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Original Article

Experimental study of the functional reserve of median nerve in rats

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Abstract: Objective: To study the functional changes of median nerve after removing a certain bundle of it and to explore the functional reserve of median nerves. Methods: 220 three-month old SD rats were randomly divided into experimental groups and sham groups. And the experimental group was further divided as 1/8 group, 1/4 group, 1/3 group, 1/2 group, and 2/3 group according to ratio of the resection portion, with 22 rats in each group. The section of the lowest level on median nerve trunks were exposed, and a certain portion of it were separated and resected in experimental group, while in sham groups, the nerve was only separated without resection. The general state of health of all rats were observed, and the α motor neurons in cornu anterior medullae spinalis were studied 1 week, 2 weeks and 2 months postoperatively. Neuro-electrophysiology and function of dominated muscles were studied 2 weeks, 2 months, 3 months, and 4 months postoperatively. Results: All rats survived without infection and obvious ulcer. The number of the α motor neurons in cornuanterior medullae spinalis didn't change (P>0.05), and obvious superstructure changes were observed in early stage in 1/2 and 2/3 group, but restored after 2 months. There was no significant changes in latencies of motor neuron evoked potentials between experimental groups and sham group (P>0.05), however, there is significant difference if the 2 week group was compared with 2 month, 3 month and 4 month group (P<0.05). Moreover, there is also significant difference in terms of the wave amplitude of evoked potential of motor neurons, the maximum wave amplitude and the persistence time of its innervated muscle if the 2 week group was compared with those in 2 month, 3 month and 4 month group (P<0.05), and there is significant difference between different proportion resection groups (P<0.05). Conclusions: Median nerve has a certain amount of functional reserve, and the quantity of the functional reserve of median nerve without compromise is the 1/3 of the whole trunk.

Keywords: Peripheral nerve injury, nerve tracing, nerve transposition, nerve functional reserve

Introduction

Brachial plexus nerve root avulsion was mainly treated with neuro-neural intraplexal transposition [1]. Since Oberlin et al reported for the first time that partial bundle branch of ulnar nerve was transferred to the musculocutaneous nerve to surgically restore the flexion function of elbow in 1994 [2], partial nerve transposition surgery has been acclaimed for its inherent advantages. In 1996, Chang et al. [3] extended the surgery to the median nerve and also achieved good results. Since then, many scholars in China and abroad continued to perform this type of surgery, and achieved good therapeutic effects [4-10]. But the significant drawback of partial nerve transposition surgery is the limited sources of the nerves. This study was designed to investigate the amount of median nerve functional reserve and to provide reliable evidence for using more nerve sources.

Materials and methods

Animals and main instruments used

Total 220 three-month-old SD rats were used in the study, including 110 females and 110 males weighing 300-350 g (Jilin University, Bethune Medicine Laboratory Animal Center). Miniature glass needle with tip outer diameter 30 μm (Corning Co. USA); BL-420E Four Channel Biological and Functional Experimental System (Thai Union Technology Co., China); ELITE ESP Flow Cytometer (Beckman-Coulter Inc., USA); JEM-1200E Transmission Electron Microscopy (Japan Electronics Corporation, Japan). All ani-

mal experiments were approved by Institutional Animal Care and Use Committee.

Grouping of animals

The 220 SD rats were randomly divided into experimental and control groups with 110 (55 females and 55 males) mice in each group. The experimental group was further divided into 5 groups (with 22 in each group) according to the proportion of nerve cut, namely 1/8 (which means 1/8 of the nerve was cut off), 1/4 group, 1/3 group, 1/2 group and 2/3 group. Rats were anesthetized with ketamine (200 mg/kg) intraperitoneal injection. Longitudinal incision was performed medially on both upper limbs, bilateral upper 1/2 segment of the median nerve was exposed under sterile condition and according to the literature [11], the lowest cut plane was measured and determined (80% of the upper arm). Under a 10× operating microscope, the nerve bundle was separated and cut at a certain percentage with a fine glass needle after cross positioning in the experimental group of mice. In the control group, two fine glass needles were inserted in the same region to isolate and expose 1.5 mm of the never bundle without dissection.

