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Is it necessary to use the entire root as a donor when transferring contralateral C₇ nerve to repair median nerve?

Kai-ming Gao^{1, 2, 3}, Jie Lao^{1, 2, 3, *}, Wen-jie Guan^{1, 2, 3}, Jing-jing Hu⁴

1 Department of Hand Surgery, Huashan Hospital, Fudan University, Shanghai, China

2 Key Laboratory of Hand Reconstruction, Ministry of Health, Shanghai, China

3 Shanghai Key Laboratory of Peripheral Nerve and Microsurgery, Shanghai, China

4 Nursing Department, Huashan Hospital, Fudan University, Shanghai, China

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Graphical Abstract



***Correspondence to:** Jie Lao, M.D., Ph.D., laojie633@sina.com.

orcid: 0000-0003-4343-6050 (Jie Lao)

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Abstract

If a partial contralateral C_7 nerve is transferred to a recipient injured nerve, results are not satisfactory. However, if an entire contralateral C_7 nerve is used to repair two nerves, both recipient nerves show good recovery. These findings seem contradictory, as the above two methods use the same donor nerve, only the cutting method of the contralateral C_7 nerve is different. To verify whether this can actually result in different repair effects, we divided rats with right total brachial plexus injury into three groups. In the entire root group, the entire contralateral C_7 root was transected and transferred to the median nerve of the affected limb. In the posterior division group, only the posterior division of the contralateral C_7 root was transected and transferred to the median nerve. In the entire root + posterior division group, the median nerve was repaired on the affected side in the three groups. At 8, 12, and 16 weeks postoperatively, electrophysiological examination showed that maximum amplitude, latency, muscle tetanic contraction force, and muscle fiber cross-sectional area of the flexor division group. No significant difference was found between the entire root and entire root + posterior division groups. Counts of myelinated axons in the median nerve were greater in the entire root group than in the entire root + posterior division group, which were greater than the posterior division group. We conclude that for the same recipient nerve, harvesting of the entire contralateral C_7 root achieved significantly better recovery than partial harvesting, even if only part of the entire root was used for transfer. This result indicates that the entire root should be used as a donor when transferring contralateral C_7 nerve.

Key Words: nerve regeneration; peripheral nerve injury; brachial plexus injury; avulsion injury; contralateral C_7 transfer; nerve root; entire root; partial root; median nerve; ulnar nerve; animal experiment; neural regeneration

Introduction

Contralateral C_7 (c C_7) nerve transfer originated with Gu et al. (1992, 1998, 2002). As c C_7 is not always involved in the injury, it has been considered as one of the most effective procedures and is commonly used in the treatment of total brachial plexus avulsion injury patients (Chuang et al., 1998,

2012; Gao et al., 2006; Chen et al., 2007; Wang et al., 2011, 2013; Lin et al., 2013; Yang et al. , 2015). Gu et al. (1998) reported that functional recovery of median nerve reached 62.5% for motor function and 75% for sensory function. Terzis et al. (2009) also reported 62% of motor function and 41% of sensory function recovery of median nerve. Neuro-

morphological investigations showed that the total axonal count for cC_7 averaged approximately 24,000 (Sungpet et al., 1990; Chuang, 1995), which was much more than any of the recipient nerves, such as median nerve and radial nerve. For this reason, some surgeons recommended using a partial cC_7 nerve, but not the entire root, as the donor. However, Gao et al. (2013) reported that harvesting of the entire cC_7 nerve root promoted significantly better recovery than partially harvested cC_7 nerve root transfer when repairing median nerve. Gao et al. (2013) also reported that if the entire cC_7 nerve root was harvested for simultaneous transfer to median nerve and the biceps branch, in which situation the donor for the median nerve was equivalent to partial cC_7 root transfer, both recipient nerves achieved good recovery.

Such a result is interesting yet seems contradictory, leading us to question what a reasonable explanation for this phenomenon may be. We supposed that the harvesting of the entire cC_7 nerve root might be the key factor for the good outcome of cC_7 nerve transfer. To examine this, we conducted the study described herein.

Materials and Methods

Animal preparation

Fifty-four adult female Sprague-Dawley rats weighing 200–250 g were provided by the Experiment Animal Center of Fudan University, China (license No. DF014). The rats were housed in plastic cages on clean sawdust, under a 12-hour light/dark cycle, with six animals per cage. The rats had free access to food and water. The right forelimb was chosen as the affected limb.

