Research article

The diameters and number of nerve fibers in spinal nerve roots

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Objective: To investigate the anatomical and histological features of spinal nerve roots and provide base data for neuroanastomosis therapy for paraplegia.

Methods: Spinal nerve roots from C1 to S5 were exposed on six adult cadavers. The diameter and the number of nerve fibers of each nerve root were measured, respectively, with a caliper and image analysis software.

Results: As for ventral roots, the diameter of C5 (2.50 ± 0.55 mm) was the largest in cervical segments. In thoracic and lumbosacral segments, the diameter gradually increased from T11 to S1 and then decreased from S1 to S5 except L3. S1 (1.43 ± 0.16 mm) was the thickest root and S5 (0.14 ± 0.02 mm) was the thinnest one. As for dorsal roots, the diameter of C7 (4.61 ± 0.87 mm) was the largest in cervical segments. From T11 to S1, the diameter increased and then decreased gradually from S1 to S5. The diameter of dorsal roots from T1 to S5 was largest at S1 (2.95 ± 0.57 mm) and smallest at S5 (0.27 ± 0.13 mm), respectively. C7 (8467 ± 1019), T12 (6538 ± 892), L3 (9169 ± 1160), and S1 (8253 ± 1419) ventral roots contained the most nerve fibers in cervical, thoracic, lumbar, and sacral segments, respectively. Similarly, C7 (39 653 ± 8458), T1 (26 507 ± 7617), L5 (34 455 ± 2740), and S1 (41 543 ± 3036) dorsal roots, respectively, contained the most nerve fibers in their corresponding segments.

Conclusion: The findings in the current study provided the imperative data and may be valuable for spinal nerve root microanastomosis surgery in the paraplegic patients.

Keywords: Anastomosis, Nerve transfer, Paraplegia, Spinal cord injuries, Spinal nerve roots

Introduction

Paraplegia caused by spinal cord injury (SCI) is a common disease in neurosurgery; nevertheless, the treatment of paraplegia remains difficult at present. According to a recent epidemiological study, the estimate of annual incidence in traumatic SCI varied from 12.1 to 57.8 cases per million worldwide.[1] Furthermore, paraplegia was found to be more common than tetraplegia (paraplegia: 58.7%; tetraplegia: 40.6%).[2] Persons with paraplegia usually experience a loss of motor and sensory function in lower extremities and excretion functions causing serious complications such as bedsores and urinary tract infection.

There is no effective method to completely recover the impaired nerve function at present. In recent decades, some scholars have partially reconstructed variable nerve function under the level of SCI by different methods of neuroanastomosis, which provides a new approach for the treatment of paraplegia.[3–6] In order to improve the therapeutic effect, the anatomical and histological features of spinal nerve roots have important clinical significance. In this study, we investigate the microstructure, diameter, and nerve fiber quantity of spinal nerve roots from C1 to S5 to provide fundamental data for spinal nerve root microanastomosis treatment.

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Methods

Specimen preparation

Spinal cord dissections were performed on 6 (12 sides) human formalin-fixed cadavers donated to the Department of Anatomy in Nanjing Medical University. Two donors were female and four were male, with an average age of 43 years at death. The cadavers were placed in the prone position. Following the removal of all

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muscles, ligaments, and vertebral laminae, the spinal cord was exposed by a longitudinal midline incision in the posterior dura and arachnoid membrane. Then, all the spinal nerve roots were identified from distal (dural sleeve) to proximal (origin at the spinal cord) in C1–S5 spinal cord segments. At each spinal cord segment, we measured the diameters of ventral and dorsal nerve roots by a caliper with a precision of 0.02 mm. Finally, short segments of these nerve roots were sectioned 3 cm adjacent to the dural sac and then placed in phosphate buffer solution in preparation for immunohistochemical study. Both nerve fibers and diameter were measured at 1 cm adjacent to nerve root outlet inside the spinal dura mater.