Experimental design

General conditions: In postoperative survival rats, the following conditions will be observed and recorded: anypost-incision infections in skin, any skin ulcers, any swelling, deformity and lameness in the affected limb.

Changes of the α motor neurons in cornu anterior medullae spinalis

The changes of number and ultra-structure of the α motor neurons in cornu anterior medullae spinalis were observed at 1 week, 2 weeks and two months after surgery in both experimental and control groups of rats. Briefly, after thoracotomy, the rats were cannulated from the left ventricle into the ascending aorta. The spinal cord was cut at C7 level, observed under light microscopy, then dehydrated, xylene and paraffin-embedded, and finally serial sections were made cross-sectionally, with thickness of 5 μm for each slice. One slice from every 20 sections was taken for HE staining. The spinal anterior horn from each side was observed under 10× optical microscope, the total number of α motor neurons on five slices was counted and averaged. Criteria for defining a α motor neuron: diameter greater than 20 μm, obvious Nissl's body, anterior horn cells with clear nucleolus. For TEM study, the rats were irrigated with 4% paraformaldehyde in PBS, the spinal cord was cut at C7 level, the anterior horn of the spinal cord tissue was trimmed to pieces with size of 1 mm^3 under the microscope, fixed in 2.5% glutaraldehyde and 1% osmium dual acid fixative solution, placed in 4°C refrigerator overnight. After dehydration, specimens were placed intoa solution mixed with same amount of acetone and embedding agent for 2 h, embedded with Epons 618, ultrathin sections were cut and double stained with uranyl acetate and lead citrate, then observed under transmission electron microscope. Fives anterior horn slices were randomly selected for each specimen and one α motor neuron was chosen to observe the changes of number and morphology of mitochondria, lysosomes, Nissl's body under electron microscopy cytoplasm.

Electrophysiological study

Electrophysiological studies were performed on five rats from each of the experimental and control groups at 2 weeks, 2, 3 and 4 months after surgery. Briefly, one channel of the BL-420E four-channel biological and functional experimental system was turned on, the latency and amplitude of the induced potential of the motor neuron were observed (Pulse stimulation: super current and voltage 5.0 V, 1 ms pulse width single square wave).

Functional test of the dominating muscle

Another channel of the BL-420E four-channel biological and functional experimental system was turned on, the dominating muscle was attached to the tension transducer, and the amplitude (mV) and duration(s) of maximum tetanic tension during isometric muscle contraction (pre-load is set to 5 mg) were measured.

Statistical analysis

Statistical analysis was performed using SPSS 10.0 software. Data were expressed as mean ± standard deviation. ANOVA was used to compare differences between the two groups, and F test was used for pairwise comparison. *P*

Table 1. The number of spinal cord α motor neurons in the anterior horn in various experimental groups (n=10, %, $\bar{X}\pm S$)

Time	1 week	2 weeks	2 months	Average
1/8 cut group	98.34±4.36	98.55±8.27	99.50±4.14	98.23±6.47
1/4 cut group	97.54±2.23	97.69±6.47	98.48±6.15	97.74±5.46
1/3 cut group	98.48±4.53	98.83±3.37	96.87±7.42	97.70±4.7
1/2 cut group	96.27±9.36	95.98±8.78	96.80±6.72	96.33±8.82
2/3 cut group	94.85±10.47	94.91±14.05	95.76±9.65	95.03±10.94

Note: "n" represents the number of specimens at a given time point in a certain experiment group.

value of <0.05 was considered statistically significant.

Results

Results of observation of general situation

All rats survived until after completion of the experiment, no incision infection, no significant limb ulceration was recorded. In 1/8, 1/4, and 1/3 cut groups, the swelling, deformity, lameness of the limb were restored two weeks after the surgery. In 1/2 and 2/3 cut group, the limb deformity and lameness were still visible two months after the surgery, alleviated and disappeared 3-4 months after the surgery. In control group, there were slight swelling and limp one week after the surgery, and back to normal two weeks after surgery.

Changes of α motor neurons in the anterior horn

Changes of the numbers of α motor neurons in the anterior horn: There is no significant difference in the numbers of α motor neurons in the anterior horn between experiment group and control group at either time point (P>0.05), **Table 1**.

