Selective control by posterior spinal nerve roots of micturition and erection in rats*

Wenting Wang, Mouwang Zhou, Genying Zhu, Tao Li, Nan Liu

Department of Rehabilitation Medicine, Third Hospital, Peking University, Beijing 100191, China

Abstract
The posterior rootlets in L6 and S1 spinal cord of adult male Sprague-Dawley rats underwent electrostimulation. The bladder pressure, urethral perfusion pressure and intracavernous pressure were recorded. When some posterior rootlets of L6 and S1 were electrostimulated, the intracavernous pressure peaked rapidly, but the bladder pressure and the urethral perfusion pressure curve did not show great change. When other rootlets were stimulated, the bladder pressure changed greatly, but the urethral perfusion pressure and the intracavernous pressure did not show great change. When different rootlets were stimulated, the urethral perfusion pressure changed maximally, but there were no great changes in bladder pressure or intracavernous pressure. Furthermore, stimulation of some rootlets produced simultaneous changes in two or three different pressure measures mentioned above. The results demonstrate that regulation by L6 and S1 posterior rootlets of the rat bladder detrusor, external urethral sphincter and penis cavernous body are significantly distinct. Different rootlets can be distinguished by electrostimulation.

Key Words
urination; penile erection; electrophysiology; Sprague-Dawley rats; neurogenic bladder; spinal cord injury; neural regeneration

Research Highlights
(1) Microsurgery and electrophysiologic studies of spinal nerve roots in the lumbosacral region of Sprague-Dawley rats revealed selective regulation by spinal anterior and posterior roots of the bladder, external urethral sphincter and penile erection tissues, providing clinical support for selective dorsal rootlet rhizotomy in treatment of bladder dysfunction after spinal cord injury.
(2) The bladder detrusor, external urethral sphincter and cavernous body of the penis were differentially controlled by different rootlets of L6 and S1 posterior spinal nerve roots.

INTRODUCTION
Spinal cord injury results not only in paraplegia or quadriplegia, but also in loss of urinary and defecation control and alteration of sexual function\(^\text{[1-4]}\). Tetraplegic and paraplegic patients with lesions above the spinal micturition center may have a high incidence of reflex urinary incontinence and detrusor/sphincter dyssynergia, resulting in chronic renal failure, which is a major cause of morbidity and mortality in patients with chronic spinal cord injury\(^\text{[5]}\). A number of therapies are used for the treatment of suprasacral spastic neurogenic bladder associated with spinal cord injury\(^\text{[6-16]}\). However, deafferentation of the sacral dorsal roots during traditional rhizotomy carries the risk of impairing superficial and deep sensation in the perineum, and may result in reflex erectile dysfunction\(^\text{[17]}\).
We sought to determine if the sacral dorsal roots could be distinguished by microanatomy into those specifically innervating the bladder, the urethra and the penile cavernous body. In our previous study, we found that the urogenital center in Sprague-Dawley rats was located in the L₆–S₁ segment of the spinal cord, and the posterior sacral root could be differentiated into three or four bundles, and then into three or four rootlets.

Do rootlets in the L₆–S₁ spinal cord segment where the urogenic center is located innervate the bladder detrusor, the external urethral sphincter, the bulbocavernosus muscle and the ischial cavernous muscle in a selective manner? This study aimed to provide a more precise electrophysiological definition of the lumbosacral posterior nerve roots and rootlets in the Sprague-Dawley rats.

RESULTS

Spinal nerve root control of bladder function and erection

When some posterior rootlets of the L₀ or S₁ nerve roots were stimulated, the bladder pressure would rise highly, but the urethral perfusion pressure and the intracavernous pressure did not exhibit much change (Figure 1). When other rootlets were stimulated, the urethral perfusion pressure rose to as much as 3 907.26 Pa, but there were no great changes in bladder pressure or intracavernous pressure (Figure 1). When some other dorsal rootlets of L₀ or S₁ were electrostimulated, the intracavernous pressure rose as high as 9 414.86 Pa in 2–3 seconds.

Figure 1  Changes in rat intravesical pressure (red fine line, upper), urethral perfusion pressure (green fine line, middle) and intracavernous pressure (blue fine line, lower) in rats when nerve tracts of the posterior root of the spinal cord underwent electrostimulation.

