The feasibility study of extradural nerve anastomosis technique for canine bladder reinnervation after spinal cord injury

Jian Tang^{1*}, Jun Ma^{2*}, Lei Yang^{1*}, Xinpeng Huang¹, Yingbin Ge³, Tao Sui¹, Zhongqing Wei⁴, Xiaojian Cao¹

¹Department of Orthopedics, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China,

²Department of Orthopedics, Affiliated Suqian Hospital of Xuzhou Medical College, Suqian, Jiangsu, China,

³Department of Physiology, Nanjing Medical University, Nanjing, Jiangsu, China, ⁴Department of Urology, the Second Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

Objective: Intradural nerve anastomosis for bladder innervation has been demonstrated to be useful. However, its clinical application remains limited because of the complex surgery, its complications and extensive bony destruction. The purpose of the current study was to demonstrate the feasibility of extradural spinal root anastomosis for bladder innervation in canines.

Methods: Ten beagle dogs were used. The length of the extradural segment of the nerve root, upper nerve root outlet (the point at which it emerges from the spinal dura mater) to S2 (dS2), the S3 (dS3) nerve root outlet distance, and the diameters of the extradural spinal roots were measured. The numbers of nerve fibers from L6 to S3 ventral roots were calculated using immunohistochemical staining.

Results: The extradural spinal roots could be divided into a ventral root (VR) and a dorsal root (DR) before the ganglionic enlargement of the dorsal root, and the extradural motor nerve roots situate ventrally to their corresponding sensory nerve roots. The extradural nerve root lengths of S1 and parts of L7 were longer than the corresponding dS2. The numbers of nerve and motor nerve fibers, and the diameters of extradural nerve roots, were gradually descending from L6 to S3.

Conclusion: The S1 VRs and parts of the L7 VRs can be extradurally anastomosed to the S2 nerves without tension. A nerve graft was needed for extradural anastomosis of L6 VRs and parts of L7 VRs to S2 VRs. This study demonstrated the feasibility of extradural spinal nerve anastomosis for treating neurogenic bladder in canines.

Keywords: Bladder dysfunction, Spinal nerve root, Nerve transfer, Spinal cord injury (SCI)

Introduction

Lower urinary tract dysfunction caused by spinal cord injury (SCI) or sacral root injury continues to have high prevalence and long-term consequences on the wellbeing of patients. Serious urinary system complications, especially chronic renal failure, due to bladder

dysfunction are always the leading causes of death.¹⁻⁴

A survey study performed in 2004 showed that patients rated restoration of bladder function as important, which was ranked second after sexual function in paraplegic patients, and third after hand and sexual function in quadriplegic patients.⁵ During the past century, many studies have focused on treatments to regain function in a decentralized bladder after SCI or spinal root injury; these treatments have included bladder reinnervation, sacral root electrostimulation, highly selective rhizotomy of sacral roots, direct reinnervation of the detrusor muscle, intermittent catheterization and creation of an

*Jian Tang, Jun Ma, and Lei Yang contributed equally to this work.

Zhongqing Wei and Xiaojian Cao are co-corresponding authors.

Correspondence to: Xiaojian Cao, Department of Orthopedics, The First Affiliated Hospital of Nanjing Medical University, NO. 300, Guangzhou Road, Nanjing City, Jiangsu Province, China. Email: xiaojiancao001@163.com

Institution of the work performed: Laboratory Animal Center of Nanjing Medical University and the Laboratory of Physiology of Nanjing Medical University

artificial reflex pathway.^{6–12} In particular, creation of an artificial reflex has been a significant breakthrough in treating neurogenic bladder.^{13,14} The use of intradural nerve transfer for bladder reinnervation has previously been demonstrated in both animal and clinical studies.^{15–20} However, in a previous canine study, Ma *et al.*²⁰ found that intradural nerve transfer caused extensive destruction, and that microanastomosis in the dural sac had high technical difficulty. The extensive destruction and the complicated surgical procedure have likely restricted further study and clinical application.