Immunohistochemistry

After routine dehydration, clarification, paraffin embedding, and sectioning (5 μm, cross section), all cross sections were stained in the laboratory. Neurofilament 200 (NF200), a specific marker of neurofilament, was used to determine the number of nerve fibers. Immunohistochemical expression of NF200 in spinal nerve roots was examined as follows: paraffin-embedded cross sections were dewaxed, rehydrated, and immersed in phosphate buffered saline for 5 minutes. After antigen retrieval by microwave, the cross sections were blocked for 20 minutes in normal goat serum, and then incubated over-night with the primary antibody against NF200 (Abcam, Cambridge, MA, USA) at 4°C. After washing, the cross sections were further incubated with secondary antibody (KIT5010, MaxVision™, Fuzhou, Fujian Province, China) for 15 minutes at room temperature, and then the reactivity was visualized with 3,3′-diaminobenzidine tetrahydrochloride hydrate (Boster, Wuhan, China). Finally, all slices were mounted for light microscopic observation. In addition, the histological structure of nerve roots was observed by HE staining in some cross sections.

Photography and measurement

All samples were photographed using a light microscope (Olympus BX50, Tokyo, Japan) connected with a CCD camera (Olympus DP70, Tokyo, Japan) which has a high resolution of 4080×3072 pixels. Then, systematic nerve fiber counts of stained cross sections were performed by the Image Pro Plus 6.0 image analysis software.

Data analysis

All data were collected and inputted into Excel 2007 (Microsoft, Seattle, WA, USA) to save for further statistical analysis conducted with SPSS 13.0 software (SPSS, Chicago, IL, USA). Statistical analysis was used to determine the average, standard deviation (SD), and differences between the left and right sides. Linear correlation analysis was used to calculate the relationship between the diameter and the number of nerve fibers of spinal nerve roots. A P value of <0.05 was considered to be statistically significant.

Results

Histological microstructure of spinal nerve roots

Spinal nerves are composed of ventral and dorsal roots in their corresponding spinal cord segments. Typically, a number of rootlets originate from the corresponding level of spinal cord, and then they are aggregated gradually to form nerve sub-bundles, bundles, and finally a nerve root.[7] As shown in Fig. 1 (Parts A, B, and C), the ventral roots adjacent to the dura usually consisted of only one nerve bundle while most dorsal roots normally comprised several nerve bundles. With varying sizes and shapes, the nerve bundles were loosely organized by small amounts of connective tissues. Each nerve bundle was wrapped by a membrane tissue with a mean thickness of 18.92 μm. Within the bundle, blood vessels were enclosed in its connective tissues.

As shown in Fig. 1 (Part D), the immunohistochemistry showed positive staining (brown granules) in the axons, but negative in the myelin sheaths of spinal nerve root. Thus, the nerve fiber counts were available by the immunohistochemical staining.

Diameters of spinal nerve roots

As shown in Table 1 and Fig. 2, different segments of spinal nerve roots had various sizes. The diameter of dorsal root was always larger than the corresponding ventral root, the diameter ratio being about 1.961.

As for ventral roots, the diameter of C5 (2.50 ± 0.55 mm) was the largest in cervical segments. In thoracic and lumbosacral segments, the diameter gradually increased from T11 to S1 and then decreased from S1 to S5 except L3. S1 (1.43 ± 0.16 mm) was the thickest root and S5 (0.14 ± 0.02 mm) was the thinnest one. Different segments of the dorsal roots also had a similar trend. As for dorsal roots, the diameter of cervical nerve roots was largest at C7 (4.61 ± 0.87 mm). From T11 to S1, the diameter increased and then decreased gradually from S1 to S5. The diameter of dorsal roots from T1 to S5 was largest at S1 (2.95 ± 0.57 mm) and smallest at S5 (0.27 ± 0.13 mm), respectively.

No meaningful differences between the left and right sides were observed ($P > 0.05$).