X-axis: Stimulus duration; Y-axis: pressure value. Green coarse line below shows stimulus duration, and the data below it are stimulus intensity.

(A) Significant changes in intravesical pressure following electrostimulation of some rootlets of L₀ and S₁ spinal cord posterior roots.

(B) Significant changes in rat urethral perfusion pressure when other rootlets were stimulated.

(C) Significant changes in intracavernous pressure when others were stimulated.

(D–F) Significant changes occurred simultaneously in two or three of these pressure values during electrostimulation. 1 cmH₂O = 98 Pa.
However, the bladder pressure and the urethral perfusion pressure curve did not exhibit a great change (Figure 1). Stimulation of some rootlets affected pressure in all three sites simultaneously (Figure 1).

**Functional differences between the L₆ and S₁ spinal cord posterior rootlets**

In terms of their effects on bladder pressure, there was no significant difference between L₆ and S₁ spinal cord segments ($P = 0.972$). Changes in urethral perfusion pressure induced by L₆ dorsal rootlet stimulation were much greater than those elicited by S₁ segment stimulation ($P < 0.001$). Changes in intracavernous pressure induced by S₁ spinal cord segment rootlet stimulation were significantly greater than those triggered by L₆ segment stimulation ($P < 0.001$); the mean change in intracavernous pressure was $3,070.34$ Pa (Table 1).

There was no significant difference between the right and left sides, either in the L₆ segment ($P = 0.887$) or in the S₁ segment ($P = 0.828$).

**DISCUSSION**

Treatment of hyperreflexic neurogenic bladder after spinal cord injury remains a challenge. Neurosurgery is generally performed to relax the detrusor, to reduce sphincter spasticity and to increase urinary bladder storage function. Some researchers have used complete sacral nerve root rhizotomy for the treatment of spastic bladder after paraplegia[18-19]. Although it is effective in relaxing the detrusor, reducing sphincter spasticity and increasing urinary bladder storage function, the surgery tends to over-relax the detrusor and sphincter. As a result, urination is frequently not significantly improved. Consequently, complete dorsal root rhizotomy has not been widely adopted. Thus, a novel alternative surgical treatment is crucially needed.

Approximately 80% of all spinal cord injuries occur in young men. Conventional cutting of the root can result in the loss of reflex penile erection, and in incomplete spinal cord injury patients, the loss of sacral sensory function[17]. For most young men who want to retain reflex erection, we must find a way to successfully restore bladder function after spinal cord injury, and at the same time enhance sexual function and fertility and improve the patient’s quality of life.

Sprague-Dawley rats, which are used extensively in neurological urology surgeries, were chosen to carry out the protocol. Our previous work had confirmed that, at least in Sprague-Dawley rats, based on the manner in which the nerve roots stretch from the spinal cord to their exit through the dura, a root can be divided into three or four bundles, then into three or four rootlets. Dissection of these rootlets throughout their entire course from their point of origin to their exit shows that they maintain their identity. This is similar to the anatomical characteristics of the sacral root in humans[20-24].

Stimulation of certain individually isolated posterior rootlets in L₆ and S₁ spinal cord segments had a great impact on bladder pressure, with only minor effects on urethral perfusion pressure and intracavernous pressure. In contrast, stimulation of some other rootlets mainly influenced urethral perfusion pressure, while the stimulation of some different rootlets predominantly affected intracavernous pressure. Functional results support this—different posterior rootlets in each segment can induce different responses in the bladder detrusor, the external urethral sphincter and the penis cavernous. Therefore, different posterior rootlets of L₆ and S₁ spinal cord segments modulate the functioning of different genitourinary tract organs. However, there is evidence that none of these posterior rootlets subserve only one target. The correlation between the posterior rootlets and the genitourinary tract organs is not “point-to-point”. Nevertheless, there is definitely a certain degree of selectivity. This might result from the extensive fiber connections among rootlets and the complexity of the nerve system.