The present study was designed to demonstrate the possibility of nerve transfer outside the spinal dural mater and modify the surgery for bladder reinnervation in canines, using anatomical and histological analyses.

Materials and methods

Anatomic dissection

Ten adult beagle dogs, weighing 10–12 kg, were studied. All procedures were approved by the Nanjing Medical University, and the animals were treated in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals that were formulated by the Ministry of Science and Technology of China. All animals were sacrificed after the administration of sodium pentobarbital anesthesia. Each dog was placed in the prone position. Following the removal of all muscles and ligaments, the thoracic and lumbosacral vertebral lamina and the articular process were fully exposed. Next, the dural sac and spinal nerve roots were exposed by removing the laminae from L6 to the sacrum (Fig. 1A).

The dissections of the nerve roots outside the dura mater were performed under a surgical microscope.

Figure 1 Overviews of the dural sac and the extradural spinal nerve roots. (A) The spinal nerve root from L6 to S2. (B) Red and black pins, the superior-most point of the nerve root outlet; blue pin, the midpoint of the superior point and inferior point of the nerve root outlet; and brown pin, the center of the spinal ganglion. (Colour online)

Measurements were performed using a caliper with a precision of 0.01 mm. Finally, short segments of samples, including intradural and extradural nerve roots, were cut and placed in phosphate buffered saline (PBS) buffer in preparation for the histological study.

The following parameters were measured:

1. The length of the extradural segment of the nerve root: from the outlet of the spinal nerve at its emergence from the spinal cord dura mater to the center of its spinal dorsal root ganglion (the distance between the midpoint of the superior and inferior points of the nerve root outlet and the center of the spinal ganglion) (Fig. 1B);
2. dS2: upper nerve root outlet to S2 nerve root outlet (the distance between the superior-most point of the upper root outlet and that of the S2 nerve root outlet) (Fig. 1B);
3. dS3: upper nerve root outlet to S3 nerve root outlet (the distance between the superior-most point of the upper root outlet and that of the S3 nerve root outlet);
4. The extradural diameters of the ventral and dorsal nerve roots;
5. The gross anatomy of the extrathecal nerve roots.

Immunohistochemistry

All samples were routinely dehydrated, clarified, paraffin embedded, and sectioned (5 μ m cross sections). Hematoxylin and eosin (HE) staining was utilized to observe the microstructure of different samples. Neurofilament 200, a specific marker of neurofilament, was used to determine the number of nerve fibers. Briefly, paraffin-slicing slides were dewaxed, rehydrated, and immersed in PBS for 5 minutes. The sections were heated in 3% H_2O_2 at 37°C for 10 minutes, washed using PBS and microwaved in boiling citrate sodium buffer. Then, the sections were blocked in normal goat serum for 20 minutes. After they were washed, the sections were incubated overnight at 4°C in mouse anti-neurofilament 200 (NF-200; Abcam Cambridge, MA, USA) monoclonal antibody and washed. Finally, the sections were further incubated with secondary antibody (horseradish peroxidase-labeled goat anti-mouse immunoglobulin G, KIT5010; MaxVision, Fuzhou, Fujian province, China) for 20 minutes at room temperature. Then, they were reacted with 3,3'-diaminobenzidine tetrahydrochloride hydrate (Boster, Wuhan, China).

