Dual-Channel Neuromodulation of Pudendal Nerve with Closed-Loop Control Strategy to Improve Bladder Functions

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Abstract

Although sacral anterior root neuromodulation (SARN) has proven to be effective in patients with neurogenic bladder, it is not widely accepted due to the need to conduct a dorsal rhizotomy, and commercially available SARN devices are not usually equipped with a closed-loop controller for the automatic regulation of bladder functions. Therefore, there is still a need for a more effective electrical neuromodulation scheme to restore bladder function. Intravesical pressure (IVP) is the major biosignal that reflects the state of bladder conditions. The present study develops a closed-loop control strategy for improving bladder emptying and verifies its performance using animal experiments. Two channel outputs of electrical currents triggered by IVP-feedback signals were set to automatically regulate a rat's pudendal nerve for selective nerve stimulation and blocking. Under this experimental design, a series of *in vivo* animal experiments were conducted on anesthetized rats, including the computational characterization of biosignals, the development of an intermittent high-frequency blocking current waveform for blocking the nerve, and verification of the control strategy. Results show that the IVP-feedback control strategy for dual-channel pudendal neuromodulation performed well in animal experiments and could be utilized to selectively stimulate and block the pudendal nerve to augment bladder contraction and restore external urethral sphincter bursting activity to improve bladder emptying. This study demonstrates the feasibility of the IVP-based feedback-control strategy with animal experiments. The results could provide a basis for developing a sophisticated neural prosthesis for restoring bladder function in clinical use or for neurophysiological study.

Keywords: Neuromodulation, Intravesical pressure, Electrical stimulation, Pudendal nerve

1. Introduction

The urinary bladder accumulates and stores urine (continence) and evacuates urine at appropriately selected times and places (voiding or micturition). These functions require
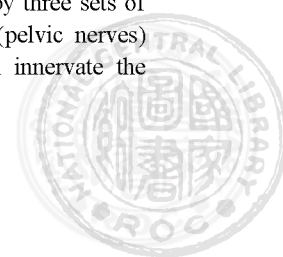
reciprocal coordination of the urinary bladder and outlet, including bladder neck, urethra, and external urethral sphincter (EUS). During urine storage, the bladder is quiescent, and the intravesical pressure (IVP) remains low. Activity in the EUS gradually increases during bladder filling to maintain continence. During voiding, the pattern of activity is reversed, and the bladder contracts while the EUS either relaxes, as in cats and humans, or exhibits phasic periods of quiescence, as rats and dogs [1]. These organs are regulated by three sets of peripheral nerves: the sacral parasympathetic (pelvic nerves) and thoracolumber sympathetic nerves, which innervate the

bladder and proximal urethra, and the sacral somatic nerves, which innervate the EUS [2].

Reciprocal activities of the outlet (bladder neck, urethra, and urethral sphincter) and bladder are coordinated by descending projections from the pontine micturition center [3-6]. Neurological disease or spinal cord injury (SCI) can lead to loss of voluntary control of bladder evacuation and detrusor-sphincter dyssynergia (DSD), where the bladder and urethral sphincter exhibit co-contractions during the micturition period, preventing the flow of urine down the urethra. DSD generates high bladder pressure, vesicourethral reflux, and renal failure in the long term [7]. Most patients must resort to daily urethral catheterization, which results in frequent urinary tract infections and a reduced quality of life [7].

Currently, electrical neuromodulations have been developed to treat patients with neurogenic bladder [8]. For example, sacral anterior root neuromodulation (SARN) was successfully performed in SCI patients with voiding dysfunction [9]. Despite its clinical efficacy, the surgical procedure required to implant the electrodes on the sacral roots is invasive and time-consuming [10]. In addition, for certain groups of patients such as neurogenic patients, SARN is not widely accepted due to the need to conduct a dorsal rhizotomy to minimize dyssynergic contractions of the sphincter. However, transection of the dorsal nerve roots can eliminate residual sensation and reflexes, including defecation, erection, ejaculation, and lubrication [11,12]. Thus, there is a need for a more effective prosthesis to restore the bladder's voiding function.

Recently, many studies on animals or humans have indicated that modulation of pudendal nerve activity by electrical stimulation or blocking techniques and local application of pharmacological agents are useful for treating voiding dysfunction, and that electrical stimulation of the pudendal nerve could engage the augmenting reflex and thereby improve bladder emptying in subjects with voiding dysfunction [13-17]. This is because the pudendal nerve contains afferent and efferent pathways. Afferent stimulation can augment urethra-bladder or somato-bladder reflexes [18,19], whereas blockade of motor pathways was utilized to reduce EUS contractions.