The clinical implications of our findings are exciting. Surgical strategies can be tailored to the specific clinical symptoms. For example, for the treatment of reflex urinary incontinence, which is associated with high reflex and high pressure caused by spinal cord injury, selective cutting of part of the sacral nerve rootlets can be performed. At the same time, the dorsal rootlets participating in penis function can be preserved to maintain reflex erection function and the patient’s quality of life can be improved. This is especially crucial for the adult males who account for 80% of patients suffering
from chronic spinal cord injury. Moreover, selective resection of the rootlets that regulate the functioning of the urethra can be performed to reduce spasticity of the external urethral sphincter, increase the coordination of micturition and lower the possibility of detrusor/sphincter dyssynergia, which is closely related with urinary tract infection and chronic renal failure. In combination with sacral anterior root stimulation, which is regarded as one of the most effective ways to treat the spastic bladder after spinal cord injury\[25-31\], selective dorsal rootlet deafferentation could be performed. Furthermore, selective stimulation of the rootlets that innervate the bladder detrusor, but not the external urethral sphincter, could also be considered to overcome the disadvantages of traditional sacral anterior root stimulation.

Highly selective dorsal rootlet rhizotomy can be readily accomplished intradurally. The surgery is highly selective and targets detrusor or sphincter spasm. The technically simple aspect, lower damage and great clinical potential of this approach make it attractive. Our experimental results demonstrate clinical practical efficacy, and may provide new strategies to improve the quality of life in patients with chronic spinal cord injury. Further basic and clinical research is required to fully reveal the potential of our findings.

**MATERIALS AND METHODS**

**Design**
A self-controlled animal experiment.

**Time and setting**
Experiments were performed in the Physiology Lab of Peking University Health Science Center in China from October 2009 to March 2010.

**Materials**
A total of 40 adult, male, 6-week-old Sprague-Dawley rats, weighing 250–300 g, were supplied by the Peking University Health Science Center Experimental Animal Center (license No. SCXX (Jing) 2002/0001). They were housed at 22 °C in standard laboratory conditions, under a 12-hour darklight cycle with free access to food and water. After each experiment, rats were housed individually and observed daily. They showed no sign of sickness or distress at anytime. In all experiments, adequate measures were taken to minimize pain and discomfort to the rats, in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology in China\[32\].

**Methods**

**Electrostimulation**
After anesthesia, all rats were placed onto a heated operating table to maintain body temperature between 37 and 38°C throughout the experiment. During the experiments, sodium pentobarbital was added when needed. After a tracheostomy with placement of polyethylene tubing (intramedic PE-200, AD Instruments Pty Ltd., Australia) to facilitate respiration, PE-50 tubing (AD Instruments Pty Ltd.) was inserted into the left external jugular vein for saline infusion\[33\]. The urinary bladder was exposed through a ventral midline incision\[34\] and bladder pressure was monitored by inserting a PE-50 catheter into the bladder dome\[35\]. A parallel PE-50 catheter was inserted through the bladder dome, positioned into the proximal urethra, and attached via a T-connector to both a perfusion pump (0.08 mL/min (AD Instruments Pty Ltd.) and a pressure transducer. The abdominal skin incision was closed with a running suture, with the muscle wall left open to diminish future increases of abdominal pressure during electrostimulation. Before closing, the bladder capacity was measured and the bladder was left empty. Capacity was determined by infusion of normal saline through the cystostomy catheter used to monitor bladder pressure and by recording the volume necessary to cause overflow incontinence or spontaneous micturition. Through a transverse perineal incision, the left penile crus was exposed by removing the overlying ischiocavernous muscle. A 23G needle filled with 250 unit/mL of heparin solution and connected to PE-50 tubing was inserted in the crus and fixed to the albuginea with a 7/0 Dermalon suture. The bladder, intracavernous and urethral pressures were measured and recorded using the ML870B80 Exercise Physiology System (AD Instruments Pty Ltd.).

Through a dorsal incision, and with the help of an operating microscope (× 10), laminectomy was performed from L1 to L3, and the dura was opened the entire length of the laminectomy. Exsiccation of the neural tissue was prevented by a continuous saline drip. With the help of saline irrigation, a short 3/0 monofilament thread was used as an atraumatic dissector. After the bladder was filled to 50% capacity, intradural electrostimulation of the dorsal roots and rootlets of L6 and S1 segments was performed with a delicate platinum-iridium bipolar hook electrode (AD Instruments Pty Ltd.). The following stimulation parameters were used: monophasic rectangular pulses; pulse amplitude 4 V; pulse width, 0.2 ms; pulse rate, 30 pulses per second. After the electrostimulation was...
completed, the animal was sacrificed and the laminectomy continued down to the sacrum to confirm the spinal segments.