Changes of the superstructure of α motor neurons in the anterior horn: As shown in **Figure 1**, in control group, one week after sham surgery, there was tiny mitochondria swelling, cristae became shallow, Nissl's body was scattered in the cytoplasm, and the number of cytosol lysosomes was normal. One week after surgery in 1/8, 1/2, and 2/3 cut group, there was mild swelling of mitochondria, flattening of the mitochondrial cristae, more sparsely distribution of cytoplasm Nissl's bodies, and no significant reduction in the number of lysosomes. Two weeks after the surgery, mitochondrial swelling subsided significantly with slight increase in the size of mitochondria, the number of Nissl's body was slightly denser than the control group with a wider distribution, and no obvious lysosomal abnormalities. All organelles were restored to normal in two months. The changes in 1/2 and 2/3 cut group were more significant than those in control group at one week after surgery, especially in 2/3 cut group, the difference was obvious. Two weeks after surgery, there was still obvious mitochondrial swelling, with increase in size and decrease in numbers, reduction in the number of Nissl's body, reduced in the range of distribution in cytoplasm, and reduction in the number and volume of lysosomes. Two months after the surgery, mitochondria, Nissl bodies and lysosomes were back to normal with increased lipofuscin content.

Functional test of the dominating muscle

Changes of muscle contractibility shown with electrophysiological study: As shown in **Figure 2**, from 1/8, 1/4, 1/3, 1/2 to 2/3, with the increase in the proportion of nerve bundles cut, the maximum contractibility of the muscle it dominated gradually declined. When the cut ratio is greater than one-third, the maximum tonic contraction declined sharply.

Changes of amplitude of maximum tetanic tension during isometric muscle contraction of Triceps: As shown in **Table 2**, two week after surgery, the amplitudes of maximum tetanic tension during isometric muscle contraction of Triceps in each experimental group were significantly decreased compared with those in control group (P<0.05 or P<0.01). While the impaired contraction of flexor carpi radialis were back to normal in 1/8 cut and 1/4 cut groups at two months after surgery, it stayed impaired in 1/3 cut, 1/2 cut and 2/3 cut groups even at four months after surgery as compared with those in control group. However, within each experimental group, compared with the flexor carpi radialis contraction amplitudes at two weeks after surgery, those at two months, three months and four months after surgery showed significant improvement (P<0.05 or P<0.01), though not back to normal yet.