Acetylcholinesterase (AChE) is found in many types of conducting tissues.²¹ The level of AChE in motor nerve fibers is significantly higher than that in sensory nerve fibers.²² In this way, the immunohistochemistry of AChE was used to determine the number of motor nerve fibers. The monoclonal antibody to AChE was

obtained from Abgent (San Diego, CA, USA). Briefly, paraffin-slicing slides were dewaxed, rehydrated, and immersed in PBS for 5 minutes. The sections were heated in 3% H₂O₂ at 37° C for 10 minutes, washed using PBS and microwaved in boiling citrate sodium buffer. Then, the sections were blocked in normal goat serum for 20 minutes. After they were washed, the sections were incubated overnight at 4°C in monoclonal antibody to AChE (Abgent, San Diego, CA, USA) and washed. Finally, the sections were further incubated with secondary antibody (horseradish peroxidase-labeled goat anti-mouse immunoglobulin G, KIT5010; MaxVision, Fuzhou, Fujian province, China) for 20 minutes at room temperature. Then, they were reacted with 3,3'-diaminobenzidine tetrahydrochloride hydrate (Boster, Wuhan, China).

Images were obtained using a light microscope (Olympus BX50, Tokyo, Japan) that was connected to a CCD camera (Olympus DP70, Tokyo, Japan). Nerve fiber and motor nerve counts of stained cross sections were performed with Image Pro Plus 6.0 image analysis software.

Data analysis

All data were input into Excel 2013 (Microsoft, Seattle, WA, USA). The statistical analysis was conducted using SPSS 20.0 software (IBM SPSS, Armonk, NY, USA) with one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test (SNK test), and $P < 0.05$ was considered to be significant.

Figure 2 A view of the extradural spinal nerve roots. (A) Demonstration of the feasibility of dividing VR from DR outside the dural sac. L6 VR and S1 VR situate ventrally relative to their corresponding sensory nerve roots. (B) An illustration of extradural nerve root anastomosis without tension (S1 VR–S2 VR). L6 VR, L6 ventral root; L6DR, L6 dorsal root; S1 VR, S1 ventral root; S1DR, S1 dorsal root; and DRG, dorsal root ganglion. (Colour online)

Results

Topographic anatomy and microstructure of spinal nerves

From L6 to S3, the nerve root outlets were not consistent with the corresponding vertebrae. The outlet of the L6 nerve root was located at the lower 1/3 of the L5 vertebra; the outlets of the L7 and S1 nerve roots were situated in the upper 1/2 and lower 1/2 of the L6 vertebra, respectively; and the S2~S3 nerve root outlets were present in the upper 1/2 of the L7 vertebra. After leaving the dural sac, the spinal nerve roots descended in the spinal canal until arriving at the corresponding intervertebral foramen. Before the ganglionic enlargement of the dorsal root, they could be divided into a ventral root (VR) and a dorsal root (DR) (Fig. 2A). Furthermore, the extradural motor nerve roots are situated ventrally relative to their corresponding sensory nerve roots (Fig. 2A). Therefore, it is feasible to separate and recognize the VR from the DR outside the dural sac.

The length of the extradural nerve roots varied at different levels, as shown in Table 1.

The average length of the extradural nerve roots gradually increased from L6 to S2. No significant differences were found between the left and the right sides.

Table 1 also shows that, for dS2 and dS3, there were no significant differences between the left and the right sides. Comparing the length of the extrathecal nerve roots with dS2 and dS3, the S1 nerve root was long enough to be extradurally anastomosed to the S2 and S3 nerves without tension (Fig. 2B), but only 60% (12/20) and 5% (1/20) of the L7 nerve roots, respectively, could be extradurally coapted with the S2 and S3 nerve roots in a tension free manner. A nerve graft was required to coapt the L6 nerve root to the S2 or S3 nerve roots extradurally.

The HE staining demonstrated the considerably different envelopes between intra and extra dural spinal nerves. Extradural VRs and DRs were both wrapped in a thick membrane, whereas only a thin layer was observed around intradural VRs and DRs. (Fig. 3E and F)

Nerve fiber diameters and counts

Table 2 shows that the diameters of the spinal nerve roots differed from L6 to S3. The DRs were always larger than the corresponding VRs. As for the VRs, the diameters of L6 to S1 were always larger than those of S2 and S3. The diameters of the DRs had an identical trend that was not statistically significant. No significant differences were observed between the left and the right sides.