**Data analysis**
The bladder pressure, urethral perfusion and the intracavernous pressure were recorded before and after stimulation. Data were expressed as mean ± SD. All data were input into Microsoft Office Excel 2003 software, and statistical analysis was conducted with SPSS 10.0 software (SPSS, Chicago, IL, USA) using independent sample t-test. A P-value of < 0.05 was considered statistically significant.

**Acknowledgments:** We thank teacher Jiandong Wang from Peking University Health Science Center Physiology Laboratory for experimental technical guidance and assistance, and for providing experimental equipment. We thank Professor Changman Zhou from the Anatomy Laboratory of Peking University Health Science Center for the experimental design and results of the audit and for valuable comments. We thank Experimental Animal Center of Peking University Health Science Center for excellent animal handling and care.

**Funding:** This project was funded by the National Natural Science Foundation of China, No. 30672096.

**Author contributions:** Wenting Wang was responsible for implementation, data collection, integration and the first draft of the manuscript. Mouwang Zhou was responsible for conception and design, performing experiments, directing research, validation of the draft, and for helping manage funds. Nan Liu and Tao Li analyzed experimental data, provided information support, and assisted with documents. All authors approved the final version of the manuscript.

**Conflicts of interest:** None declared.

**Ethical approval:** The project obtained Animal Ethical Approval from Ethics Committee of Third Hospital, Peking University, China.

**Author statements:** The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

**REFERENCES**

[1] Simpson LA, Eng J, Hsieh JT, et al. The health and life priorities of individuals with spinal cord injury: a systematic review. J Neurotrauma. 2012;29(8):1548-1555.

[2] Cameron AP, Rodriguez GM, Schomer KG. Systematic review of urological followup after spinal cord injury. J Urol. 2012;187(2):391-397.

[3] Hagen EM, Faerestrand S, Hoff JM, et al. Cardiovascular and urological dysfunction in spinal cord injury. Acta Neurol Scand Suppl. 2011;(191):71-78.

[4] Jeong SJ, Cho SY, Oh SJ. Spinal cord/brain injury and the neurogenic bladder. Urol Clin North Am. 2010;37(4):537-546.

[5] Francis K. Physiology and management of bladder and bowel continence following spinal cord injury. Ostomy Wound Manage. 2007;53(12):18-27.

[6] Li JS, Hassouna M, Sawan M, et al. Electrical stimulation-induced right lateral hypothalamic and striatal reward responses. J Neurotrauma. 2012;29(8):1548-1555.

[7] Li JS, Hassouna M, Sawan M, et al. Long-term effect of sphincteric fatigue during bladder neurostimulation. J Urol. 1995;153(1):238-242.

[8] Haleem AS, Boehm F, Legatt AD, et al. Sural root stimulation for controlled micturition: prevention of detrusor-external sphincter dyssynergia by intraoperative identification and selective section of the sacral nerve branches. 1993;149(6):1607-1612.

[9] Tanagho EA, Schmidt RA, Orvis BR. Neural stimulation for control of voiding dysfunction: a preliminary report in 22 patients with serious neuropathic voiding disorders. J Urol. 1989;142(2 Pt 1):340-345.

[10] Brindley GS, Polkey CE, Rushton DN, et al. Sural anterior root stimulators for bladder control in paraplegia: the first 50 cases. J Neurol Neurosurg Psychiatry. 1986;49(10):1104-1114.

[11] Robinson LQ, Grant A, Weston P, et al. Experience with the brindley anterior sacral root stimulator. Br J Urol. 1988;62(6):553-557.

[12] Koldewijn EL, Rijkhoff NJM, van Kerrebroeck PhEV, et al. Selective sacral root stimulation for bladder control: acute experiments in an animal model. J Urol. 1994;151(6):1674-1679.

[13] Rijkhoff NJM, Koldewijn EL, van Kerrebroeck PhEV, et al. Acute animal studies on the use of an anodal block to reduce urethral resistance in sacral root stimulation. IEEE Trans Rehab Eng. 1994;2(2):92-99.

[14] Brindley GS, Rushon DN. Long-term follow-up of patients with sacral root stimulator implants. Paraplegia. 1990;28(8):469-475.