The frequency of the electrical current is an important parameter for modulating nerve activity. A low-frequency stimulation current (LFSC) applied to afferent axons of the pudendal nerve in humans or cats was demonstrated to initiate reflex bladder contractions and voiding [20,21]. On the other hand, a high-frequency blocking current (HFBC) for modulating pudendal nerve efferent axons or local application of a 5% phenol solution effectively blocks axonal conduction, suppresses EUS contractions, and in turn decreases intraurethral pressure [22,23]. Complex methods for controlling pudendal nerve activity have been proposed. One scheme involves the use of bladder sensory nerve activity recorded on sacral nerve roots to trigger pudendal nerve stimulation and thereby create an artificial circuit to modulate voiding function [24,25]. However, electroneurogram signals suffer from low signal-to-noise (S/N) ratios, which dramatically reduce the feasibility of utilizing

neural signals for feedback control of neuroprostheses. In contrast to electroneurograms, IVP and EUS electromyograms (EMGs) usually have relatively high S/N ratios and can be acquired via non-invasive means in the clinic [26].

The present study develops a reliable closed-loop control strategy by electrically modulating pudendal nerve activity to improve bladder emptying in animal experiments. The IVP and EUS EMG signals were initially characterized to determine whether these biosignals could be used for the closed-loop control of pudendal neuromodulation. On the basis of the characterization results, a dual-channel feedback control strategy and an intermittent HFBC pattern were designed. Finally, the control strategy combined with the designed electrical current pattern was applied to the pudendal nerve in acute animal experiments to assess its performance on bladder function. The results could provide a basis for developing a new type of bladder controller for clinical use.

2. Materials and methods

The Institutional Animal Care and Use Committee of Taipei Medical University and Hospital approved the experimental protocols involving the use of animals in this study. In total, 16 female Sprague-Dawley rats weighing 290~360 g were used in this study.

2.1 Computational characterization of physiological signals

Several normal rats (n = 10) were used in an experiment to computationally characterize IVP and EUS EMG signals. Rats were anesthetized with urethane (1.2 g/kg, s.c.). 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, and a PE-50 tube was inserted into the bladder lumen for bladder pressure measurements. 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 with a purse-string suture. Two insulated silver wire electrodes (0.05 mm in diameter) with exposed tips were inserted into the lateral aspects of the mid-urethra to record the EMG from the EUS (Fig. 1). Finally, the abdominal wall was closed with nylon sutures.
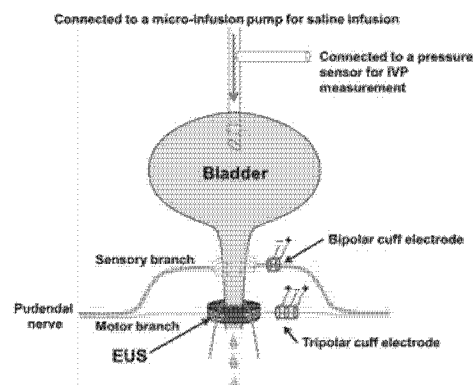

Figure 1. Schematic diagram of experimental setup used to modulate pudendal nerve and record IVP and EUS EMG activities in rats.

The PE tube was connected via a three-way stopcock to an infusion pump and a pressure transducer (P23XL-1, Becton Dickinson, Franklin Lakes, NJ, USA) to measure the IVP (Fig. 1). The IVP and EUS EMG were simultaneously recorded during continuous-infusion cystometry with an open urethra. The bladder was filled at 0.2 ml/min with physiological saline at room temperature via the superpubic catheter until voiding occurred; this process was repeated until each trial included at least three voiding contractions. The recorded signals were used for computational characterization.

2.2 Setup of experimental systems

A flowchart of the entire experimental system is shown in Fig. 2. There are several operating procedures in this flowchart. The IVP and EUS EMG signals were simultaneously recorded (sampling rate of 5 kHz) during continuous-infusion cystometry, and the recorded signals were delivered to an analog input of the LabVIEW program written by our lab (National Instruments, Austin, TX, USA) via an analog-to-digital (A/D) converter (Biopac MP 36, BIOPAC Systems, Santa Barbara, CA, USA). The IVP signal was amplified by the A/D converter by 100 × and filtered by a 1 kHz low-pass filter, and the EUS EMG signal was amplified by 1000 × and filtered by a 10-3k Hz band-pass filter. The program sampled and analyzed the bladder pressure signal and generated a voltage signal to each of the stimulators via a digital-to-analog converter (USB-6212, National Instruments, Austin, TX, USA). The stimulator was triggered when the IVP reached a preset threshold and stopped when the real-time IVP feedback signal returned to below this threshold. Specifications of signal processing for the hardware systems are tabulated in Table 1.
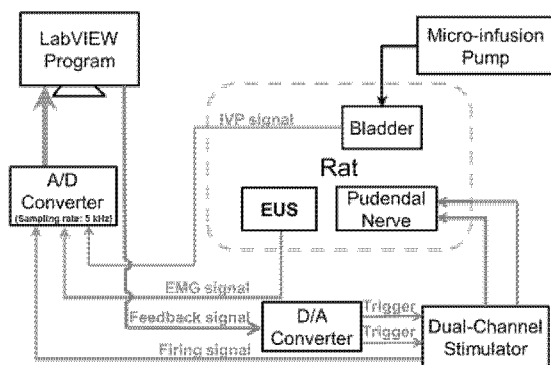