Table 1 Nerve root outlet-ganglion center (mm), dS2 (mm) and dS3 (mm)

	Nerve root outlet-ganglion center (mm)	dS2 (mm)	dS3 (mm)
L6	16.75 ± 1.36	29.22 ± 2.19	34.63 ± 2.36
L7	19.27 ± 1.20*	17.77 ± 1.86	23.48 ± 1.97
S1	20.75 ± 1.62 [†]	8.19 ± 1.07	13.94 ± 1.40
S2	21.63 ± 1.98		5.66 ± 0.61

dS2: upper nerve root outlet to S2 nerve root outlet; dS3: upper nerve root outlet to S3 nerve root outlet.

*vs dS2 of L7 P < 0.05. [†]vs dS2 and dS3 of S1 P < 0.05.

Figure 3 Micrographs of the intradural or extradural nerve root cross sections. (A) Cross-section of extradural L6 ventral root (NF200 stain; 10×Magnification). (B) The axons of extradural spinal nerve root are stained brown. (C) Cross-section of extradural S3 ventral root (monoclonal antibody to AChE stain; 10× Magnification). (D) Cross-section of extradural S2 dorsal root (monoclonal antibody to AChE stain; 10× Magnification). (E) HE staining of the extradural spinal nerve root (10×magnification). (F) HE staining of the intradural dorsal nerve root (10× magnification). (Colour online)

Table 2 Measurements of the spinal nerve roots

	Diameter (mm)		No. of nerve fibers		No. of motor nerve fibers Ventral	No. of motor nerve fibers Dorsal
	Ventral	Dorsal	Ventral	Dorsal		
L6	1.05 ± 0.05	2.03 ± 0.05	10213 ± 540	28012 ± 1482	8830 ± 435	2550 ± 261
L7	0.92 ± 0.04	1.82 ± 0.03	8845 ± 218	22463 ± 751	7598 ± 314	2093 ± 111
S1	0.58 ± 0.02	0.80 ± 0.02	5679 ± 249	10953 ± 217	4850 ± 254	1444 ± 194
S2	0.48 ± 0.02*	0.70 ± 0.02	4843 ± 195 [†]	8619 ± 271	3596 ± 254 [#]	1021 ± 187
S3	0.39 ± 0.03	0.58 ± 0.02	3295 ± 227	6702 ± 313	2794 ± 165	679 ± 92

* vs diameters of L6-S1 P < 0.05. [†] vs No. of nerve fibers of L6- S1 P < 0.05. [#] vs No. of Motor Nerve Fibers of L6-S1 P < 0.05.

As shown in Fig. 3A and B, the NF-200 immunohistochemical staining showed positive staining of the axons (brown granules); as Fig. 3C and D show, the AChE protein was detected in the VRs and at low levels in the DRs.

The number of nerve and motor nerve fibers gradually decreased from L6 to S3 (Table 2). The number of nerve fibers in the DR was more than that in the corresponding VR, but the motor nerve fiber count of the VR was significantly higher than that of the corresponding DR. No statistically significant differences were observed between the left and right sides.

Discussion

Neurogenic bladder dysfunction after SCI or spinal nerve root injury is a serious threat to the quality of life, and recovery from neurogenic bladder remains a worldwide challenge. In the 1990s, Xiao *et al.*^{13,14} proposed the concept of “the artificial reflex”, which provided a novel method for treating neurogenic bladder. In previous clinical studies,^{17,18,23} the procedure has achieved satisfactory results.