[15] Talalla A, Bloom JW, Ngugen Q. Successful intra spinal.extradural sacral nerve stimulation for bladder emptying in a victim of traumatic spinal cord transaction. Neurosurgery. 1986;19(6):955-961.

[16] Tanagho EA, Schmidt RA. Electrical stimulation in the clinical management of the neurogenic bladder. J Urol. 1988;140(6):1331-1339.

[17] Francios G, Olivier R. Neural control of erection. Physiol Behav. 2004;83(2):189-201.

[18] van Ophoven A, Pannek J. The future of invasive neuromodulation: new techniques and expanded indications. Urologe A. 2012;51(2):212-216.
[19] Pannek J, Göcking K, Bersch U. Sacral rhizotomy: a salvage procedure in a patient with autonomic dysreflexia. Spinal Cord. 2010;48(4):347-348.

[20] Zhou MW, Zhou CM, Wang WT, et al. Microsurgical anatomy of lumbosacral nerve rootlets for highly selective sacral root rhizotomy in chronic spinal cord injury. Anat Rec (Hoboken). 2010;293(12):2123-2128.

[21] Arslan M, Çömert A, Açıar H, et al. Lumbosacral intrathecal nerve roots: an anatomical study. Acta Neurochir (Wien). 2011;153(7):1435-1442.

[22] Hauck EF, Wittkowski W, Bothe HW. Intradural microanatomy of the nerve roots S1-S4 at their origin from the conus medullaris. J Neurosurg Spine. 2008;9(2):207-212.

[23] Fehlings MG, Tighe A. Anatomy of the sacral nerve roots: clinical implications for neural repair. J Neurosurg Spine. 2009;11(3):253-254.

[24] Dahms SE, Tanagho EA. The impact of sacral root anatomy on selective electrical stimulation for bladder evacuation. World J Urol. 1998;16(5):322-328.

[25] Brindley GS, Polkey CE, Rushton DN. Sacral anterior root stimulators for bladder control in paraplegia. Paraplegia. 1982;20:365-381.

[26] Tanagho EA and Schmidt RA. Electrical stimulation in the clinical management of the neurogenic bladder. J Urol. 1988;140(6):1331-1339.

[27] Martens FM, den Hollander PP, Snoek GJ, et al. Quality of life in complete spinal cord injury patients with a Brindley bladder stimulator compared to a matched control group. Neurol Urodyn. 2011;30(4):551-555.

[28] Martens FM, Heesakkers JP. Clinical results of a brindley procedure: sacral anterior root stimulation in combination with a rhizotomy of the dorsal roots. Adv Urol. 2011;2011:709708.

[29] Vignes JR, Seze M, Sesay M, et al. Anterior sacral root stimulation with dorsal rhizotomy (Brindley technique). Neurochirurgie. 2003;49(2-3 Pt 2):384-394.

[30] Differential effects of sacral anterior root stimulation on anal sphincter and colorectal motility in spinally injured man. Br J Surg. 1986;73(6):478-482.

[31] Zhou MW, Chen YP, Huang HS, et al. Observation of effect of Brindley’s technique on the bladder function of SCI patients. Zhonghua Wuli Yixue yu Kangfu Zazhi. 2007;29(10):695-697.

[32] The Ministry of Science and Technology of the People’s Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

[33] Martinez-Piñeiro L, Trigo-Rocha F, Hsu GL, et al. Response of bladder, urethral and intracavernous pressure to ventral lumbosacral root stimulation in Sprague-Dawley and Wistar rats. J Urol. 1992;9(148):925-929.

[34] Kamo I, Torimoto K, Chancellor MB, et al. Urethral closure mechanisms under sneeze-induced stress condition in rats: a new model for evaluation of stress urinary incontinence. Am J Physiol Regul Integr Comp Physiol. 2003;285(2):R356-365.

[35] Kaiho Y, Kamo I, Chancellor MB, et al. Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol. 2007;292(2):F639-646.

[36] Hou CL, Zhang SM, Xu RS, et al. Functional selective innervations of sacral roots to pelvic detrusors and sphincters: a canine electrostimulation study. Zhongguo Linchuang Jiepouxue Zazhi. 2000;18(3):244-247.

(Edited by He XJ, Yu ZJ/Qiu Y/Song LP)