Figure 2. Flowchart of entire experimental system. The feedback control for dual-channel neuromodulation of the pudendal nerve in the rat was determined by the amplitude of the IVP signals.

Table 1. Specifications for signal processing in experimental system.

Type of signal	Gain	Filter for signals		
		Low-pass	High-pass	Band-stop
IVP	100×	1 kHz	None	60 Hz
EUS-EMG	1000×	3 kHz	10 Hz	60 Hz
Trigger signals for stimulators	5×	NA	NA	NA

IVP: intravesical pressure; EUS EMG: external urethral sphincter electromyogram;
NA: not applicable

2.3 Verification of closed-loop control strategy with animal experiments

Several anesthetized rats ($n = 6$) with acute SCI were used in this experiment to verify the performance of the developed device. Acute spinal cord transection was initially carried out in rats at the $T_9 \sim T_{10}$ vertebral level under anesthetized condition. After the $T_9 \sim T_{10}$ laminectomy, the dura matter and spinal cord were transected with fine scissors. The severed ends of the spinal cord typically retracted 1~2 mm and were inspected under a surgical microscope to ensure complete transection. The overlying muscle and skin were sutured closed after spinal transection. Rats further underwent midline abdominal surgery to insert a PE-50 tube into the bladder lumen and two insulated silver wire electrodes into the mid-urethra for IVP and EUS EMG measurements. The IVP and EUS EMG measurements in the rats usually began 8-24 h after the conduction of spinal transection, which was determined by the re-emergence of the micturition reflex.

Subsequently, the sensory and motor branches of the pudendal nerve were unilaterally exposed via a posterior approach by incising the distal portions of the gluteus major muscles. The pudendal nerve was mounted with a bipolar cuff electrode on the sensory branch to augment reflex bladder contractions and with a tripolar cuff electrode on the motor branch to block intermittent EUS (Fig. 1). Selective stimulation and blocking of the pudendal nerve were performed under continuous-infusion cystometry to quantify its effects on bladder emptying. The bladder was filled via the superpubic catheter until voiding occurred; this process was repeated until each trial included at least three voiding contractions. The trigger and stop of the dual-channel neuromodulation were determined by the real-time IVP feedback signal.

2.4 Data and statistical analyses

The following cystometric parameters were measured to quantify the effects of pudendal afferent stimulation on voiding: (1) the micturition volume threshold (VT), defined as the infused volume of saline sufficient to induce 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 inter-contraction interval (ICI), which was the interval between two consecutive voiding contractions (Fig. 3). The voiding efficiency (VE) was the ratio
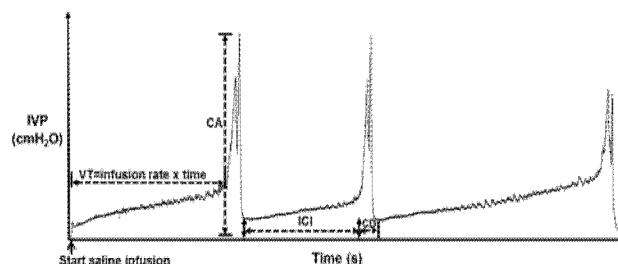

Figure 3. Typical pattern of cystometrographic (CMG) measurements with a constant rate of infusion in the rat. The CMG parameters included the micturition volume threshold (VT), contraction duration (CD), contraction amplitude (CA), and inter-contraction interval (ICI).

between the voided volume (VV) and the VT. The VV was calculated as VT less the residual volume (RV) of saline withdrawn through the intravesical catheter after the final voiding contraction.