However, neuroanastomosis within the dural sac has some significant disadvantages. First, when performed in the rat, the procedure is associated with extensive damage of the bone structure,²⁴ which may result in postoperative spinal deformity. Second, it is necessary to open the dural sac for the surgery; therefore, the risks of cerebrospinal fluid leakage and myelitis increase, and these are occasionally deadly.^{25–27} Third, because of the absence of epineurium, surgeons could accidentally damage spinal nerve roots and cauda equine when differentiating between VRs and DRs and stitching the nerves. To avoid or reduce the aforementioned defects, we tried to modify the surgical procedure by performing the spinal root anastomosis extradurally instead of performing an intradural procedure in canines.

In past decades, some researchers have extradurally performed the surgery of neuroanastomosis in animal models.^{24,28,29} Although some results were satisfactory, there have been few detailed descriptions of the anatomical structures of the extradural nerve root used in neurotization, which is important for replicating the animal models and further study.

Our study showed that VR and DR could be easily and independently separated extradurally. In addition, the intradural spinal nerve root was only encircled by a thin membrane, which might be spinal pia mater, making it vulnerable to mechanical stretch. Conversely, the extradural root was encased by a thick, considerable mechanical strength layer that

might consist of spinal pia mater, arachnoid mater and spinal dura mater. Therefore, with minor bony destruction and without opening the dura mater, extradural nerve anastomosis could not only facilitate the surgery, but also strengthen the nerve suture which is beneficial for nerve axon regeneration.

The identification of spinal nerve roots is critical for accurate anastomosis of motor-to-motor nerve and sensory-to-sensory nerve.³⁰ Previous researchers^{31–33} reported that identification and proper determination of the donor (receptor) nerve roots in the dural sac could be facilitated by anatomical-electrical features. Conversely, the study results showed that it is easier to identify the nerve roots based on the relative positions of motor and sensory nerve roots outside the dural sac. Furthermore, a piezoelectric quartz crystal immunosensor, which was invented by Sui *et al.*,³⁴ makes it possible to quickly differentiate between VRs and DRs in canines. Therefore, the use of the anatomical features and the piezoelectric quartz crystal immunosensor may simplify the procedure for achieving an accurate anastomosis of the nerve roots to rebuild bladder function.

The number of donor nerve fibers is another key point for determining the efficacy of nerve transfer. Previous studies of the brachial plexus have shown that reconstruction of the normal target organ function in neuroanastomosis does not require restoration of all receptor nerve fibers.³⁵ Further studies with somatic motor to somatic motor nerve transfer have demonstrated that the smallest proportion of nerve fibers required to maintain the target organ function is 37 to 40%.^{36,37} In the aforementioned results, our data revealed the possibility of spinal nerve root transfer to achieve bladder reinnervation with respect to the nerve fiber number. On the other hand, the crossover of the donor nerve normally causes loss of function in the original organs that are innervated by it, which should be minimized as much as possible. As a result, choosing nerve tract anastomosis over entire nerve transfer could be more ideal for preserving parts of donor nerve function.

It is well known that the growth rate of regenerative axons is very slow. When performing an anastomosis, we should consider the amount of time required to rebuild the bladder reflex before the target organ is degenerated. It is obvious that an extradural neuroanastomosis is closer to the target organ than an intradural neuroanastomosis.

Although we tried to modify the surgery for bladder reinnervation, the surgery was not easy, and it required professional, technical expertise. And in the next experiments, we will establish the animal models and evaluate the effect of the surgery. Based on the limited the

number of dogs evaluated, the data presented here only should be used as a reference.

Conclusion

Our study provides anatomic basis of extradural spinal nerve anastomosis which demonstrates the feasibility of extradural nerve anastomosis technique for treating neurogenic bladder in canines. And in the future studies, we will evaluate the effect of the surgery and conduct a systemic comparison between intradural and extradural nerve transfer for bladder reinnervation.

Disclaimer statements

Contributors None.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81371968, No. 81371969, No. 81401791) and the Foundation of Jiangsu Province Public Health Bureau of China (No. BK2012718).

Conflicts of interest None.

Ethics approval None.

