Different functional reorganization of motor cortex after transfer of the contralateral C7 to different recipient nerves in young rats with total brachial plexus root avulsion

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HIGHLIGHTS
► Rat models were constructed of different ways for contralateral C7 transfer.
► Contralateral C7 to upper trunk made faster cortex reorganization than to median nerve.
► Synergic motions were favorable to transhemispheric functional reorganization.

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ABSTRACT
Clinically, contralateral C7 transfer is used for nerve reconstruction in brachial plexus injuries. Postoperatively, synchronous motions at the donor limb are noteworthy. This study studied if different recipient nerves influenced transhemispheric functional reorganization of motor cortex after this procedure. 90 young rats with total root avulsion of the brachial plexus were divided into groups 1–3 of contralateral C7 transfer to anterior division of the upper trunk, to both the musculocutaneous and median nerves, and to the median nerve, respectively. After reinnervation of target muscles, number of sites for forelimb representations in bilateral motor cortices was determined by intracortical microstimulation at 1.5, 3, 6, 9, and 12 months postoperatively. At nine months, transhemispheric reorganization of nerves neurotized by contralateral C7 was fulfilled in four of six rats in group 1, one of six in group 2 and none in group 3, respectively; at 12 months, that was fulfilled in five of six in group 1, four of six in groups 2 and 3, respectively. Logistic regression analysis showed that rate of fulfilled transhemispheric reorganization in group 1 was 12.19 times that in group 3 (95% CI 0.006–0.651, p = 0.032). At 12 months, number of sites for hindlimb representations which had encroached upon original forelimb representations on the uninjured side was statistically more in group 3 than in group 2 (t = 9.5, p < 0.0001). It is concluded that contralateral C7 transfer to upper trunk or to both the musculocutaneous and median nerves induces faster transhemispheric functional reorganization of motor cortex than to that median nerve alone in rats.

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1. Introduction
Transfer of the contralateral C7 spinal nerve as a nerve source for nerve reconstruction has become one of the major options for treatment of root avulsion of the brachial plexus [6,20], since Gu et al. reported in 1992. In this operation, the C7 spinal nerve from the healthy side is divided and transferred, by nerve grafting, to the recipient nerve in the injured upper limb. The cutting of C7 will not leave long-range neurological deficit for the donor upper limb because of cross-innervation by the upper and lower trunks. At the initial stage of recovery for muscle power, the function of the repaired nerve will be activated by some movements on the healthy side. The rationale of that phenomenon lies in the fact that the motor cortex for the contralateral C7 is located on the injured side, so its activation needs powerful movements of shoulder adduction, elbow and wrist extension or finger flexion from the healthy side, which are originally innervated mostly (or partially) by the transferred C7. As time goes on, some patients regain independent motions, but in others, synchronous motions at the donor limb are still noteworthy [19], making patients unable to perform motions independently with the nerve neurotized by the...
contralateral C7. Our experience in operations for children with brachial plexus injuries, showed that contralateral C7 transfer to the upper trunk of the brachial plexus, which mainly innervate muscles in the upper arm, or to both the musculocutaneous and median nerves made little synchronous motions at the donor limb. But this phenomenon still existed in considerable extent if that source of axons was transferred to either of the median and radial nerves [5]. These findings imply that mode of nerve reconstruction affects functional reorganization of brain cortex.

