJ, USA) for monitoring the IVP. Two insulated silver wire electrodes (diameter, 0.05 mm) with exposed tips were inserted into the lateral aspects of the mid–urethra to record the EUS-EMG signals. Finally, the abdominal wall was closed using nylon sutures. An ultrasonic flow probe (Probe #ME2PXN; Transonic Systems, Ithaca, NY, USA) connected to a flow meter (TS410; Transonic Systems) was used to measure the UFR. The flow probe was placed around the most distal part of the urethra [14–16].

All rats underwent urodynamic and EUS-EMG examinations, generally beginning three to four h after anesthesia induction. After the bladder was manually voided, the IVP, EUS-EMG, and UFR of the NC rats were simultaneously recorded during continuous-infusion cystometry at an infusion rate of 0.2 ml min\(^{-1}\) (0.3 ml min\(^{-1}\) for the diabetic rats) by using physiological saline at room temperature with an open urethra. The infusion pump was turned off after two or three voiding contractions. The recorded signals (sample rate, 1 kHz) were delivered to a computer through an analog-to-digital (A/D) converter (Biopac MP 36, BIOPAC Systems, Santa Barbara, CA, USA). The IVP and UFR signals were amplified 100× by using the A/D converter, and the EUS-EMG signal was amplified 1000× (figure 2).

The analyst who examined the IVP, EUS-EMG, and UFR was blind to the status of the rats. The following IVP parameters were measured to quantify the effects of chronic pudendal neuromodulation on bladder function: (1) the micturition volume threshold (VT), defined as the infused volume of saline sufficient for inducing the first voiding contraction;
(2) the contraction amplitude (CA), defined as the maximum pressure during voiding; (3) the bladder contraction duration (CD) during voiding; (4) the residual volume (RV) of saline withdrawn through the intravesical catheter after the final voiding contraction; and (5) the voiding efficiency (VE), the ratio between the voided volume (VV) and the VT. The VV was obtained by subtracting the RV value from the VT value [17–19].

For the EUS-EMG and UFR recordings, two parameters were measured (figure 5(B)) [14]: (1) the duration of the EUS burst period (BP), defined as the interval between the point at which the tonic EMG is converted into a burst discharge and the point at which the EMG is converted into a tonic EMG, and (2) the mean UFR of the flow duration, defined as the mean UFR of the interval between the point at which the baseline UFR is converted into a burst discharge and the point at which the UFR is converted into the baseline.

Histological examination

After urodynamic examinations, all rats were euthanized by administering an overdose of urethane. The unilateral pudendal nerve along with the cuff electrode was completely removed, fixed in 10% buffered formalin, and embedded in paraffin. The middle segment of the nerve tissue was then cut into 2 μm-thick slices, which were stained using H&E [18]. To examine the tissue morphology, digital images of the tissue samples were captured using an optical microscope (Carl Zeiss AG, Oberkochen, Germany) at 100× or 400× magnification. For quantifying the chronic neuromodulation on the pudendal nerve, Axiovision software (Carl Zeiss AG, Oberkochen, Germany) was used to digitally measure the cross-sectional area and axonal density of the sensory branch of the pudendal nerve of all the rats.

Statistical analysis

Data are presented as the mean ± SD. A two-way analysis of variance (ANOVA) was used to compare the parameters obtained through the IVP, EUS-EMG, UFR, and histological examinations. The ANOVA was followed by Tukey’s honest significant difference test to assess post hoc paired comparisons (SigmaStat, SPSS, Chicago, IL, USA). For all analyses, \( P < 0.05 \) was considered statistically significant.

Results

Basic structure of the implantable microstimulation system

Figure 3 shows the configuration of the implantable microstimulation system, which is comprised of a single-channel microstimulator module and cuff electrode. The size of the microstimulation system facilitates chronic implantation in rats. The microstimulator was equipped with a magnetic power switch and red light-emitting diode (LED). The power switch of the implanted microstimulation system could be controlled in vivo by using a magnetic field from an extracorporeal magnet. When power to the stimulator was switched on by the magnetic field, the LED was activated to...
indicate that the system was operating (figure 3(B)). To stimulate the pudendal nerve, the microstimulator module was connected to a bipolar nerve cuff electrode with a length of 3 mm and distance of 1 mm between electrode rings. The system specifications and components are detailed in table 1.