References

- Frankel HL, Coll JR, Charlifue SW, Whiteneck GG, Gardner BP, Jamous MA, *et al.* Long-term survival in spinal cord injury: a fifty year investigation. *Spinal Cord* 1998;36(4):266–74.
- Watt JW, Wiredu E, Silva P, Meehan S. Survival after short- or long-term ventilation after acute spinal cord injury: a single-centre 25-year retrospective study. *Spinal Cord* 2011;49(3):404–10.
- Hackler RH. A 25-year prospective mortality study in the spinal cord injured patient: comparison with the long-term living paraplegic. *J Urol* 1977;117(4):486–8.
- Capoor J, Stein AB. Aging with spinal cord injury. *Phys Med Rehabil Clin N Am* 2005;16(1):129–61.
- Anderson KD. Targeting recovery: priorities of the spinal cord-injured population. *J Neurotrauma* 2004;21(10):1371–83.
- Torrens M, Hald T. Bladder denervation procedures. *Urol Clin North Am* 1979;6(1):283–93.
- Ninkovic M, Stenzl A, Hess M, Feichtinger H, Schwabegger A, Colleselli K, *et al.* Functional urinary bladder wall substitute using a free innervated latissimus dorsi muscle flap. *Plast Reconstr Surg* 1997;100(2):402–11; discussion 12–4.
- Senjaya F, Midha R. Nerve transfer strategies for spinal cord injury. *World Neurosurg* 2013;80(6):e319–26.
- Cameron AP. Pharmacologic therapy for the neurogenic bladder. *Urol Clin North Am* 2010;37(4):495–506.
- Xiao CG. Reinnervation for neurogenic bladder: historic review and introduction of a somatic-autonomic reflex pathway procedure for patients with spinal cord injury or spina bifida. *Eur Urol* 2006;49(1):22–8; discussion 28–9.
- Gasparini ME, Schmidt RA, Tanagho EA. Selective sacral rhizotomy in the management of the reflex neuropathic bladder: a report on 17 patients with long-term followup. *J Urol* 1992;148(4):1207–10.
- Brindley GS. The first 500 patients with sacral anterior root stimulator implants: general description. *Paraplegia* 1994;32(12):795–805.
- Xiao C-G, De Groat WC, Godec CJ, Dai C, Xiao Q. “Skin-Cns-Bladder” reflex pathway for micturition after spinal cord injury and its underlying mechanisms. *J Urol* 1999;162(3):936–42.
- Xiao CG, Godec CJ. A possible new reflex pathway for micturition after spinal cord injury. *Paraplegia* 1994;32(5):300–7.
- Nouhaud FX, Caremel R, Leroi AM, Grise P. [Bladder reinnervation with creation of a “somo-autonomic” reflex pathway in spinal cord injured or spina bifida, a new way for treatment?]. *Prog Urol* 2011;21(8):501–7.
- Tuite GF, Storrs BB, Homsy YL, Gaskill SJ, Polsky EG, Reilly MA, *et al.* Attempted bladder reinnervation and creation of a scratch reflex for bladder emptying through a somatic-to-autonomic intradural anastomosis. *J Neurosurg Pediatr* 2013;12(1):80–6.
- Xiao CG, Du MX, Dai C, Li B, Nitti VW, de Groat WC. An artificial somatic-central nervous system-autonomic reflex pathway for controllable micturition after spinal cord injury: preliminary results in 15 patients. *J Urol* 2003;170(4 Pt 1):1237–41.
- Lin H, Hou C, Zhen X, Xu Z. Clinical study of reconstructed bladder innervation below the level of spinal cord injury to produce urination by Achilles tendon-to-bladder reflex contractions. *J Neurosurg Spine* 2009;10(5):452–7.
- Lin H, Hou C, Chen A. Reconstructed bladder innervation above the level of spinal cord injury to produce urination by abdomen-to-bladder reflex contractions. *J Neurosurg Spine* 2011;14(6):799–802.