Studies have demonstrated that transhemispheric functional reorganization of the motor cortex does occur after contralateral C7 transfer. With a rat model of transferring the contralateral C7 to the median nerve in the injured limb, Lou et al. [14] found that the ipsilateral motor cortex activated the injured forepaw initially, and after a transitional period of bilateral activation of cortices, the contralateral cortex controlled the injured forepaw exclusively. Using functional magnetic resonance imaging, Beaulieu et al. [1] studied cerebral plasticity in seven adult patients who underwent transfer of the contralateral C7 to the musculocutaneous nerve due to total root avulsion of the brachial plexus. They showed that flexion of the elbow was associated with a bilateral network activity, and the contralateral cortex originally involved in control of the injured arm did participate in control of that motion. This suggested a tendency of transhemispheric functional reorganization of motor cortex in adults undergoing contralateral C7 transfer. Based on our clinical findings in treatment of children’s plexopathy, we hypothesized that transfer of the contralateral C7 to the upper trunk or to both the musculocutaneous and median nerves is more favorable to transhemispheric functional reorganization of motor cortex than that to the median nerve alone. As structure of the brachial plexus of rats is similar to that of humans in source, branching [2], and representative peripheral nerve and muscles for each spinal nerve [4], this study, therefore, used young rat models to test our hypothesis.

2. Materials and methods

2.1. Grouping of animals

90 young Sprague-Dawley rats weighing 50–70 g at the time of surgery, were divided randomly into three groups of 30 each, according to different neurotized nerves on the injured side, i.e., group 1 in which the contralateral C7 was transferred to the anterior division of the upper trunk, group 2 in which the contralateral C7 was transferred to both the musculocutaneous and median nerves, and group 3 in which the contralateral C7 was transferred to the median nerve alone.

2.2. Construction of rat models simulating operations of contralateral C7 transfer

After anesthesia with intraperitoneal injection of sodium pentobarbital (0.5%, 40 mg/kg; Shanghai Reagent Company, Shanghai, China) and by an aseptic technique, the rat was placed in a prone position and a longitudinal posterior-middle incision about 2.5 cm in length was made between levels of occiput and scapular angulus superior. The left longissimus capitis cervicis, semispinalis cervicis, digastricus, and complexus muscles were retracted laterally, and muscles on the processus spinosus and lamina arcus vertebrae were detached to expose the left C5–T1 spinal nerves. The lamina arcus vertebrae from C5 to T1 on the left side were removed with a rongeur. The spinal cord was gently pulled right using a hook, and the dorsal roots of the left C5–T1 spinal nerves were then exposed and avulsed. The ventral roots of C5–T1, therefore, could be seen, and were also avulsed. The distal ends of C5–T1 roots were embedded, by some stitches with 11/0 nylon (Nürnberg, Germany), in the surrounding tissue to prevent nerve regeneration. The incision was closed. The rat was then turned over to a supine position. A 15 mm supraclavicular incision was made, on the right side, to expose the brachial plexus. The C7 spinal nerve was cut proximal to its divisions. Another incision was made on the left forelimb, and the left ulnar nerve and brachial vessels were exposed. The brachial vessels were ligated under the inferior ulnar collateral vessels, and the whole length of the ulnar nerve based on brachial vessels was freed. The ulnar nerve was divided at the wrist and its proximal end was taken to the right neck incision, through a thoracic subcutaneous tunnel, to be coapted to the contralateral C7 spinal nerve with 11/0 nylon. The left ulnar nerve was also cut at its origin, and the recipient nerve neurotized by the contralateral C7 through the ulnar nerve graft varied with grouping, that is, the distal end of the ulnar nerve at the axilla coapted to the anterior division of the upper trunk in group 1, to both the musculocutaneous and median nerves in group 2, and to the median nerve alone in group 3 (Fig. 1).

2.3. Intracortical microstimulation mapping

Six normal adult rats were taken for intracortical microstumulation to identify representative areas of the motor cortex for both forelimbs, which were referential to assess functional motor cortex reorganization in experimental groups. And then at each interval of 1.5, 3, 6, 9 and 12 months after operations, six rats were taken respectively from each group for intracortical microstimulation to map the representations of bilateral motor cortices for the forelimb on the injured side, which had been innervated successfully by the contralateral C7.