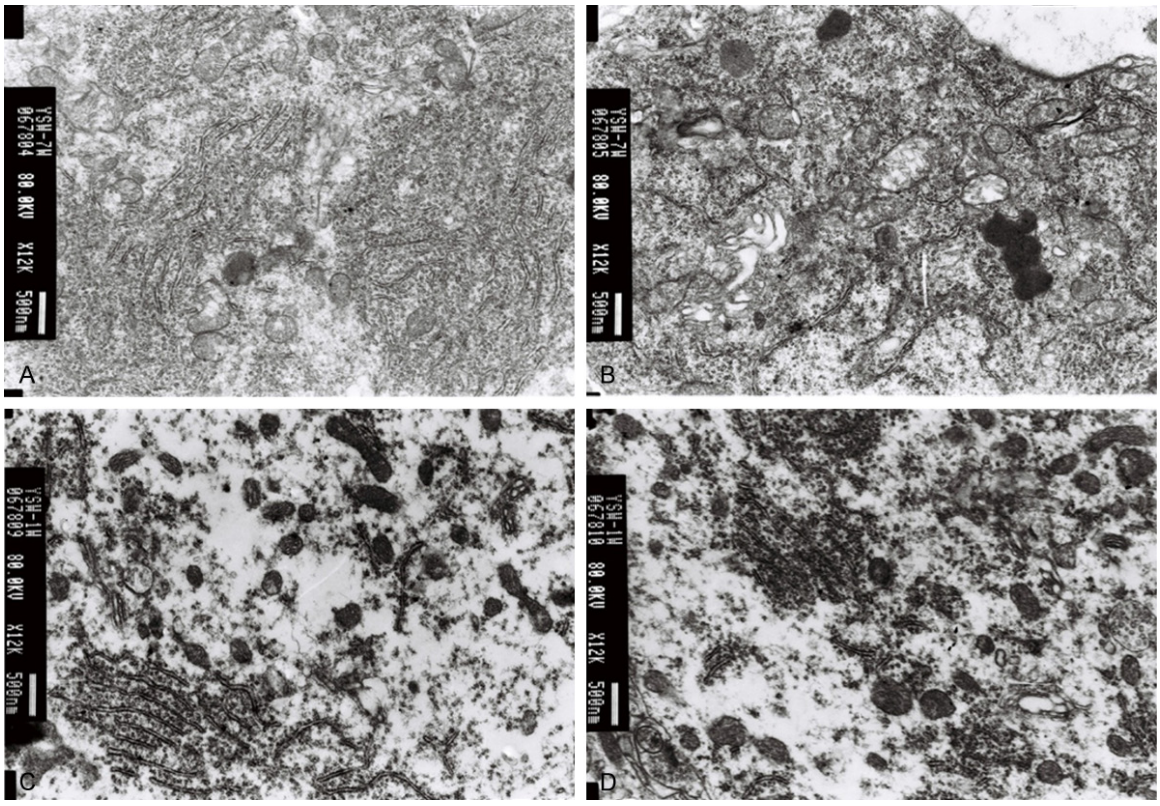

Figure 1. Observation of the super-structural changes of α motor neurons in cornu anterius medullae spinalis one week after surgery (TEM ×12000). A. Control group. B. 1/8 cut group. C. 1/2 cut group. D. 2/3 cut group. Images are representatives of experiments repeated at least three times.

Changes of duration of maximum tetanic tension during isometric muscle contraction of flexor carpi radialis

As shown in **Table 3**, two week after surgery, the durations of maximum tetanic tension during isometric muscle contraction of flexor carpi radialis in each experimental group were significantly decreased compared with those in control group ($P<0.05$ or $P<0.01$). Except those of 1/2 cut and 2/3 cut groups at two months after surgery, the durations of maximum tetanic tension during isometric muscle contraction of flexor carpi radialis of all the rest groups were back to normal at each time point. Consistently, within each experimental group, compared with the flexor carpi radialis contraction durations at two weeks after surgery, those at two months, three months and four months after surgery all showed significant improvement ($P<0.05$ or $P<0.01$), and most of them were back to normal as compared with control group.

Discussions

In this study, based on changes of the distribution, number and ultrastructure of the anterior horn motor neurons, we concluded that the partial damage of neural stem had some influences on them [12-13]. However, this effect is reversible: the recovery is good with time, not causing widespread death of the anterior horn motor neurons. Possible reasons are: first, the neural stem is the peripheral axon of the neuron and its partial damage has little effect on neuron itself; second, there is connection between nerve branches above and below the damage level to ensure an adequate supply of neurotrophic factors; third, partial nerve damage caused only neurons in a segment of the spinal cord to lose contact with each other, however, its neighboring neurons and glial cells are healthy and fully capable to ensure its survival through synaptic contacts. This is consistent with the findings of previous studies about