- Ma J, Sui T, Zhu Y, Zhu A, Wei Z, Cao XJ. Micturition reflex arc reconstruction including sensory and motor nerves after spinal cord injury: urodynamic and electrophysiological responses. *J Spinal Cord Med* 2011;34(5):510–7.
- Satoh K, Armstrong DM, Fibiger HC. A comparison of the distribution of central cholinergic neurons as demonstrated by acetylcholinesterase pharmacohistochemistry and choline acetyltransferase immunohistochemistry. *Brain Res Bull* 1983;11(6):693–720.
- McNeill ME, Norvell JE. Acetylcholinesterase activity of primary sensory neurons and dorsal root fibers in the cat. *Anat Rec* 1978;190(1):155–9.
- Lin H, Hou CL, Zhong G, Xie Q, Wang S. Reconstruction of reflex pathways to the atonic bladder after conus medullaris injury: preliminary clinical results. *Microsurgery* 2008;28(6):429–35.
- Chuang DC, Chang PL, Cheng SY. Root reconstruction for bladder reinnervation: an experimental study in rats. *Microsurgery* 1991;12(4):237–45.
- Shaffrey CI, Spotnitz WD, Shaffrey ME, Jane JA. Neurosurgical applications of fibrin glue: augmentation of dural closure in 134 patients. *Neurosurgery* 1990;26(2):207–10.
- Narotam PK, Qiao F, Nathoo N. Collagen matrix duraplasty for posterior fossa surgery: evaluation of surgical technique in 52 adult patients. *Clinical article. J Neurosurg* 2009;111(2):380–6.
- Tachibana E, Saito K, Fukuta K, Yoshida J. Evaluation of the healing process after dural reconstruction achieved using a free fascial graft. *J Neurosurg* 2002;96(2):280–6.
- Lin H, Hou C, Chen A, Xu Z. Reinnervation of atonic bladder after conus medullaris injury using a modified nerve crossover technique in canines. *World Neurosurg* 2010;73(5):582–6.
- Ruggieri MR, Braverman AS, D’Andrea L, McCarthy J, Barbe MF. Functional reinnervation of the canine bladder after spinal root transection and immediate somatic nerve transfer. *J Neurotrauma* 2008;25(3):214–24.
- Dai CF, Xiao CG. Electrophysiological monitoring and identification of neural roots during somatic-autonomic reflex pathway procedure for neurogenic bladder. *Chin J Traumatol* 2005;8(2):74–6.
- Arslan M, Comert A, Acar HI, Ozdemir M, Elhan A, Tekdemir I, *et al.* Lumbosacral intrathecal nerve roots: an anatomical study. *Acta Neurochir (Wien)* 2011;153(7):1435–42.
- Wall EJ, Cohen MS, Abitbol JJ, Garfin SR. Organization of intrathecal nerve roots at the level of the conus medullaris. *J Bone Joint Surg Am* 1990;72(10):1495–9.
- Schalow G. The problem of cauda equina nerve root identification. *Zentralbl Neurochir* 1985;46(4):322–30.
- Sui T, Ge Y, Liu W, Zhao ZK, Zhang N, Cao X. An acetylcholinesterase antibody-based quartz crystal microbalance for the rapid identification of spinal ventral and dorsal roots. *PLoS One* 2013;8(7):e69049.

- 35 Chen L GY, Li DC. An experimental study of the least nerve roots of the brachial plexus for maintaining the normal limb function. *Chin J Hand Surg* 1998;14(4):234–8.
- 36 Kalantarian B, Rice DC, Tiangco DA, Terzis JK. Gains and losses of the XII-VII component of the "baby-sitter" procedure: a morphometric analysis. *J Reconstr Microsurg* 1998;14(7):459–71.
- 37 Wei L. An experimental study on the functional orientation and micro-structure of the C7 nerve root and its branches [dissertation]. Shanghai: Fudan University; 2003.