Fig. 1. Diagrams showing different surgical approaches of contralateral C7 transfer. Group 1: contralateral C7 transfer to the anterior division of the upper trunk, with the aim of re-innervating the biceps muscle. Group 2: contralateral C7 transfer to both the musculocutaneous and median nerves, with the aim of re-innervating the biceps and flexor digitorum muscles. Group 3: contralateral C7 transfer to the median nerve, with the aim of re-innervating flexor digitorum muscles. Double arrows point to the connecting position where the ulnar nerve graft is bridged between contralateral C7 and recipient nerve on the injured side.
The rat was anesthetized with a single injection of sodium pentobarbital (0.5%, 40 mg/kg ip) and maintained under this anesthetic (2.0–4.0 mg/kg h iv). The rat’s body temperature was monitored with a rectal probe and maintained at 37 °C using an electric heating pad. With the rat mounted in a stereotaxic apparatus (Huamu Factory, Shanghai, China), a craniotomy was performed over the bilateral parietal and frontal bones corresponding to the forelimb representation areas in motor cortex. The dura was cut and retracted, and 37 °C mineral oil was poured over the cortex to prevent the cortex from dehydration. Varnish-insulated tungsten microelectrodes with tip impedances of 0.2–1.0 MΩ were used for cortical stimulation. The cortical surface covering the area between 5 mm anterior to and 1 mm posterior to Bregma and from 0.5 to 4 mm lateral to the midline, which had been reported to be the forelimb representation area in normal rat’s motor cortex [7], was tested to map motor representations in steps of 0.5 mm for rats in both normal control and experimental groups. With a stimulus electrode penetrated perpendicularly to a depth of 1.8 mm below the cortical surface that was defined to be layer V of the frontal cortex [14], monophasic cathodal pulses (75 ms train length, pulse duration at 250 Hz, 200 μm pulse duration) of a maximum of 60 μA were passed through the electrode, being generated by a constant current stimulator (Model: U-ML180-OG-02A, MacLab, Powerlab, AD Instruments, Castle Hill, New South Wales, Australia). Motor cortical maps were then constructed by sites which located the area of the penetrations in which electrical stimuli evoked a visible joint movement of the forelimb at a current intensity of 60 μA or less. If no movement could be detected with this current intensity, that site was defined as “nonresponsive” one. The motor cortex map of other body parts such as vibrissae, neck, mouth, hindlimb, which were elicited in forelimb representation areas, were also constructed, and this might be used to be an auxiliary parameter to evaluate functional motor cortex reorganization in experimental groups.

All surgical procedures and protocols in this study were consistent with the Guidelines for Ethical Care of Experimental Animals approved by the International Animal Care and Use Committee.

2.4. Statistical analysis

For recovery time of rats’ joint movement and number of sites for representations in motor cortex, values were expressed as mean ± standard deviation. Logistic regression analysis was used to analyze the difference of the number of cases that had fulfilled transhemispheric functional reorganization in the motor cortex among the three groups. Two-tailed t test was applied to compare the number of sites for motor cortical areas of the hindlimb which had encroached upon forelimb representations. All statistical analyses were made using Stata version 10.0 (Stata Corporation, College Station, TX, USA) software, and statistical significance was set at 0.05.

3. Results

3.1. Efficacy of contralateral C7 transfer

In the experimental groups, the forelimbs on the injured side were entirely paralyzed after avulsion of the C5–T1 spinal nerves. The recovery time of elbow flexion in group 1 was 48 ± 7.8 days postoperatively, that of elbow flexion and of finger flexion in group 2 was 45 ± 6.4 days and 58 ± 8.2 days respectively, that of finger flexion in group 3 was 54 ± 8.9 days. These results showed that all the target muscles in the three groups were re-innervated, and most of them regained function of muscle contraction at around 1.5 months after contralateral C7 transfer.

3.2. Forelimb representations in motor cortex of normal rats

In the six normal rats, the forelimb representations in motor cortex were located in 0.5–4 mm anterior to Bregma and from 2 to 4 mm lateral to the midline, which were lateral to vibrissae representation and in front of hindlimb representation. The stimulation of forelimb representations on one side evoked visible motion of the forelimb on the opposite, rather than on the same side. The number of sites in forelimb representations was 29.6 ± 2.3 on the left side and 31.5 ± 2.5 on the right side, which was not statistically different (t = 1.35, p = 0.207) (Fig. 2A).