Before the developed microstimulation system can be considered for long-term in vivo implantation, its performance must be verified. Our results show that all the microstimulators steadily delivered an approximately 10 μA regulated cathodic current in monophasic pulses (pulse width, 0.1 ms) with a fixed stimulation frequency (20 Hz). The device can thus provide continuous electrical current output for at least 48 h, meeting the requirement for 30 min daily neuromodulation for six weeks. In output resistance tests, all the microstimulators steadily produced a 10 μA current output when the output resistive load was increased from 1 to 200 kΩ (figure 4).

In addition, to test the waterproof ability of the system, six microstimulators were soaked in a beaker with normal saline for one month. Our results show that all devices remained physically and functionally intact. We observed no corrosive phenomena on the devices, and they continued to output a stable 10 μA electrical current. Thus, the system demonstrated satisfactory waterproof ability.

**Current threshold for the activation of pudendal axons**

Our results revealed that the minimum current amplitude for eliciting a reflex activation of the EUS by stimulating the pudendal sensory nerve ranged from 0.01 to 0.028 mA in normal rats (0.017 ± 0.008 mA, n = 6).

**Effects of chronic pudendal neuromodulation on NC rats**

Figure 5 presents examples of a typical pattern of simultaneous recordings of the IVP, EUS-EMG, and UFR during the continuous transvesical saline infusion in the NC rats with sham ES for three weeks (i.e. without neuromodulation). The bladder contracted and voiding occurred when the VT was reached. During filling, contractions occurred at earlier times compared with the first contraction because the RV after the first contraction was added to the infused volume (figure 5).

The EUS exhibited small-amplitude tonic activity during the initial filling phase and between micturition contractions; no urine flow was detected in the UFR measurement. However, during bladder contractions, the EUS activity exhibited a marked increase in amplitude, coinciding with the occurrence of urine flow. Detailed features of the EUS-EMG activity during a single micturition contraction clearly showed a long BP (figure 5(B)). The IVP and UFR were accompanied with EUS burst activity, and both were superimposed on a series of high-frequency oscillations.

Our results show that the basic pattern of the IVP, EUS-EMG, and UFR was similar between sham and real ES in all the NC subgroups; thus, no quantifiable difference was observed in any IVP, EUS-EMG, or UFR parameter (table 2).
Compared with the diabetic rats with sham ES, the diabetic rats with real ES for three or six weeks exhibited several changes, namely a decreased bladder VT, CD, RV, and EUS BP and an increased VE of 51%–57% (P < 0.05). Moreover, a significant increase was observed in the mean UFR of the diabetic rats with six weeks of real ES. However, no change was observed in the diabetic rats with three weeks of real ES.

The UFR patterns of the diabetic rats with sham ES for three or six weeks formed several clusters of discontinuous transient spikes during a single bladder voiding (n = 81/81 among 24 rats), which was in contrast to the long, steadily continuous UFR of the NC rats (figures 6(C) and 7(C) compared with figure 5(B)). The transient spikes in the UFR pattern also formed one or two long segments of a steadily continuous UFR in the diabetic rats after three or six weeks of real ES (figures 6(D) and 7(D); n = 70/78 among 24 rats).

**Histological examination of the sensory branch of the pudendal nerve**

The pudendal nerves of all the rats were histologically examined. Figure 8 presents examples of transverse sections of the sensory branch of the pudendal nerve of the NC and diabetic rats with six weeks of sham and real ES. Although the basic morphological pattern of the pudendal nerve of the NC and diabetic rats was similar, the cross-sectional area and axonal density differed markedly (figure 9). Our results revealed no significant difference in the cross-sectional area and axonal density among the four NC subgroups. Similarly, no significant difference was observed in the diabetic rats with sham ES for three weeks compared with the corresponding NC rats. However, the area and density values of the diabetic rats with six weeks of sham ES were significantly lower than those of the corresponding NC rats. Conversely, the area and density values of the diabetic rats increased significantly after six weeks of real ES.