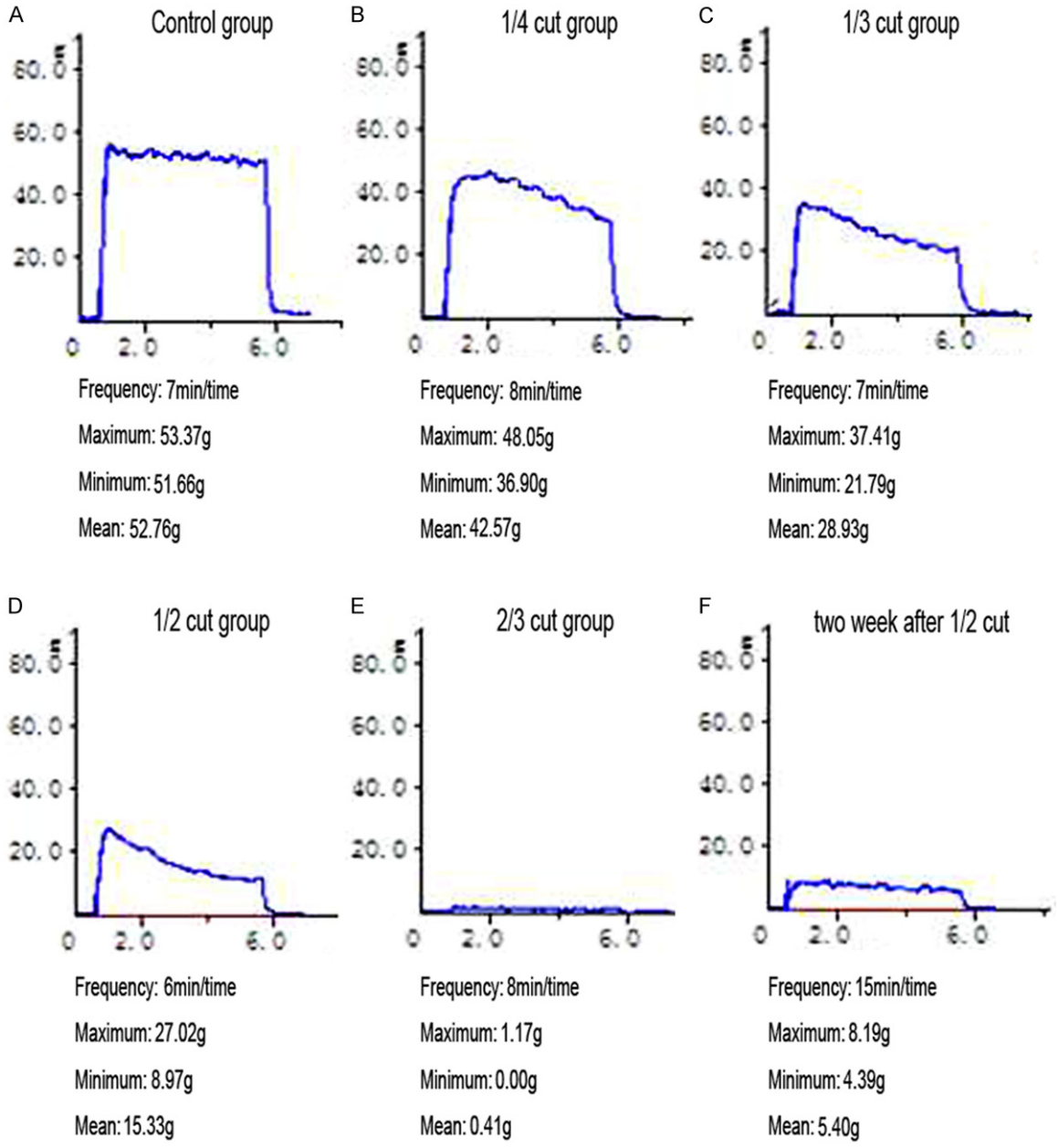

Figure 2. Representative electrophysiological pictures from each group. A. Control group. B. 1/4 cut group; C. 1/3 cut group; D. 1/2 cut group; E. 2/3 cut group; F. Two week after 1/2 cut. Images are representatives of experiments repeated at least three times.

the effect of peripheral nerve damage on the central nervous system [5].

The amplitude of maximum tetanic tension during isometric muscle contraction represents muscle contractility, ie, the ability of muscle to perform work externally. This study shows that, with the increase of proportion of the cut of neural stem, the amplitude of maximum tetanictension contraction of muscle reduces, but is not proportional to the ratio of the cut. From the point of view of the impact of the ratio of cut on the ability of muscle contraction, the muscle contraction ability in 1/8 cut and 1/4 cut group recovered completely within the two months after surgery. When ratio of cut increased to 1/3, it took longer time to recover, it reached certain degree of recovery two months after surgery, but not completely recovered even three months after the surgery, and there is no significant further recovery after three months, where the amplitude of maximum tetanic ten-

Table 2. Changes of amplitude (g) of maximum tetanic tension during isometric muscle contraction of flexor carpi radialis ($\bar{X}$±S) (n=10)