Nerve fiber counts

As shown in Table 1 and Fig. 3, C7 (8467 ± 1019), T12 (6538 ± 892), L3 (9169 ± 1160), and S1 (8253 ± 1419) ventral roots contained the most nerve fibers in cervical, thoracic, lumbar, and sacral segments, respectively. S5

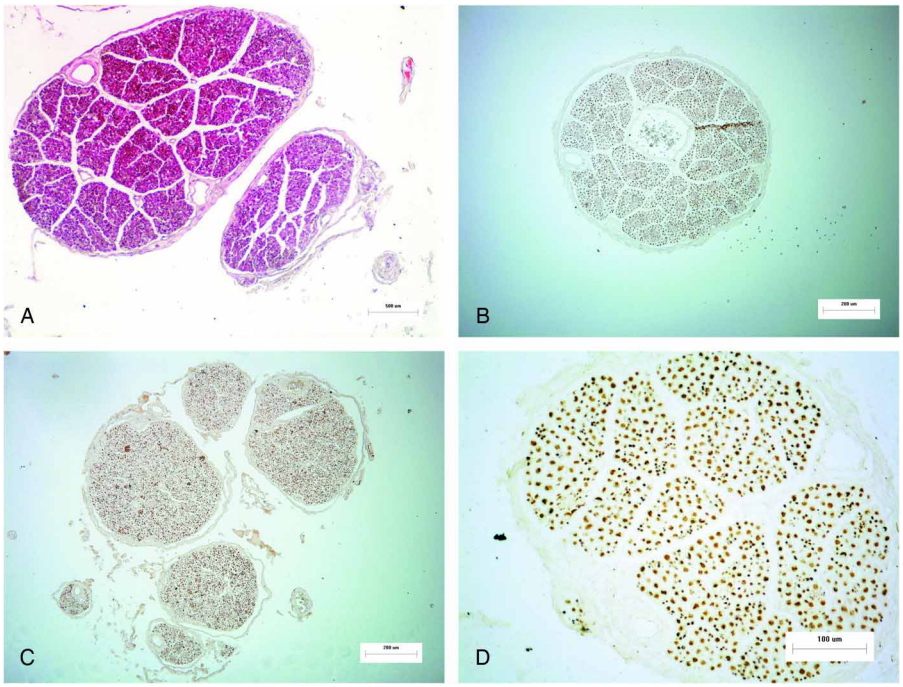

Figure 1 Overviews of ventral and dorsal roots. A: Cross-section of C3 dorsal root (HE stain; Magnification 100×); B: Cross-section of T12 ventral root (NF200 stain; Magnification 100×); C: Cross-section of T2 dorsal root (NF200 stain; Magnification 100×); D: Cross-section of C2 ventral root (NF200 stain; Magnification 200×).

Table 1 The diameters and number of nerve fibers in spinal nerve roots

	Diameter (mm)		Number of nerve fibers	
Segment	Ventral root	Dorsal root	Ventral root	Dorsal root
C1	0.97 ± 0.16	1.21 ± 0.28	2751 ± 639	6430 ± 1606
C2	1.34 ± 0.30	2.61 ± 0.51	3116 ± 724	11 947 ± 2977
C3	0.80 ± 0.23	2.87 ± 0.52	2460 ± 471	21 876 ± 1916
C4	1.39 ± 0.24	2.49 ± 0.34	3833 ± 408	10 647 ± 887
C5	2.50 ± 0.55	3.43 ± 0.77	7841 ± 1020	23 300 ± 2856
C6	2.23 ± 0.73	3.99 ± 0.75	7048 ± 1157	36 353 ± 7451
C7	2.22 ± 0.50	4.61 ± 0.87	8467 ± 1019	39 653 ± 8458
C8	1.71 ± 0.60	3.92 ± 0.62	5883 ± 1000	31 156 ± 8273
T1	1.03 ± 0.23	2.18 ± 0.31	5788 ± 1186	26 507 ± 7617
T2	0.75 ± 0.11	1.30 ± 0.14	3576 ± 398	10 234 ± 1728
T3	0.78 ± 0.10	1.35 ± 0.16	5499 ± 1126	14 888 ± 2514
T4	0.77 ± 0.17	1.13 ± 0.13	5485 ± 973	10 849 ± 1832
T5	0.64 ± 0.08	1.21 ± 0.30	5326 ± 1314	8355 ± 1390
T6	0.71 ± 0.18	1.07 ± 0.16	3666 ± 1407	10 015 ± 1666
T7	0.78 ± 0.15	1.25 ± 0.27	4297 ± 1130	9123 ± 1178
T8	0.83 ± 0.25	1.29 ± 0.18	3643 ± 1340	7619 ± 903
T9	0.81 ± 0.15	1.33 ± 0.25	5209 ± 704	8369 ± 967
T10	0.72 ± 0.08	1.27 ± 0.15	5269 ± 963	11 329 ± 2724
T11	0.69 ± 0.08	1.26 ± 0.16	4870 ± 895	9713 ± 1824
T12	0.76 ± 0.14	1.45 ± 0.19	6538 ± 892	10 420 ± 802
L1	0.81 ± 0.07	1.55 ± 0.28	5384 ± 833	16 820 ± 3456
L2	0.96 ± 0.16	1.93 ± 0.27	7374 ± 720	18 615 ± ±3284
L3	1.19 ± 0.07	2.24 ± 0.30	9169 ± 1160	26 191 ± 2772
L4	1.04 ± 0.07	2.48 ± 0.38	7878 ± 1386	31 175 ± 2686
L5	1.37 ± 0.16	2.66 ± 0.40	8657 ± 1396	34 455 ± 2740
S1	1.43 ± 0.16	2.95 ± 0.57	8253 ± 1419	41 543 ± 3036
S2	0.93 ± 0.11	2.02 ± 0.53	4766 ± 1035	18 642 ± 1716
S3	0.55 ± 0.07	1.32 ± 0.60	2233 ± 299	11 971 ± 964
S4	0.34 ± 0.03	0.52 ± 0.17	1356 ± 193	3402 ± 304
S5	0.14 ± 0.02	0.27 ± 0.13	906 ± 111	2206 ± 197