**Discussion**

In the present study, we successfully developed an implantable microstimulation system by using SMD components. The system provided a stable current output for chronic pudendal neuromodulation in diabetic rat models. *In vivo* experiments showed that the bladder VT, CD, and RV were significantly higher in the diabetic rats with three or six weeks of sham ES than in the corresponding NC rats; thus, the VE decreased significantly (table 2). These results are consistent with typical symptoms observed in patients with diabetes, namely increased bladder capacity, decreased bladder contractility, and elevated postvoid residual urine volumes. These abnormal results in the diabetic rats with three or six weeks of real ES substantially were reversed; thus, the VE increased significantly from 17%–45% to 51%–57%. Although the histological examination revealed no change in the diabetic rats with three weeks of sham ES compared with the corresponding data of the NC rats, significant decreases were observed in the cross-sectional areas and axonal density of the

**Effects of chronic pudendal neuromodulation on diabetic rats**

Examples of the effects of chronic pudendal ES on the IVP, EUS-EMG, and UFR in the diabetic rats are shown in figures 6 and 7, and the corresponding results are summarized in table 2. The bladder VT, CA, CD, and RV increased significantly in the diabetic rats with three or six weeks of sham ES compared with those in the NC rats (table 2). Their VE decreased significantly to 17%–45% (P < 0.05). Moreover, the EUS BPs of the diabetic rats with three or six weeks of sham ES increased significantly, whereas the mean UFR of the diabetic rats with six weeks of sham ES decreased significantly.
<table>
<thead>
<tr>
<th></th>
<th>Volume threshold (ml)</th>
<th>Contraction amplitude (cmH$_2$O)</th>
<th>Contraction duration (s)</th>
<th>Residual volume (ml)</th>
<th>Voiding efficiency (%)</th>
<th>Burst period (s)</th>
<th>Mean UFR (ml min$^{-1}$)</th>
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<tbody>
<tr>
<td>NC with 3 wk of</td>
<td></td>
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</tr>
<tr>
<td>sham ES</td>
<td>0.62 ± 0.09</td>
<td>35.4 ± 3.4</td>
<td>22.5 ± 2.3</td>
<td>0.20 ± 0.04</td>
<td>66.9 ± 6.0</td>
<td>4.5 ± 1.0</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>real ES</td>
<td>0.68 ± 0.09</td>
<td>35.4 ± 2.0</td>
<td>20.0 ± 2.1</td>
<td>0.24 ± 0.04</td>
<td>64.4 ± 6.2</td>
<td>4.0 ± 0.6</td>
<td>4.6 ± 0.4</td>
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<tr>
<td>Diabetic with 3</td>
<td></td>
<td></td>
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<tr>
<td>wk of Sham ES</td>
<td>1.42 ± 0.34$^{b}$</td>
<td>40.4 ± 1.2$^{b}$</td>
<td>30.8 ± 3.0$^{b}$</td>
<td>0.77 ± 0.20$^{b}$</td>
<td>45.3 ± 8.0$^{b}$</td>
<td>7.7 ± 0.5$^{b}$</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>real ES</td>
<td>0.72 ± 0.18$^{a}$</td>
<td>39.1 ± 3.0</td>
<td>20.2 ± 2.9$^{a}$</td>
<td>0.31 ± 0.11$^{a}$</td>
<td>57.1 ± 8.4$^{a}$</td>
<td>4.3 ± 0.3$^{a}$</td>
<td>4.5 ± 0.7</td>
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<td>NC with 6 wk of</td>
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<tr>
<td>sham ES</td>
<td>0.65 ± 0.07</td>
<td>32.2 ± 2.6</td>
<td>22.3 ± 2.4</td>
<td>0.24 ± 0.05</td>
<td>62.7 ± 7.7</td>
<td>5.1 ± 1.1</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>real ES</td>
<td>0.66 ± 0.09</td>
<td>34.9 ± 3.5</td>
<td>24.6 ± 2.0</td>
<td>0.24 ± 0.06</td>
<td>63.3 ± 7.6</td>
<td>4.0 ± 0.5</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>Diabetic with 6</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>wk of Sham ES</td>
<td>1.61 ± 0.23$^{b}$</td>
<td>43.7 ± 2.5$^{b}$</td>
<td>31.3 ± 3.0$^{b}$</td>
<td>1.33 ± 0.21$^{b}$</td>
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<tr>
<td>real ES</td>
<td>0.85 ± 0.19$^{a}$</td>
<td>36.3 ± 2.7$^{a}$</td>
<td>27.2 ± 4.4$^{a}$</td>
<td>0.42 ± 0.14$^{a}$</td>
<td>51.2 ± 7.2$^{a}$</td>
<td>4.6 ± 0.3$^{a}$</td>
<td>4.4 ± 0.7$^{a}$</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SD, n = 12.