The fifty-four rats were randomly assigned to the entire root group, the posterior division group and the entire root + posterior division group. In each group, the 18 rats were equally divided to three subgroups: the 8-week post-operation group, the 12-week post-operation group, and the 16week post-operation group.

The study protocol was approved by the Animal Ethics Committee of Fudan University of China (20150629A335). The experimental procedure followed the *United States National Institutes of Health Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85–23, revised 1985). All rats were intraperitoneally injected preoperatively with 10% chloral hydrate solution (3 mL/100 g; Shanghai Reagent Company, Shanghai, China) for general anesthesia. All efforts were made to minimize the number and suffering of the animals used in the experiment.

Model of total brachial plexus injury

After general anesthesia, experimental rats were fixed in the supine position. The fur of the surgery area was shaved and the skin was prepared with iodine. As per the procedure introduced by Wang et al. (2014) and Jiang et al. (2016), a supraclavicular transverse incision was made on the right side to expose C_5 to T_1 nerve roots and then the nerve roots from C_5 to T_1 were completely transected at the intervertebral foramen level after blocked by 2% lidocaine to imitate total brachial plexus injury (BPI) (**Figure 1**). No active motion of

the affected limb after surgery was considered as successful establishment of the BPI model.

cC₇ nerve transfer

The cC_7 nerve transfer was conducted in two stages. In the first stage, the normal contralateral plexus (left side) was explored. It was exposed to the trunk-to-division level and a longitudinal epineurotomy was performed in the division to separate the anterior and posterior division. Afterwards, the ulnar nerve was harvested from the affected limb and passed across the chest through a subcutaneous tunnel then transferred to the normal neck. The donor cC₇ nerve root was harvested in three different ways and the rats were separated into three different groups depending on the method of harvesting. In the entire root group, the entire root of the cC₇ nerve was transected and coapted with the ulnar nerve using 11-0 microsutures (Figure 2A). In the posterior division group, only the posterior division of the cC_7 nerve was transected and coapted with the ulnar nerve (Figure 2B). In the entire root + posterior division group, the entire root of the cC₇ nerve was transected but only the posterior division was coapted with the ulnar nerve, and then the anterior division was transferred to the pectoralis major, bridged by the medial antebrachial cutaneous nerve on the affected side (Figure 2C).

The second stage was performed 4 weeks after the first stage in all groups. The ulnar nerve and median nerve on the affected side were transected at the level of the middle of the arm and then the distal stump of the ulnar nerve was coapted to the proximal stump of the median nerve with 11-0 microsutures.

Electrophysiological examination of the median nerve, a muscle tetanic contraction force test and measurement of the muscle fiber cross-sectional area of the flexor digitorum superficialis (FDS), as well as neuromorphology of the median nerve were performed for the three groups at 8, 12, and 16 weeks postoperatively, with 6 rats from each group at each time point. All examinations were performed at 24°C and warm saline was used to keep the muscles and nerves moist.

Electrophysiological examination

Electromyograms were conducted using Neuromatic 2000M keypoint 4-channel electrophysiological apparatus (Dantec, Skovlunde, Denmark) at 8, 12, and 16 weeks postoperatively. An electrode was used to stimulate the cC_7 nerve with single square wave shocks, 2.5 mA super pulse current, 0.2 ms pulse width, and 1 Hz stimulus frequency. The FDS was selected as the representative muscle of the median nerve and a concentric needle recording electrode was inserted to obtain the electrical signal. The maximum amplitude and latency of compound muscle action potentials were recorded from the FDS.

Muscle tetanic contraction force test

At 8, 12, and 16 weeks postoperatively, the FDS tendon was transected at the wrist level and the muscle belly was then dissected to its origin at the medial epicondyle of the humerus. The distal end of the FDS was then attached to a force displacement transducer (JZJ101, Chengdu Instruments, China), with an optimal initial length, by a 4-0 silk suture and the transducer was then linked to a multichannel physiology signal collection system (RM6240BD, Chengdu Instruments, China). The electrodes were placed on the surface of the muscle belly and the maximal value of the tetanic contraction force of the muscle was evaluated with 2.0 V amplitude, 20 Hz frequency, and 1 ms wave width stimulation.

Muscle fiber cross-sectional area

The muscle fiber cross-sectional area (CSA) was evaluated at 8, 12, and 16 weeks postoperatively. The FDS muscle was harvested after the muscle tetanic contraction force test. The muscle was then fixed with 10% formalin, dehydrated through a graded alcohol series and embedded with paraffin. Afterwards, muscle sections of 5 μ m thickness were cut from the middle of the belly and stained with hematoxylin and eosin. The average muscle fiber CSA of the FDS was evaluated by measuring five random areas at 200× magnification by light microscopy (LeicaDWLB2, Leica, Heidelberg, Germany), using digital image analysis software (Leica-Qwin, Leica).