Values represent mean ± SD.

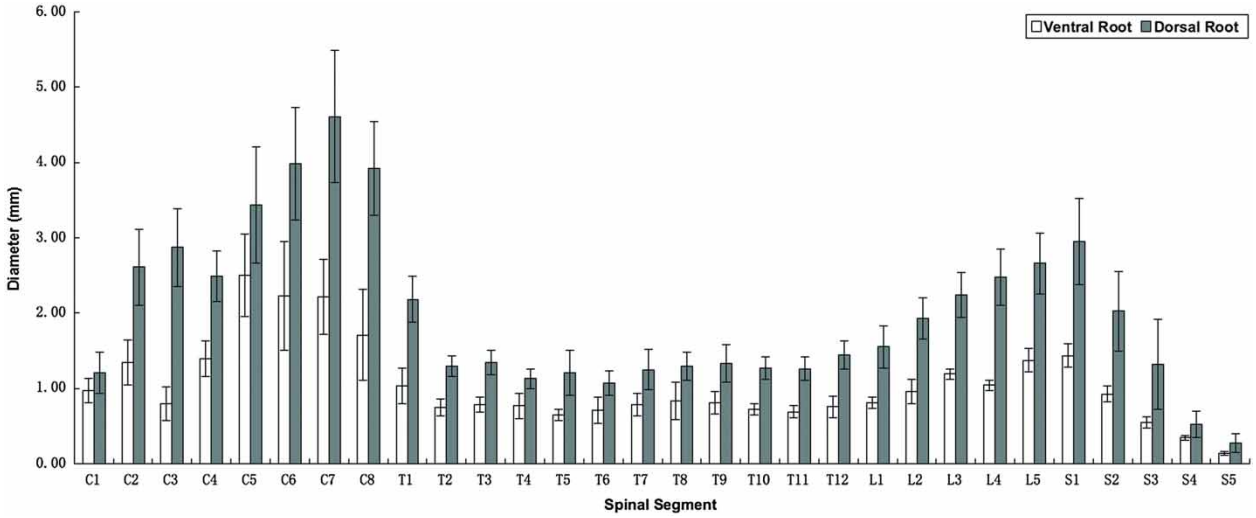

Figure 2 Diameters of spinal nerve roots.