* $p < 0.05$ indicates a significant difference in the diabetic or NC rats between the sham and real ES for the same duration.

$^{b}$ $p < 0.05$ indicates a significant difference between the diabetic and NC rats with the sham ES for the same duration.
Figure 6. Typical patterns of the IVP (top) under continuous transvesical infusion, EUS-EMG (middle), and UFR (bottom) simultaneously recorded in the diabetic rats with sham ES (A) and real ES (B) for three weeks. The parentheses in (A) and (B) represent the UFR recording periods in (C) and (D) at faster timescales, respectively.

Figure 7. Typical patterns of the IVP (top) under continuous transvesical infusion, EUS-EMG (middle), and UFR (bottom) simultaneously recorded in the diabetic rats with sham ES (A) and real ES (B) for six weeks. The parentheses in (A) and (B) represent the period of UFR recordings in (C) and (D) at faster timescales, respectively.

diabetic rats with six weeks of sham ES (figure 9). Both the area and density values increased after six weeks of real ES.

Significant differences in the CA were also found in diabetic rats between three and six-week groups. The bladder CA increased significantly in the diabetic rats with real ES for six weeks (no change was observed in the three-week group). The significant increase in CA suggests that inducing diabetes increased the urethral resistance, causing significant attenuation of the VE. This assumption is indirectly supported by a previous study [20] that suggested that when the pudendal nerves in diabetic rats were crushed, the amplitude of voiding pressure was reduced, causing significant acceleration in bladder emptying by shortening the voiding duration. This speculation is also partially supported by our results, as evidenced by the detected decrease in the CA and increase in the mean UFR in the diabetic rats with six weeks of real ES.

Few studies have explored the effects of chronic ES on pudendal nerve models in animal studies because of a lack of a suitable implantable microstimulation devices. The implantable device proposed in the present study has two advantages over the nonimplantable devices used in previous acute neuromodulatory experiments [21–23]. First, applying ES on peripheral nerves by using a cuff electrode not only provides a small current amplitude, but also delivers the electrical current precisely to the target nerve tissue. Second, the device-implanted rats were awake and allowed to move without restriction; thus, our experimental data reflect physiological changes compared with the data obtained from anesthetized rats. Our results reveal that at an output resistance of 200 kΩ, the microstimulator steadily produced a 10 μA regulated current of 20 Hz monophasic pulses. The nerve impedance value appears to depend on the fiber density, myelin content, cell structure, and electrolyte concentration [24, 25], and the value was generally no more than 130 kΩ over various anatomical regions on the nerve tissues [24, 26]. According to the aforementioned findings, our device might be feasible for use in other neuromodulation experiments and as a novel tool for associated neuroscience studies. Notably, a
recently reported implantable neurostimulation system with a four-channel stimulator with wireless programmable and rechargeable functions was developed for animal experiments [27]. Compared with our device, the merit of this four-channel system is that it can be more flexibly utilized in various neuromodulatory experiments. However, the challenges of the four-channel system are its complexity of construction and high cost of fabrication.