Time	2 w	2 m	3 m	4 m
1/8 cut group	29.81±3.64+	33.99±5.25	34.10±3.68	34.45±1.75
1/4 cut group	26.46±2.87*	33.18±5.77	34.60±4.32	34.64±2.63
1/3 cut group	21.44±5.73*	23.79±5.56*	25.06±5.40*	31.14±6.46+
1/2 cut group	8.97±1.26*	22.94±6.04*,-	25.03±6.31*	25.95±5.87*
2/3 cut group	1.56±1.85*	11.75±4.09*,-	12.15±3.33*	12.11±3.52*
F value	13.93	8.52	9.53	11.08
$F_{0.05}$=2.47	p<0.05	p<0.05	p<0.05	p<0.05
Control group	33.54±1.98	34.27±2.10	36.16±1.41	34.60±1.83
F value	181.94	64.33	55.68	39.57
$F_{0.05}$=3.94	p<0.05	p<0.05	p<0.05	p<0.05

Note: Compared control group: *p<0.01, +p<0.05 (n=10); Compared 2 w group: -P<0.01 (n=10).

Table 3. Changes of duration (s) of maximum tetanic tension during isometric muscle contraction of flexor carpi radialis ($\bar{X}$±S) (n=10)

Time	2 w	2 m	3 m	4 m
1/8 cut group	3.87±1.14*	4.86±1.30	4.98±1.03	4.95±0.19
1/4 cut group	1.84±1.09*	3.70±1.06*	4.79±1.27	4.80±0.71+
1/3 cut group	1.96±0.82*	4.31±1.47	4.68±2.04	4.79±1.63
1/2 cut group	0.3±0.75*	2.99±1.46*	4.54±1.84	4.85±0.96
2/3 cut group	0.68±0.26*	1.73±1.34*	4.42±2.01	4.56±1.49
F value	8.93	4.68	0.11	0.14
$F_{0.05}$=2.47	p<0.05	p<0.05	p>0.05	p>0.05
Control group	4.95±0.32	4.98±0.11	5.00±0.14	5.00±0.46
F value	312.51	43.49	1.6	1.44
$F_{0.05}$=3.94	p<0.05	p<0.05	p>0.05	p>0.05

Note: Compared control group: *p<0.01, +p<0.05 (n=10).

sion contraction recovered up to nearly 90% of that of the control group. As the proportion of cut increases, the loss of muscle contraction is more obvious. Cutting 1/2 and 2/3 of the neural stem caused a complete loss of muscle strength at two weeks after surgery, especially in the 2/3 cut group, there was no muscle contractile response at all; and it was difficult to recover also, the ultimate recovery extent was only 75% and 35% of that in the control group. In terms of the recovery time, the muscle contractility recovered the fastest in the first two months, and reached to the maximum degree of recovery at three months, after which, there was no significant change.

The duration of the maximum tetanic tension contraction of a muscle represents the endurance of that muscle. Cutting certain percentage of neural stem did affect the muscle contraction endurance, especially in the early stage of damage, the greater the proportion of cut, the shorter the maximum tetanic tension contraction duration. With the extension of time after injury, although there was a delayed recovery in the bigger cut ratio group, the durations of the maximum tetanic tension contraction in all groups were significantly increased, with most of them reaching normal level within three months after surgery. But in the recovery process after injury, the length of the durations of the maximum tetanic tension contraction did not completely reflect the extent of restoration of muscle function. Because, although the muscle in each group could perform maximum tetanic tension contraction, the maximum tetanic force was significantly reduced. Therefore, to assess the recovery of muscle function, both the amplitude and duration of the maximum tetanic tension during isometric contraction should be comprehensively evaluated, with the amplitude being a more sensitive indicator. This phenomenon has not been reported in previous studies.

The changes in histology of the neuron and the function indexes of its dominated muscle in the groups with damage less than 1/3 cut are far less than those with damages of 1/2 and 2/3 cut. This suggests that if the extent of nerve damage surpasses the compensation limit of the living organism, permanent neurological damage can be caused. In this sense, we can infer that the median nerve function reserve is 1/3 of the whole nerve. Our study expands the proportion of the nerve cut when performing partial bundle branch displacement [2, 3], which may help to develop a richer nerve resources for intraplexal transposition surgery.