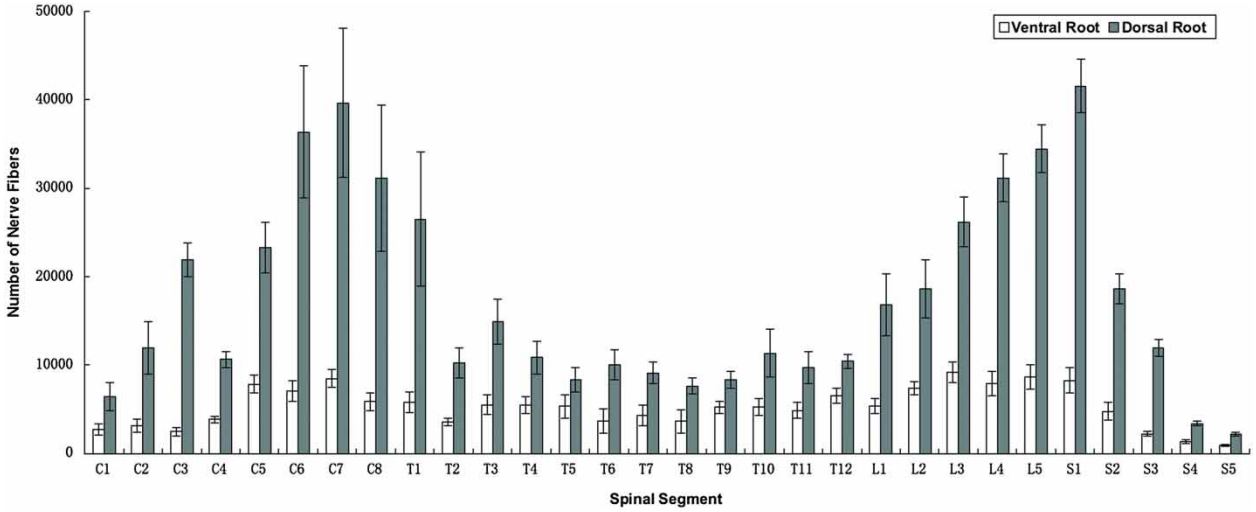

Figure 3 The number of nerve fibers in spinal nerve roots.

(906 ± 111) ventral root contained the least nerve fibers in sacral segments. As for dorsal roots, similarly, C7 (39 653 ± 8458) also contained the most nerve fibers among the cervical nerve roots. T1 (26 507 ± 7617), L5 (34 455 ± 2740), and S1 (41 543 ± 3036) contained the most nerve fibers in thoracic, lumbar, and sacral segments, respectively. From T1 to S5, S5 (2206 ± 197) contained the least nerve fibers.

In addition, compared with the corresponding ventral roots, the dorsal roots always contained more nerve fibers. There were no significant differences between right and left sides ($P > 0.05$).

Relationship between the diameter and number of nerve fibers

The results of Spearman's rank correlation analysis indicated a significantly positive correlation between the number of nerve fibers and diameters of roots ($r = 0.573$, $P < 0.01$ for ventral roots; $r = 0.803$, $P < 0.01$ for dorsal roots).

Discussion

Treatment of paraplegia caused by SCI is always a difficult challenge. With the unceasing study of the regeneration capacity of the spinal nerve root axon,[8–12] spinal nerve root microanastomosis has been explored to recover the impaired nerve function in paraplegia patients. Furthermore, some researchers have partially restored muscle and pelvic organs function in paraplegia patients by various surgical procedures,[13–15] providing a new therapeutic approach for paraplegia. However, the optimum choice of donor nerve for anastomosis remains uncertain because of the lack of anatomical and histological data of related spinal nerve roots. So,

we conducted the anatomical observations, measurements, and statistical analysis of six adult spine specimens.

Nerve fiber counts

Previous studies by Arnell,[16] Davenport and Bothe,[17] Ingbert,[18] Schalow,[19] and Hauck *et al.*[20] have provided some information regarding the number of nerve fibers in only a few spinal segments using silver or osmium staining. In our study, the detailed data regarding the number of nerve fibers of both the ventral and dorsal root in entire C1–S5 segments were investigated. Moreover, as a specific marker of neurofilament, NF200 staining used in our study is also a relatively current method for the staining of nerve tissue. With a better staining of small diameter axon as shown in Fig. 1 (Part D), this method ensured an accurate and convenient quantitative analysis of nerve fibers.

Clinical significance of our research on spinal nerve roots

The curative effect of nerve transfer procedure greatly depends on the choice of the donor nerve. The following key factors should be considered: the nerve diameter, the number of nerve fibers, relatively secondary function, and the level of SCI.