However, the status of electrode–tissue connections during the chronic pudendal neuromodulation is likely a critical factor determining the effects of pudendal neuromodulation. During the three to six-week animal study, we randomly measured the changes in the electrode–nerve impedance by using a 1 kHz sinusoidal wave (personal data). The results of that measurement showed that the increase in the maximal impedance magnitude was no more than 50% of the initial value among all the animals. The results are consistent with those in a previous study [26] that reported that the maximal electrode–nerve impedance was approximately 160% of the initial impedance for an implantation of a cuff electrode at the sciatic nerve for 42 days. However, on the basis of the histological data collected in this study, all specimens of the sensory branch of the pudendal nerve were localized within the cuff electrode canal. Thus, the electrode–tissue connection in this study was maintained under normal conditions during the three to six weeks of in vivo experiments.

Chronic diabetes mellitus can cause peripheral polyneuropathy, which may extensively affect the autonomic and somatic sensory and motor nerves [5]. Previous studies have found no sympathetic (hypogastric) control in rats subjected to diabetes for four weeks [28]. Furthermore, the diameter of...
hypogastric and pelvic nerve fibers in diabetic rats significantly decreased, and this decrease was accompanied by nerve demyelination [29]. Our results further demonstrate a significantly decreased cross-sectional area and axonal density of the pudendal sensory nerves in the diabetic rats with sham ES for six weeks. Thus, peripheral polynueopathy of the LUT system, including the hypogastric, pelvic, and pudendal nerves, might contribute to diabetic cystopathy in rat models.

The mean value of the cross-sectional area and the axonal density of the pudendal nerve in the diabetic rats increased significantly after six weeks of real ES (figure 9); the values were extremely similar to those of the NC rats. These findings imply that chronic neuromodulation in diabetic rats has neural repair effects, thereby reducing the severity of pudendal neuropathy during three and six weeks of ES. This speculation is based on past animal studies, in which the application of ES with 20 Hz rectangular waveforms on transected or crushed sciatic nerves has yielded highly enhanced recovery of motor function and an increase in the number of neuronal cells [21, 30]. By contrast, our results revealed that the current threshold for activating pudendal axons was approximately 0.17 μA in normal rats. This finding indicated that the diabetic rats in our study underwent chronic subthreshold stimulation, because the fixed 10 μA intensity utilized in this study was lower than the threshold. Several studies have reported that monophasic stimulation pulses combined with a subthreshold stimulation intensity (1–10 μA) constituted an optimal ES parameter combination that successfully facilitated neuronal cell growth in transected sciatic nerves, as evidenced by a more mature nerve structure with a smaller cross-sectional area, more myelinated fibers, higher axon density, and a higher ratio of blood vessels to total nerve area compared with that in control groups [21, 30–32]. Although the pudendal nerve was not crushed or transected in our study, the same stimulation parameters (i.e. a 20 Hz stimulation frequency with 10 μA stimulation intensity) were applied. Thus, the neural repair effects on the pudendal nerve may have contributed to the improved VE in the diabetic rats (table 2).

The UFR measurement approach adopted in this study enabled us to observe the urine flow pattern in the diabetic rats during voiding. This flow pattern was an outcome of the functional integration of the bladder and EUS activity. Our results reveal that a long, steady continuous UFR was typically observed in the NC rats, whereas the UFR of the diabetic rats (sham ES) showed clusters of intermittent transient spikes. Moreover, the EUS BP was prolonged in the diabetic rats. These findings reveal that DSD occurred in the diabetic rats. DSD may increase voiding time and residual urine and reduce VE, leading to chronic bladder overdistention in patients with diabetes [33]; these clinical symptoms were detected in our results.