Neuromorphology

Neuromorphology of the median nerve was also conducted at 8, 12, and 16 weeks postoperatively in each group. After electrophysiological examination, a 2 mm length of the median nerve of the affected limb was harvested from a point 5 mm distal to the elbow. The nerve segment was fixed with 2.5% glutaraldehyde for 72 hours, dehydrated through a graded alcohol series, and embedded in epoxy resin. The median nerve segment was then cut into 0.5 μ m sections and stained with 5% toluidine blue. The cross-section of the nerve was examined by microscopy at 400× magnification (LeicaDWLB2, Leica) and the total number of myelinated axons was quantified using Leica FW2000 analysis software (Leica-Qwin) by measuring the area of view and myelinated axon counts and calculating the mean density of myelinated axons.

Statistical analysis

All analyses were conducted with Stata 13.0 software (STATA Data Analysis and Statistical Software, Baltimore, Maryland, USA). Data were expressed as the mean \pm SD. Comparisons among postoperative groups were performed using a one-way analysis of variance test followed by Student-Newman-Keuls *post hoc* tests, and *P* values less than 0.05 were considered statistically significant.

Results

Electrophysiological examination

All 54 rats survived and no wound infection or self-mutilation occurred. The electrophysiological examination showed that there was no significant difference in maximum amplitude or latency of compound muscle action potentials of the median nerve (recorded in the FDS) between the entire root

Muscle tetanic contraction force test

The muscle tetanic contraction force test of the FDS showed that there was no significant difference between the entire root group and the entire root + posterior division group (P > 0.05), which were significantly greater than the posterior division group (P < 0.05; **Table 3**).

Muscle fiber cross-sectional area

The muscle fiber CSA evaluation of the FDS showed that there was no significant difference in cross-sectional area between the entire root group and the entire root + posterior division group (P > 0.05), but they were significantly larger than that of the posterior division group (P < 0.05) (**Table 4**). Representative light photomicrographs of the morphology of the FDS are provided in **Figure 3**.

Neuromorphology

Axon counts showed that the number of myelinated fibers of median nerve in the entire root group was significantly increased compared with those in the posterior division group and the entire root + posterior division group (P < 0.05) and the counts of entire root + posterior division group were significantly greater than in the posterior division group (P < 0.05) (**Table 5**). Representative light photomicrographs showing the morphology of the median nerve in the three groups are provided in **Figure 3**.

Discussion

At present, nerve transfer is recognized as the most successful method to restore affected limb function after total brachial plexus injury (Gu et al., 1987; Narakas et al., 1988; Panupan et al. 1995; El-Gammal et al., 2002; Hou et al., 2002; Shin et al., 2005; Gao et al., 2013a, b c). However, the numbers of donors are limited and sometimes involved in the injury. The contralateral C7 nerve has been considered as one of the most useful donors for the reconstruction of brachial plexus function (Muhetidier et al., 2011; Wang et al., 2013; Hu et al., 2014; Tu et al., 2014; Zhang et al., 2015) as it is not involved in the initial injury and contains a large number of axons, averaging approximately 24,000. Further investigation showed that the posterior division of the C_7 nerve root contains relatively more motor fascicles, while the anterior division contains more sensory fascicles (Gu et al., 1994; Lao et al., 1995). Based on this neuromorphological result for the cC7 root, some surgeons recommended using a partial cC7 nerve as the donor nerve and not the entire root in order to retain more sensory function in the healthy limb (Hierner et al. 2007; Gao et al., 2010; Zou et al., 2010) but the results were not always satisfactory. Waikakul et al. (1999) and Panupan et al. (2001) both reported a 29% effective motor function recovery rate of the median nerve and Sammer et al. (2012) reported no effective recovery of the median nerve when harvesting half of a cC₇ nerve root as the donor.

Table 1 Maximum amplitude (mV) of compound muscle action potentials of median nerve when recorded in flexor digitorum superficialis

Post-operation (week)	Entire root group	Posterior division group	Entire root + posterior division group
8	5.47±0.83*	2.18±0.61	5.18±0.71*
12	$9.68 {\pm} 0.88^{*}$	5.8±0.81	$11.2 \pm 1.83^*$
16	$21.9 \pm 2.45^{*}$	12.5±2.07	$20.6 \pm 3.02^*$

*P < 0.05, *vs.* posterior division group. Data are expressed as the mean \pm SD (n = 6, one-way analysis of variance test followed by Student-Newman-Keuls *post hoc* test). Entire root group: The entire contralateral C₇ root was transected and transferred to the median nerve of the affected limb. Posterior division group: Only the posterior division of the contralateral C₇ root was transected and transferred to the median nerve. Entire root + posterior division group: The entire contralateral C₇ root was transected, while only the posterior division was transferred to the median nerve.