All data are presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for overall comparisons between groups and was followed by the Student-Newman Keuls post hoc test using SigmaStat (SPSS, Chicago, IL, USA). Individual comparisons in a group were made using Student's t test. For all tests, a value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Characteristics of IVP and EUS EMG in normal control and SCI rats

The typical pattern of IVP and EUS EMG measurements in both normal control (NC) and SCI rats are shown in Fig. 4, and cystometric parameters are summarized in Table 2. Patterns of the IVP during the continuous intravesical infusion of saline did not significantly differ between NC and SCI rats. After the acute SCI intervention, the volume threshold and bladder contraction amplitude increased and the inter-contraction interval and voiding efficiency decreased compared to corresponding values for the NC rats (Table 2). The lower voiding efficiency appeared to decrease the inter-contraction interval (Fig. 4), since there was a larger residual volume after the impaired contractions and at a constant filling rate, the bladder again reached the threshold volume in less time.
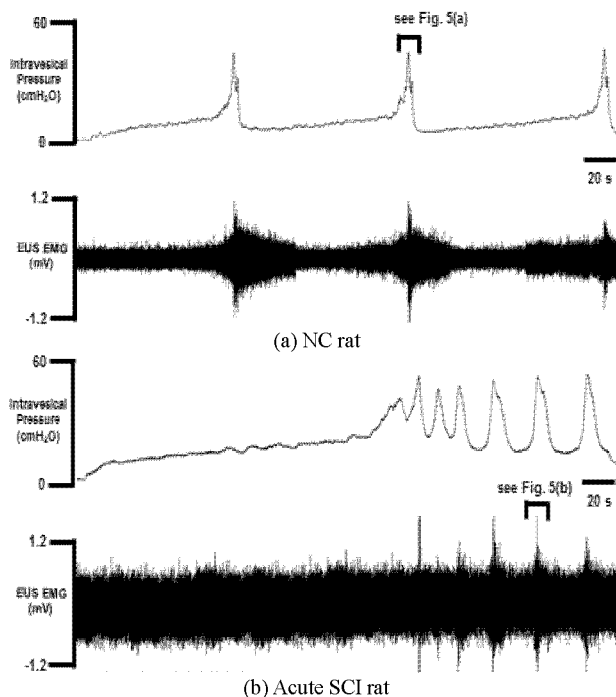

Figure 4. Comparison of IVP (top traces) and EUS EMG activity (bottom traces) under transvesical infusion (0.2 ml/min) between (a) NC and (b) SCI rats. The parentheses in (a) and (b) are the respective recording periods of micturition in Fig. 5(a) and (b) at faster time scales.

Table 2. Parameters of intravesical pressure (IVP) in normal control (NC) and acute spinal-cord-injured (SCI) rats.

Groups	NC rat	Acute-SCI rat
VT (ml)	0.41 ± 0.55	0.83 ± 0.04 *
CA (cmH$_2$O)	29.64 ± 1.05	38.46 ± 1.24*
CD(s)	27.92 ± 0.99	17.91 ± 1.56*
ICI(s)	158 ± 11	16 ± 2*
VV(ml)	0.32 ± 0.05	0.11 ± 0.03*
RV(ml)	0.09 ± 0.02	0.72 ± 0.06*
VE(%)	77.18 ± 5.32	12.77 ± 3.99*

VT: volume threshold; CA: contraction amplitude; CD: contraction duration; ICI: inter-contraction interval; VV: void volume; RV: residual volume; VE: void efficiency = VV/VT. Values are Mean ± SD, n = 10 rats. *$p < 0.05$ indicates a statistically significant difference compared with NC values.

3.2 Computational characterization of IVP and EUS EMG

A single micturition contraction of the bladder can be divided into three phases of IVP: phase 1, rising phase, from point A to B; phase 2, high-frequency oscillation (HFO) and rebound phase, from point B to C; and phase 3, falling phase, from point C to D, as shown in Fig. 5(a) [27]. During a single micturition contraction, the EUS EMG clearly showed a long bursting period (BP), which lasted from the end of phase 1 throughout the entire duration of phase 2 (Fig. 5(a)), which was characterized by clusters of high-frequency spikes (in the active period; AP) separated by periods of quiescence (in the silent period; SP) (Fig. 5(c)).