Like other tissues and organs, there is a certain amount of functional reserve in peripheral nerve. This functional reserve, *per se*, is the result of the cross-mixing of a variety of functional nerve fibers at the proximity of peripheral nerve [14]. And it is this functional reserve that lays down the foundation for reasonable and effective clinical application of neural transposition to maximize the recovery of limb motor dysfunction. This network structure changed the one to one model of domination for nerve-muscle relationship, so that it reduces the amount of neural fibers needed for dominating the same amount of muscle through continuous distribution and integration in the proximal site as well as enabling the synergy between muscles, meanwhile, it also provides the opportunity and space for compensation after nerve injury. The significance of the study of peripheral nerve function reserve was that it enables us to discover, explore and apply the valuable resources within the peripheral nerve to achieve a perfect integration through surgical approach to redistribute the residual neuronal function after neurological damage. Nerve transposition [15] is a genius use of such attributes of nerves, and partial nerve transposition is a precise and scientific adjustment of the former: it not only saves the valuable neural resources, but also reduces the side injury.

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Disclosure of conflict of interest

None.

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References

[1] Narakas A, Herzberg G. Neuro-neural intraplexal transfers in traumatic radicular avulsions of the brachial plexus: Report on fifteen cases. Ann Chir Main 1985; 4: 211-218.

[2] Oberlin C, Béal D, Leechavengvongs S, Salon A, Dauge MC, Sarcy JJ. Nerve transfer to biceps muscle using a part of ulnar nerve for C5-C6 avulsion of the brachial plexus: Anatomical study and report of four cases. J Hand Surg 1994; 19: 232-237.

[3] Chang WS, Chu Y, Zhou L. Partial median and median nerve transfer for treatment of root avulsion of C5 and C6. J Hand Surg 1996; 12: 137-139.

[4] Al-Qattan MM. Obstetric brachial plexus palsy associated with breech delivery. Ann Plast Surg 2003; 51: 257-264.

[5] Li YC, Chang WS. The long-term efficacy of Median nerve and median nerve bundle branch transposition in restoration of elbow flexion. J Hand Surg 2001; 17: 22-24.

[6] Sungpet A, Suphachatwong C, Kawinwonggowit V, Patradul A. Transfer of a single fascicle from the ulnar nerve to the biceps muscle after avulsions of upper roots of the brachial plexus. J Hand Surg Br 2000; 25: 325-328.

[7] Sungpet A, Suphachatwong C, Kawinwonggowit V. One-fascicle median nerve transfer to biceps muscle in C5 and C6 root avulsions of brachial plexus injury. Microsurgery 2003; 23: 10-13.

[8] Tung TH, Mackinnon SE. Brachial plexus injuries. Clin Plast Surg 2003; 30: 269-87.

[9] Bertelli JA, Ghizoni MF. Contralateral motor rootlets and ipsilateral nerve transfers in brachial plexus reconstruction. J Neurosurg 2004; 101: 770-778.

[10] Gu YD, Cai PQ, Xu F, Peng F, Chen L. Clinical application of ipsilateral C7 nerve root transfer for treatment of C5 and C6 avulsion of brachial plexus. Microsurgery 2003; 23: 105-108.

[11] Cui JL, Lu LJ, Xue J. Experimental study of tracer methodology in microdissection of brachial plexus. Surgery Journal 2007; 30: 136-137.

[12] Kurimoto T, Miyoshi T, Suzuki A, Yakura T, Watanabe M, Mimura O, Fukuda Y. Apoptotic death of beta cells after optic nerve transection in adult cats. J Neurosci 2003; 23: 4023-4028.

[13] Imaizumi K, Benito A, Kiryu-Seo S, Gonzalez V, Inohara N, Lieberman AP, Kiyama H, Nuñez G. Critical role for DP5/Harakiri, a Bcl-2 homology domain 3-only Bcl-2 family member, in axotomy-induced neuronal cell death. J Neurosci 2004; 24: 3721-3725.

[14] Lin HD, Chen DS, Fang YS. Sensation of the fingers dominated by the brachial plexus. China Reparative and Reconstructive Surgery 2007; 21: 604-606.

[15] Yang MJ, Shi QL, Gu YD. Recent development of experimental neurotization as a treatmen T for brachial plexus injuries. China Reparative and Reconstructive Surgery 2005; 19: 902-905.