Table 3 Muscle tetanic contraction force test (g) of flexor digitorum superficialis

Post-operation (week)	Entire root group	Posterior division group	Entire root + posterior division group
8	3.48±0.39*	1.78±0.21	3.07±0.14*
12	$6.21 \pm 0.54^{*}$	3.46 ± 0.39	$6.05 \pm 0.79^{*}$
16	$11.21{\pm}1.68^{*}$	$6.19 {\pm} 0.86$	$10.08 \pm 1.07^{*}$

*P < 0.05, *vs.* posterior division group. Data are expressed as the mean \pm SD (n = 6, one-way analysis of variance test followed by Student-Newman-Keuls *post hoc* test). Entire root group: The entire contralateral C₇ root was transected and transferred to the median nerve of the affected limb. Posterior division group: Only the posterior division of the contralateral C₇ root was transected and transferred to the median nerve. Entire root + posterior division group: The entire contralateral C₇ root was transected, while only the posterior division was transferred to the median nerve.

Table 5 Axon counts of myelinated fibers in median nerve

Post-operation (week)	Entire root group	Posterior division group	Entire root + posterior division group
8	1,955±109*#	1,191±111	1,766±101*
12	2,565±97 ^{*#}	1,897±55	2,443±58 [*]
16	3,251±89*#	2,387±64	3,053±66*

**P* < 0.05, *vs.* posterior division group; #*P* < 0.05, *vs.* entire root + posterior division group. Data are expressed as the mean \pm SD (*n* = 6, one-way analysis of variance test followed by Student-Newman-Keuls *post hoc* test). Entire root group: The entire contralateral C₇ root was transected and transferred to the median nerve of the affected limb. Posterior division group: Only the posterior division of the contralateral C₇ root was transected and transferred to the median nerve. Entire root + posterior division group: The entire contralateral C₇ root was transected, while only the posterior division was transferred to the median nerve.

On the contrary, Gao et al. (2013) reported that harvesting and transfer of the entire cC_7 nerve root resulted in significantly better recovery than partial harvest of the cC_7 nerve root when repairing median nerve. In his investigation, the motor function recovery of the median nerve was 81.82% in the entire cC_7 group, while it was only 40% in the partial cC_7 group. Gao et al. (2013) also reported that if the entire

 Table 2 Latency (ms) of compound muscle action potentials of median nerve recorded in flexor digitorum superficialis

Post-operation (week)	Entire root group	Posterior division group	Entire root + posterior division group
8	3.83±0.59	3.97±0.62	3.80±0.58
12	2.02 ± 0.28	2.37±0.16	2.17±0.36
16	$1.37{\pm}0.11^{*}$	1.61±0.14	$1.42 \pm 0.11^{*}$

*P < 0.05, *vs.* posterior division group. Data are expressed as the mean \pm SD (n = 6, one-way analysis of variance test followed by Student-Newman-Keuls *post hoc* test). Entire root group: The entire contralateral C₇ root was transected and transferred to the median nerve of the affected limb. Posterior division group: Only the posterior division of the contralateral C₇ root was transected and transferred to the median nerve. Entire root + posterior division group: The entire contralateral C₇ root was transected, while only the posterior division was transferred to the median nerve.

Table 4 Muscle fiber cross-sectional area (μm^2) of flexor digitorum superficialis

Post- operation (week)	Entire root group	Posterior division group	Entire root + posterior division group
8	10,268.60±886.45*	6,431.71±776.67	9,613.6±675.11*
12	13,836.84±1,204.78*	9,408.73±494.16	12,855.88±571.65*
16	$20,754.74 \pm 1,239.52^*$	11,116.59±984.14	18,838.19±853.87*

*P < 0.05, vs. posterior division group. Data are expressed as the mean \pm SD (n = 6, one-way analysis of variance test followed by Student-Newman-Keuls *post hoc* test). Entire root group: The entire contralateral C₇ root was transected and transferred to the median nerve of the affected limb. Posterior division group: Only the posterior division of the contralateral C₇ root was transected and transferred to the median nerve. Entire root + posterior division group: The entire contralateral C₇ root was transected, while only the posterior division was transferred to the median nerve.