(a) NC rat

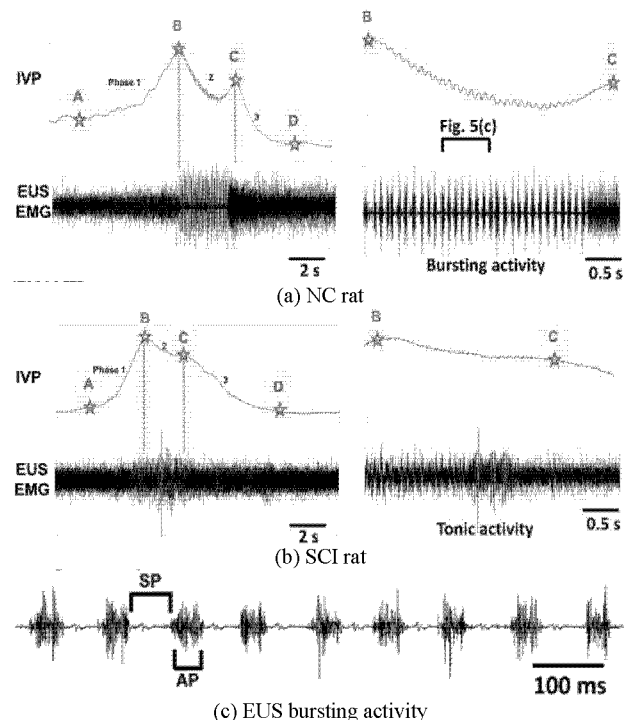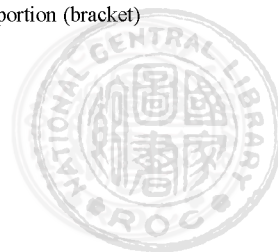(b) SCI rat

(c) EUS bursting activity

Figure 5. Computational characterization of a micturition contraction in (a) NC and (b) SCI rats. A micturition contraction was divided into three phases of IVP, which were characterized by four critical pressure points. Traces on the right are expansions of sections of traces on the left between points B and C. The EUS bursting activity occurred during phase 2 of IVP in NC rats, whereas EUS tonic activity was detected throughout the entire phase 2 in SCI rats. (c) Individual bursts were composed of active periods (APs) and silent periods (SPs), shown in the expanded EUS bursting portion (bracket) in (a).

In contrast, in SCI rats, EUS bursting activity disappeared and transformed into tonic activity, and thus long-lasting EUS tonic activity was observed throughout the three phases of IVP (Fig. 5(b)). Note that no rebound wave was detected in phase 2 of IVP (n = 10 out of 10 rats). Loss of EUS bursting activity and the rebound wave of the IVP were attributed to acute SCI, which induced detrusor-sphincter dyssynergia. Therefore, the EUS tonic activity, represented by the closing of the urethra, appeared during phase 2 of the micturition contraction, which prevented evacuation of urine from the urinary bladder. This could be the reason why acute-SCI rats were characterized by urinary retention, high voiding pressures, and a large bladder capacity during cystometric measurements (Table 2).

The IVP and EUS signals were computationally characterized by measuring four critical IVP points that divide the micturition contraction into three IVP phases (Fig. 5). Since urine evacuation and EUS bursting activity simultaneously occurred during phase 2 of IVP (IVP points B to C), the characterization results could feasibly be used to exactly predict the voiding phase of IVP for pudendal neuromodulation. Our results show that the four calculated pressure points of a micturition contraction in acute-SCI rats were all significantly higher than those in NC rats (Table 3). The IVP between points B and C (the period of voiding occurrence, phase 2) in SCI rats ranged from 34 to 38 cmH$_2$O, which was higher than the 26~34 cmH$_2$O in NC rats. IVP points A and D respectively represent the pressure-threshold- induced bladder contraction and the baseline pressure after bladder contraction; both were also significantly higher than control values. These results imply that a large amount of residual urine was retained in urinary bladder after micturition, and thus a higher IVP appeared in SCI rats. EUS EMG measurements in NC rats showed that the SP was 99.2 ± 2.2 ms, which was much longer than the 59.8 ± 2.8 ms of the AP. No EUS bursting activity was observed in acute-SCI rats (Table 3).

Table 3. Computational calculation of IVP and EUS EMG signals.

Parameters	NC rat	Acute-SCI rat
IVP Point A	9.22 ± 1.33	16.25 ± 0.68 *
IVP Point B	29.64 ± 1.05	38.46 ± 1.24*
IVP Point C	25.97 ± 1.47	34.73 ± 1.68*
IVP Point D	4.78 ± 1.07	15.69 ± 0.60*
EUS AP	59.8 ± 2.8	NA
EUS SP	99.2 ± 2.2	NA

Values are mean ± SD, n = 10 bladder contractions. * p < 0.05 indicates a statistically significant difference compared with NC value.

3.3 Design of intermittent HFBC pattern

Previous studies demonstrated that LFSC and HFBC modulation of the pudendal nerve could improve bladder emptying by stimulation of pudendal afferents for augmenting the bladder contraction amplitude [13-15] and blockade of pudendal efferents with HFBC for temporarily denervating the EUS [28,29]. In the present study, EUS bursting activity was replaced by tonic activity in acute-SCI rats, which prevented urine evacuation. EUS bursting activity represents the relaxation and opening of the outlet, which is essential to achieving efficient voiding [30]. Therefore, LFSC and HFBC were respectively applied to the sensory and motor branches of

the pudendal nerve to modulate bladder and EUS activities during micturition contractions.