Nerve diameter

Variable diameters of nerve roots may cause difficulties for anastomosis between them and affect nerve function recovery, so it is necessary to choose a donor nerve with similar diameter of receptor nerve. The measurements of diameters shown in Table 1 could provide the correlative data for surgeons.

Worthy of note, as we mentioned in Fig. 1, the dorsal roots adjacent to the dural sac usually contained several nerve bundles loosely organized by little connective tissues, so we could easily separate or combine these nerve bundles to anastomose the nerve roots with different diameters.

In addition, owing to the limited operative visual field and the dense arrangement in the narrow subdural space, especially in lumbosacral segments, it is difficult to identify the spinal nerve roots. The detailed measurements shown in Table 1 could provide some morphological bases for the identification during neuroanastomosis surgery. Our results in all cadavers indicate that, both S1 ventral root (1.43 ± 0.16 mm) and S1 dorsal root (2.95 ± 0.57 mm) were the thickest in lumbosacral level, which could be a reliable landmark for the location of other nerve roots. While in cervical segments, C5 ventral root (2.50 ± 0.55 mm) and C7 dorsal root (4.61 ± 0.87 mm) could also be anatomical landmarks.

Number of nerve fibers

The number of donor nerve fibers is one of the key factors determining the efficacy of nerve transfer.[21] However, clinical and animal experiments showed that it is not required to restore all the original nerve fibers of receptor nerve to recover the normal function of the target muscle or organ in neuroanastomosis.[22] That is to say, in nerve transfer, just a certain proportion of nerve fibers could restore the impaired function of the muscle or organ instead of 100% of the receptor nerve fibers. Further studies conducted by Kalantarian *et al.*[23] showed that the smallest proportion of nerve fibers to maintain the target muscle function is about 40%. By animal experiments, Wei[24] found that 37% may be the smallest proportion of nerve fibers to maintain the basic function of muscle. Based on the above-mentioned facts, it can be concluded that the donor nerve fibers must outnumber at least about 40% of the receptor nerve fibers. As we mentioned above, C7, T12, L3, and S1 ventral roots relatively consisted of the most motor fibers in their corresponding segments. While C7, T1, L5, and S1 dorsal roots, respectively, contained the most sensory fibers. These spinal roots should be given prior consideration in the neuroanastomosis as donor nerves.

On the other hand, the transfer of donor nerve normally causes the loss of motor and sensory function in original regions innervated by it, which should be minimized as far as possible. Especially in the dorsal roots, selective nerve bundle anastomosis instead of entire nerve root transfer could also be an option to preserve a part of donor nerve.

In a word, understanding about the number of nerve fibers will contribute to the optimal selection of donor nerve.

Linear relationship between the diameter and the number of nerve fibers

The significantly positive correlation between the diameter of nerve roots and the number of nerve fibers indicates that a thicker root normally contains more nerve fibers, and vice versa. Knowledge of this anatomical relationship may be helpful to roughly estimate the number of nerve root fibers during neuroanastomosis surgery.

Due to the different methods of neuroanastomosis, the selection of donor nerve for each specific spinal segment was not explained in detail in this paper. In clinical practice, similar diameter with the receptor

nerve, sufficient nerve fibers (at least 40% of the receptor nerve fibers), and relatively subordinate nerve function (such as T7–T12 innervating abdominal muscles and skins) should be satisfied simultaneously in the selection of donor nerve. It is noteworthy that this paper mainly described the selection principle of donor nerve from the diameter and the number of nerve fibers perspectives. Some other factors such as the level of SCI and the distance between donor and receptor nerve should also be considered in practical application.

Conclusion

In this study, we investigated in detail the anatomical and histological information of intradural spinal nerve roots from C1 to S5. The data regarding the diameter of nerve roots and the number of nerve fibers were obtained. Correlation analysis indicated a significantly positive correlation between them. All these findings in our study may be valuable for spinal nerve root microanastomosis surgery in paraplegic patients.

Disclaimer statements

Contributors There are no other contributors in the study.

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Conflicts of interest None.

Ethics approval Ethical approval was not required in our study.

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