3.3. Dynamic changes of forelimb representations in motor cortex of experimental groups

At 1.5 months after the operation, the injured forelimbs in all experimental groups showed evoked visible movements innervated by nerves which were neuritized by the contralateral C7, only when cortical representations of forelimbs in the hemisphere on the injured side were stimulated. At three and six months, the stimulation of bilateral forelimb representations could evoke movements of forelimbs on the injured side in all experimental groups, with the number of sites in the forelimb representations on the uninjured side gradually increased (Table 1).

At nine months, four of six rats in group 1, one of six in group 2 and none in group 3 had visible forelimb movements on the injured side which were only evoked by stimulation of forelimb representations on the uninjured side, implying that transhemispheric functional reorganization of the repaired nerve had been fulfilled for some rats in groups 1 and 2 (Fig. 2B–D). The number of sites in the forelimb representations on the uninjured side increased continuously in groups 1 and 3 but not in group 2 (Table 1).

At 12 months, five of six rats in group 1, four in group 2 and four in group 3 had visible forelimb movements on the injured side, which were only evoked by stimulation of forelimb representations on the uninjured side. The number of sites in the forelimb representations on the uninjured side had a tendency of decrease in each group (Table 1).

Logistic regression analysis was applied to compare the differences of the number of cases that had fulfilled transhemispheric functional reorganization in the motor cortex between the three groups from six to 12 months postoperatively. It was demonstrated that the rate of fulfilled transhemispheric functional reorganization in group 1 was 12.19 times that in group 3, which was statistically significant (95% CI: 0.006–0.651, p = 0.032); the rate of fulfilled transhemispheric functional reorganization in group 2 was 1.68 times that in group 3, which was not statistically different (95% CI: 0.225–12.524, p = 0.612).

On the other hand, we counted the number of sites for hindlimb representations which had encroached upon original forelimb representations on the uninjured side, for groups 2 and 3 at 12 months.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Sites</th>
<th>1.5 m</th>
<th>3 m</th>
<th>6 m</th>
<th>9 m</th>
<th>12 m</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>20.8 ± 2.5</td>
<td>22.3 ± 2.0</td>
<td>26.0 ± 3.0</td>
<td>18.8 ± 1.7</td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>10.8 ± 1.5</td>
<td>28.8 ± 2.6</td>
<td>27.3 ± 2.4</td>
<td>19.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7.0 ± 1.4</td>
<td>22.7 ± 2.5</td>
<td>25.5 ± 3.1</td>
<td>18.0 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

* Group 1: contralateral C7 transfer to the anterior division of the upper trunk; group 2: contralateral C7 transfer to both the musculocutaneous and median nerves; group 3: contralateral C7 transfer to the median nerve.

* m, month.
postoperatively. That number of sites was 5.7 ± 0.8 in group 2 and 9.5 ± 0.6 in group 3, respectively, and the difference was statistically significant (\( t = 9.5, p < 0.0001 \)).