According to the results of computational characterization of IVP (Table 3), two pressure thresholds of IVP were set to trigger and stop the stimulators via a LabVIEW graphical user interface for dual-channel pudendal neuromodulation (LFSC and HFBC outputs), as shown in Fig. 6(a). LFSC, a monophasic waveform with the frequency, amplitude, and pulse width fixed at 2 Hz, 0.05 mA, and 0.1 ms, respectively, was applied to the sensory branch of the pudendal nerve [14] to augment bladder contractions. The current was delivered when the bladder pressure exceeded 20 cmH$_2$O and was stopped when the pressure dropped below this threshold. An intermittent HFBC, a monophasic waveform with the frequency, amplitude, and pulse width fixed at 20 kHz, 2 mA, and 0.01 ms, respectively, was applied to the motor branch of the pudendal nerve to restore EUS bursting activity with a 25 cmH$_2$O pressure threshold. The pattern of the intermittent HFBC was characterized by clusters of a 100/60-ms stimulation-on/off ratio, as shown in Fig. 6(b). These selected nerve stimulation and blocking parameters were further verified in animal experiments to assess their effects on bladder emptying.
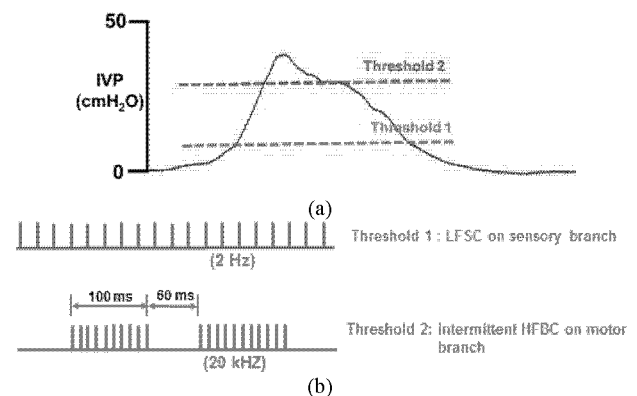

Figure 6. IVP-feedback control strategy for dual-channel neuromodulation of the pudendal nerve. (a) A computer program written in LabVIEW was used to detect the IVP signal and trigger and stop two stimulators. The stimulators were triggered when the IVP reached a preset threshold and were stopped when the IVP returned to below the threshold. (b) LFSC (2 Hz with 0.05 mA) delivered to the sensory branch of the pudendal nerve was determined by pressure threshold 1, whereas HFBC (20 kHz with 2 mA) delivered to the motor branch was determined by pressure threshold 2.

3.4 Verification in acute animal experiments

In animal experiments, it was first accessed whether the LFSC and intermittent HFBC were delivered in the correct IVP phase. Our results showed that the LFSC and the intermittent HFBC were delivered and stopped with correct timing (Fig. 7), in which the LFSC output began from phase 1 and stopped before the end of phase 3 and the HFBC was delivered during phase 3. Our statistical data showed that both LFBC and HFBC were delivered with the correct timing in 49 of 50 experimental trials. The results demonstrated that the control feedback strategy provided satisfactory performance for pudendal neuromodulation since the accuracy of current output timing was approximately 100%.
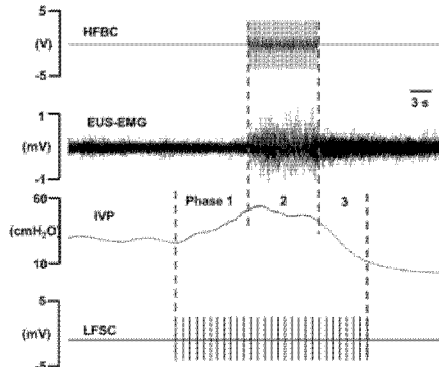
Figure 7. Example of the timing of LFSC (2 Hz with 0.05 mA) and HFBC (20 kHz with 2 mA) delivery for pudendal neuromodulation during a micturition contraction. LFSC was output throughout IVP phases 1 to 3, while the output of HFBC was in phase 2.

After the evaluation of the timing of the current outputs, the independent application of LFSC and the simultaneous application of LFSC and HFBC on the pudendal nerve were separately performed under continuous-infusion cystometry to quantify their effects on bladder emptying in acute-SCI rats ($n = 6$). Results showed that after acute SCI, rats exhibited impaired coordination of the detrusor and EUS during micturition, and thus SCI rats before neuromodulation exhibited a significant increase in the bladder contraction amplitude and residual volume and a significant decrease in the contraction duration, inter-contraction interval, and voided volume (Figs. 8(a) and 8(b); Figs. 9(b)-9(e)). Thus, the voiding efficiency was significantly reduced from 77% to 12% (Fig. 9(f)).