 cC_7 nerve root was harvested to simultaneously repair the median nerve and the biceps branch, both recipient nerves achieved good recovery, which was 75% for median nerve motor function recovery and 66.7% for elbow flexion. Terzis et al. (2009) also successfully used the entire cC_7 nerve root to selectively transfer to multiple recipient nerves in 56 cases. These previous results seem contradictory. If the entire cC_7 nerve root was transferred to two or more recipients, only partial cC_7 fascicles (equivalent to partially harvested cC_7 root transfer) could be the donor for the median nerve, but the result was much better than transfer of partially harvested cC_7 nerve root would be a key factor for the cC_7 nerve transfer.

Although the regeneration of the median nerve and the recovery of the FDS improved with prolonged time, the consistent results at 8, 12, and 16 weeks post-operation supported our viewpoint that harvest of the entire cC_7 nerve root, whether or not the entire root or only the posterior division was used as the donor, eventually resulted in significantly better functional recovery than harvest only of the cC_7 posterior division for transfer.

The result of this experiment is interesting, but the mech-



Figure 1 C_5 - T_1 nerve roots (arrows) on the right side were exposed and blocked by 2% lidocaine and then transected at the level of the intervertebral foramen to simulate total brachial plexus injury.



re root group Posterior division group Entire root + p

Figure 3 Morphology of the FDS and neuromorphology of the median nerve in the affected limb.

The upper panels show the muscle morphology of the FDS, stained with hematoxylin and eosin, of the three groups at 16 weeks post-operation at 200× magnification. The bottom panels show the neuromorphology of the median nerve, stained with 5% toluidine blue, of the three groups at 400× magnification at 16 weeks post-operation. The muscle fiber cross-sectional area of the FDS of the entire root group and the entire root + posterior division group was larger than that of the posterior division group and significantly greater axon numbers, with better morphology of the myelinated fibers can be seen in the entire root group. In the entire root group the entire contralateral C7 root was transected and transferred to the median nerve of the affected limb. In the posterior division group, only the posterior division of the contralateral C₇ root was transected and transferred to the median nerve. In the entire root + posterior division group, the entire contralateral C₇ root was transected but only the posterior division was transferred to the median nerve. FDS: Flexor digitorum superficialis.

anism is still unknown. We hypothesize that functional reorganization of cerebral cortex might play an important part in the process (Jiang et al., 2010; Hua et al., 2012; Stephenson et al., 2013; Li et al., 2015; Yu et al., 2017). Functional reorganization of cerebral cortex will occur secondary to nerve transfer, but what may be more important for contralateral C₇nerve transfer is the post-operative trans-hemispheric functional reorganization of the motor cortex (Lou et al., 2006; Pan et al., 2012; Yang et al., 2017). The efficiency of these two types of functional reorganization largely determines the recovery of the recipient nerve (Sanes et al., 1997; Chen et al., 1998). Wei et al. (2011) reported that the expression levels of BDNF and GAP43 mRNA were significantly increased in brain samples after cC7 nerve transfer, which indicates that BDNF and GAP43 may play an important role during dynamic trans-hemispheric functional reorganization. We hypothesize that the different methods of harvesting of cC₇ nerve root may lead to different expression of BDNF and GAP43 mRNA owing to different functional reorganization of the cerebral cortex, eventually resulting in different functional recovery. However, this hypothesis



Figure 2 Transfer of the entire root, posterior division and entire root + posterior division.

(A) The entire root of contralateral C_7 (cC_7) nerve (\downarrow) was transected at the trunk-to-division level and coapted with the ulnar nerve (*) on the affected side, with no tension, by 11-0 microsutures. (B) The anterior division (\rightarrow) and posterior division (\blacktriangle) of the cC7 nerve were separated at the division level and only the posterior division (\bigstar) was transected and coapted with the ulnar nerve (*) on the affected side, with no tension, by 11-0 microsutures. (C) Both the anterior (\rightarrow) and posterior (\bigstar) division of the cC₇ nerve was transected at the division level, while only the posterior division (\bigstar) was coapted with ulnar nerve (*) on the affected side, with no tension, by 11-0 microsutures. The anterior division was transferred to the pectoralis major bridged by the medial antebrachial cutaneous nerve (#) on the affected side.

needs to be investigated and proven in further research. Despite the unknown mechanism, we strongly recommend that the entire root of the cC_7 nerve be used as the donor.

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