4. Discussion

Peripheral nerve injury which removes sensory inputs and blocks motor output activity, will result in the corresponding cortical representation of this nerve to be functionless, and then to be occupied by functional expansion of neighboring cortical areas [15]. If this nerve is repaired and nerve regeneration is successful, that reorganization of cerebral cortex can be reversed totally or partially, depending upon the extent of nerve injury and the accuracy of re-innervating its primary targets [17]. A previous study of transhemispheric cortical reorganization in adult rats with contralateral C7 transfer to the median nerve, showed that this cortical remodeling underwent two processes, i.e., initially bilateral control and late one-side control from the hemisphere which was contralateral to the injured forelimb [14]. In this study with pup rats that underwent different procedures of contralateral C7 transfer, group 1 where the contralateral C7 was transferred to the upper trunk of the brachial plexus, had a faster course of transhemispheric functional cortical reorganization than group 3, where the contralateral C7 was transferred to the median nerve alone. Although the course of transhemispheric functional cortical reorganization was not faster in group 2, where the contralateral C7 was transferred to both the musculocutaneous and median nerves, than in group 3, the number of invading sites of the hindlimb in the motor cortex of the forelimb was less in the former than in the latter. This implies that group 2 had greater potential of transhemispheric functional cortical reorganization, as compared with group 3. Data of this experiment are in accordance with our clinical findings suggesting that transfer of the contralateral C7 to the upper trunk or to both the musculocutaneous and median nerves results in much less synchronous motions of the donor limb than transfer of contralateral C7 to the median nerve alone.

Previous studies have proved that functional reorganization of the forelimb motor cortex occurs in response to the development of skilled forelimb movements [11]. Lotze et al. [13] indicated that the daily use of a myoelectric hand prosthesis in acute and chronic amputees could prevent the shrinking of the motor and somatosensory hand cortical areas, as well as further expansions of non-deafferented surrounding regions such as lips and face. Clinically, the functional recovery of shoulder and elbow after contralateral C7 transfer to the upper trunk can be good, while improvement of the median nerve alone is far from gaining functional recovery of a paralyzed hand [5], and this may be the reason for which transhemispheric functional cortical reorganization is faster in patients with contralateral C7 transfer to the upper trunk than in those with contralateral C7 transfer to the median nerve alone. If the contralateral C7 is transferred to both the musculocutaneous and median nerves, the functional combination of elbow flexion and finger flexion can yield some coordinated motions in daily activities. As different muscles contributing to synergies may have common neural structures in the central nervous system [18], we conjecture that the easily obtainable transhemispheric functional cortical reorganization for the musculocutaneous nerve may simultaneously induce transhemispheric reorganization for the median nerve by some preexisting interneuronal connections between the cortical area of the musculocutaneous nerve and that of the median nerve. That may be the mechanism with which transfer of the contralateral C7 to both the musculocutaneous and median nerves gains faster transhemispheric functional reorganization than that to the median nerve alone. It must be realized, however, that results of this experiment are not directly applicable to the human situation; in the latter immediate nerve reconstruction after root avulsion of the brachial plexus is not always available, while delay of operative timing may affect re-innervation of motor and sensory targets of the reconstructed nerve.

Neuroplasticity is the changing of neurons and of the organization of their networks, which may happen through increase of new cells or change of the strength of the connections between the
neurons [12]. Previous studies have disclosed that neuroplasticity in the cerebral cortex following peripheral nerve injury undergoes two processes, i.e., the short-time plasticity and the long-time plasticity which are partly overlapped [3]. Mechanisms of the former included unmasking of previously inactive connections [10], strengthening or weakening of existing synapses activity [9] and changes in neuronal membrane excitability [8], which are mainly functional. Mechanisms of long-time plasticity refer to sprouting of new axons from surviving neurons to form new synapses [21] and even adding of new neurons [22], which are fundamentally structural. In our study, the number of sites for forelimb representations in the motor cortex on the uninjured side peaked at six months postoperatively in group 2, at nine months in groups 1 and 3, and then decreased at 12 months in all the three groups (Table 1). This tendency was also observed in other study of neuroplasticity in the cerebral cortex for peripheral nerve injury [16]. The mechanism, we speculate, is that in the process of structural reorganization, the corresponding cortical motor representations are increasingly matured by feedback of improved function of the nerve which has been repaired successfully, making other functionally compensative cortical areas inactive. It is concluded that transfer of the contralateral C7 to the upper trunk of the brachial plexus or to both the musculocutaneous and median nerves can make faster transhemispheric functional reorganization of motor cortex than that to the median nerve alone in rats.

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References

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