Our neuromodulation approach in the diabetic rats has some limitations. First, the neural reorganization might be one possible mechanism underlying the improved VE of the diabetic rats after chronic pudendal neuromodulation. This is indicated by our results that showed that chronic pudendal neuromodulation for six weeks significantly altered the urodynamic and EUS-EMG patterns in the diabetic rats, as evidenced by the significant changes in the bladder VT, CA, EUS BP, and mean UFR. Although we did not provide further direct evidence to support this possibility, several recent studies have consistently indicated that SNM substantially induced neuroplastic effects on micturition reflex pathways at the spinal and supraspinal levels [3, 34–36]. Blok et al. [35] reported different effects on brain neuroplasticity in patients with acute and chronic SNM. Chronic neuromodulation specifically involves central structures in micturition and continence control, whereas acute SNM predominantly modulates brain areas that are involved in sensorimotor learning [35]. Moreover, pudendal sensory feedback is crucial for bladder emptying [13, 37]. Recent studies have indicated that acute ES of the pudendal nerve may immediately produce positive afferent feedback to the detrusor (i.e. an augmenting reflex) during bladder emptying, thus improving the VE of humans or animal models with voiding dysfunction [12, 37, 38]. Therefore, we cannot exclude the possibility that the central neural reorganization affected the lumbosacral level in the chronic alteration of the micturition pattern in the diabetic rats.

Second, the use of monophasic current pulses for chronic neuromodulation may induce nonreversible Faradaic reactions [39, 40]. In this study, platinum, a noble metal that is commonly used as a stimulating electrode material for neural prostheses, was utilized to fabricate the bipolar cuff electrodes. Although the body is adequately equipped for buffering pH changes in the vicinity of the electrode [41], physiological changes resulting from monophasic stimulation remain a potential concern in studies of the response to ES. This is because platinum electrodes have a safe charge-carrying capacity. If the charge density exceeds 30 μC cm$^{-2}$ under in vivo conditions [42], it may damage surrounding tissues and the platinum electrode itself [40]. Thus, in our future research, we must further explore the feasibility of chronic neuromodulation with biphasic pulses on diabetic urinary retention.

Third, H&E staining was used to determine the histological changes of the pudendal nerve after chronic neuromodulation. Although H&E staining is useful for determining the gross changes of the number and cross-sectional area of pudendal axons, this approach may complicate the determination of micromorphological alterations, such as those in the myelinated layers or growth factor receptors of pudendal axons. Therefore, this study lacked detailed morphological information associated with the neuromodulatory effects on nerve healing.

Nerve conduction velocity is generally sensitive to many pathological changes associated with diabetic neuropathies, and a pronounced decrease in this velocity suggests demyelinating neuropathy [43]. We did not conduct electrophysiological experiments, and thus it remains unclear whether our diabetic animal model contained substantially destroyed pudendal reflex pathways.

Another limitation is the use of rats with diabetes for only three to six weeks as our animal model. Some clinical studies
have indicated that diabetic cystopathy is mainly attributed to progressive axonal atrophy of automatic and peripheral nerves [44]; the symptoms of bladder dysfunction may vary depending on the severity of the neuropathy. Thus, whether the present results can be generalized to all diabetic patients with cystopathy is unclear.

Although the present results demonstrate the feasibility of using chronic pudendal nerve modulation for improving voiding function in diabetic animals, the mechanisms underlying the improved VE were not ascertained. In addition, whether the chronic neuromodulation with biphasic pulses could improve diabetic urinary retention is unclear. Thus, future studies must further explore the mechanisms of chronic pudendal nerve stimulation with animal models of diabetic-induced urinary retention. In addition, the severity of the neuropathy of diabetic animal models should also be considered.

Acknowledgments

This study was supported by grants from the Ministry of Science and Technology (NSC100-2320-B-038-003-MY3, NSC102-2321-B-038-008, MOST103-2321-B-038-013, and MOST103-2221-E-038-007-MY3) provided to C W Peng and by the R&D Foundation of Urological Medicine, Taiwan.

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