(a) NC rat

(b) Acute SCI rat

(c) Acute SCI rat with concomitant LFSC

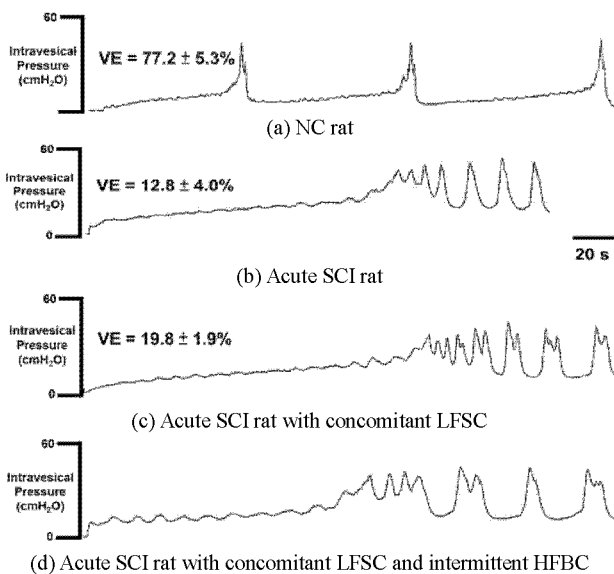(d) Acute SCI rat with concomitant LFSC and intermittent HFBC

Figure 8. Examples of the effects of the stimulation and blocking of the pudendal nerve on bladder emptying during continuous bladder infusion in SCI rat. The IVP values recorded from (a) a control rat and a rat with acute SCI (b) without any neuromodulation, (c) with independent stimulation of the pudendal nerve, and (d) with simultaneous stimulation and blocking of the pudendal nerve during reflex bladder contractions are shown. The voiding efficiency (VE) in the acute-SCI rat was significantly increased by LFSC (2 Hz with 0.05 mA), and further increased by the simultaneous

application of LFSC and HFBC (20 kHz with 2 mA).
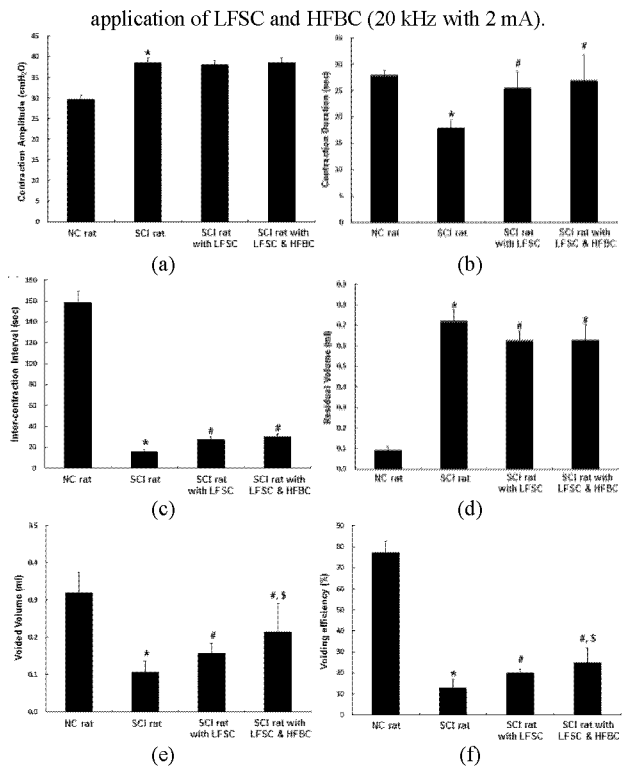

(a)

(b)

(c)

(d)

(e)

(f)

Figure 9. Summary of changes in cystometric parameters in NC rats, and SCI rats with concomitant LFSC (2 Hz with 0.05 mA) and concomitant LFSC combined with intermittent HFBC (20 kHz with 2 mA) on the pudendal nerve during reflex bladder contractions. The (a) maximum bladder contraction amplitude, (b) bladder contraction duration, (c) inter-contraction interval, (d) residual volume, (e) voided volume, and (f) voiding efficiency are shown. Each bar represents mean ± SD ($n = 6$). * $p < 0.05$ indicates a significant difference compared to NC rats. # $p < 0.05$ indicates a significant difference compared to acute-SCI rat without any neuromodulation. $ $p < 0.05$ indicates a significant difference between acute-SCI rats with concomitant LFSC and concomitant LFSC combined intermittent HFBC.

Subsequently, the acute-SCI rat applied independently with LFSC on the pudendal nerve, the voiding efficiency was significantly increased to ~20%; and this was further increased to ~25% by the simultaneous application of LFSC and intermittent HFBC. In addition, the voided volume and voiding efficiency were further increased by the simultaneous application of LFSC and HFBC when compared to the values of the independent application of LFSC (Figs. 9(e) and 9(f)). Note that the bladder contraction duration, inter-contraction interval, and voided volume were all increased by either the independent application of LFSC or the simultaneous application of LFSC and HFBC but no significant difference was found in these cystometric parameters between the two neuromodulation schemes (Fig. 8; Figs. 9(b)-9(d)).

4. Discussion and conclusion

A closed-loop strategy to control pudendal neuro-modulation was successfully implemented via IVP feedback signals. Our results show that pudendal neuro- modulation performed well under the control strategy. Pudendal nerve

stimulation and blocking were accurately timed using IVP feedback signals in 49 of 50 experimental trials, and the voiding efficiency in acute-SCI rats was significantly increased using the proposed control strategy. The IVP feedback control strategy can provide a basis for the design of novel neural prostheses to improve bladder emptying in patients with voiding dysfunction. Although the experimental system was temporarily built using commercial equipment, the design principles and animal experience gathered from this research can serve as a basis for developing an implantable dual-channel microcontroller-based microstimulator in the future.

Under the schemes of simultaneous stimulation and blocking of the pudendal nerve, our results reveal that the voiding efficiency in acute-SCI rats was significantly increased to 2-fold that of the control, and thus the inter-contraction interval increased due to the decrease in residual urine (Fig. 8). The pudendal nerve in the rat originates from the L6 to S1 spinal cord, and contains both sensory and motor branches [2,31-33]. Previous studies demonstrated that applying lower-frequency stimulation (2~20 Hz) with a low stimulation intensity (0.05~0.2 mA) to the sensory branch of the pudendal nerve could improve bladder emptying by augmenting the bladder contraction duration in rats [13,15]. Similar results were obtained for acute-SCI rats in this study.

Several studies have demonstrated the importance of phasic EUS bursting activity for efficient voiding in rats [34,35]. Studies indicated that transection of the pudendal nerve or chemical blockade of neuromuscular transmission to the striated urethral sphincter decreased the voiding efficiency in rats [34,36-40]. Our study indicates that acute SCI caused long-lasting EUS tonic activity which was observed throughout three phases of IVP due to detrusor-sphincter dyssynergia, and thus the EUS contraction closed the urethra and prevented bladder evacuation. The intermittent HFBC was designed to mimic the bursting pattern of EUS activity at phase 2 of IVP in SCI rats by intermittently blocking the EUS. The voiding efficiency increased in acute-SCI rats, which was partially attributed to the effects of intermittent HFBC. To the best of our knowledge, our study is the first to report the application of intermittent HFBC to improve voiding function in SCI rats. Nevertheless, the effects of intermittent HFBC for producing mimic EUS bursting activity on voiding efficiency warrant further exploration.

There are important limitations to the use of acute-SCI rats as the voiding dysfunction model. The present study used rats to investigate lower urinary tract function. Although rats are widely used for physiological and pharmacological studies of the lower urinary tract, rats exhibit phasic patterns of EUS activity during voiding, in contrast to the complete relaxation of the sphincter observed in humans. Therefore, it is not clear whether the present results will translate to humans.

The voiding efficiency in intact anesthetized rats before acute spinal cord transection was only 77%, which is lower than the 98%~99% voiding efficiency measured in conscious animals [41,42]. These results reveal that the anesthetic effect is another limitation in the present study. The physiology of the bladder and EUS appears to be preserved under urethane,

which is the most suitable anesthetic for physiological experiments that require demonstration of reflex micturition [43]. Although anesthetic effects are a limitation of the present study, the facilitative effects of electrical pudendal neuromodulation on bladder emptying were still readily detected in acute-SCI rats.

In summary, a model of IVP and EUS EMG signals was established and an intermittent HFBC pattern combined with LFSC was designed for pudendal neuromodulation. The dual-channel current outputs to the pudendal nerve were finely controlled via a real-time IVP feedback scheme. The control feedback strategy was verified using acute animal experiments and gave satisfactory performance. The voiding efficiency in acute-SCI rats significantly increased to 25% by concomitant dual-channel pudendal neuromodulation. The preliminary results in acute-SCI rats show that the IVP feedback control strategy is a feasible approach for improving bladder emptying in animal experiments, and can be a basis for developing a novel neural prosthesis for restoring bladder function in patients with voiding dysfunction.

Acknowledgments

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