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研究报告

Dynamically Functional Reorganization in Somatosensory Cortex Induced by The Contralateral Peripheral Nerve Transfer to an Injured Arm*

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Abstract Peripheral nerve injury of a limb usually causes functional reorganization of the contralateral somatosensory cortex. However, the patients with an operation of the contralateral seventh cervical nerve (C7) transfer to an injured arm with brachial plexus root avulsions usually have the sole tactile sensibility of the healthy hand when the injured hand is touched at the early stage after the operation. Then, at later stage they gradually get normal sense from the injured and the normal hands independently. Mimicked the process in a rat model based on the above operation, representations of the injured forepaw and the healthy forepaw in the somatosensory cortex were studied by means of somatosensory evoked potential (SEP) recording. Somatosensory function shown in SEP response amplitude and peak latency of the injured forepaw gradually recovered with time after the operation due to the contralateral C7 regeneration toward the injured limb, accompanied with the recovery process of limb movement. The somatosensory representation of the injured forepaw was observed exclusively in the ipsilateral somatosensory cortex since the 5th month after the operation. Accordingly, the overlapped representation of the injured and healthy forepaws emerged in the ipsilateral somatosensory cortex of 13 rats studied except one with separated representation though the SEP latency and response amplitude were different in responding to stimuli on the two forepaws. It is concluded that the contralateral peripheral nerve transfer to the injured arm can cause dynamically functional reorganization in the ipsilateral somatosensory cortex suggesting a remarkable plasticity of the brain function induced by an alteration of sensory input between two sides of the body in adult rats.

Key words functional reorganization, neuronal plasticity, sensory cortex, peripheral nerve, nerve transfer

Many studies have demonstrated that following a peripheral nerve cut or amputation, the functional reorganization appeared significantly in the primary somatosensory cortex as a result in change of somatotopic representation[1～4] showing an expansion of the adjacent body surface representations into the denervated area. These previous studies mainly focused on the mechanism of reorganization of the somatosensory cortex contralateral to the side of deafferentation.

Paralysis of the injured arm caused by brachial plexus root avulsions is usually permanent, since there is almost none of root regeneration. The operation of the contralateral healthy seventh cervical (C7) nerve root transfer to connect to nerves of the injured hand, initially developed by Gu in 1986[5,6], has been widely accepted and recognized as one of the best procedures for the treatment of brachial plexus root avulsions. Functionally, at the early stage after anastomosis, the patients felt that their healthy hands were touched when their injured hands were touched in fact and could move the injured arm only with a coordinative movement of the other hand. As time passed, some of the patients started to feel tactile sense separately from

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either hand when the contralateral hand was touched and the movement of either hand became independently[5,6], somehow as normal condition. We hypothesize that there must be a re-organizational process that occurs in the somatosensory cortex after this operation. To test the hypothesis above, we used rats to establish an animal model, based on the clinical treatment described above, to study the mechanism of functional reorganization in the somatosensory cortex in adulthood. The aim of study is to determine (1) where the representation of the injured forepaw lies in the somatosensory cortex after the operation, (2) how the representation changes in the somatosensory cortex following the operation, and (3) discuss what mechanism underlies the functional reorganization.

1 Materials and methods

1.1 Animal preparation

Experiments were conducted in 40 Sprague-Dawley (SD) rats. There were three groups of animals designed for the study. In addition to the first group of normal control, the following two groups were prepared for experiments.

Ⅰ. Group of 6 normal adult rats.

Ⅱ. Group of 6 rats with a unilateral nerve root avulsion of the left brachial plexus. All the adult rats were anesthetized with intraperitoneal sodium pentobarbital (0.5%) of a dose of 40～50 mg/kg. All the rats were fixed in a prostrate position. An incision was made in the middle of the neck and back from the occiput to scapular angulus superior, and the length of the incision was about 4 cm. The longissimus capitis cervicis muscle, semispinalis cervicis muscle, digastricus muscle, and complexus muscle were drawn to one side. The muscles on the processus spinosus and lamina arcus vertebrae were removed and the processus of the nerves from the cervical fourth to thoracic first (C4-T1) were exposed distinctly. The lamina arcus vertebraes of the C4-T1 on the left were removed using special pliers. The spinal cord was drawn to the left side by a special hook. The left radix dorsalis and radix ventralis of C5-T1 were exposed and all the roots of the C5-T1 were avulsed from the spinal cord and cut about 2 mm off under a microscope.

Ⅲ. Group of 14 rats with a nerve root avulsion of the left brachial plexus and the contralateral C7 nerve transfer. Anesthesia process and root avulsion of the brachial plexus operation were identical to those of Group Ⅱ. Then the median nerve and the proximal rular ulnar nerve in the left side were cut off under axilla and the distal rular nerve was dissected in wrist on the elbow. The ulnar nerve with the collateralis ulnaris superior artery was regarded as the transplantation nerve. The root of the C7 nerve was cut off on the right side. The distal terminal of the left ulnar nerve with the vascular pedicel was moved through the subcutaneous tissue of the front chest to the contralateral body and coapted with the terminal of the right C7 nerve root. At the same time, another terminal of the ulnar nerve was coapted with the median nerve of the injured limb under the left axilla. The operation was done under a microscope and is shown schematically in Figure 1.

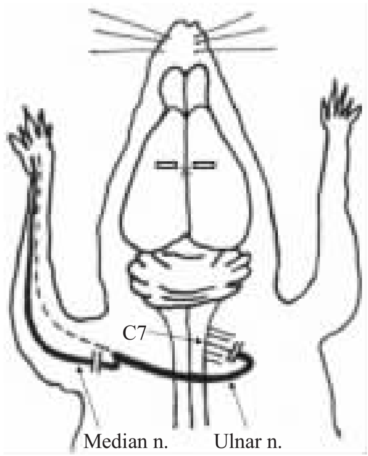

Fig. 1 Diagram showing the animal model of the operation of the contralateral (right) C7 nerve root transfer to connect to nerves of the injured hand in a rat like previously reported [6~8]

The thin dashed line denotes the original position of the ulnar nerve of the left side before the C7 transfer operation. The thick solid line from the left to the right side denotes that the distal terminal of the left ulnar nerve was shifted and connected with the contralateral C7 nerve terminal after the operation. The two rectangular areas on the brain surface indicate the areas we recorded SEPs in the somatosensory cortex for searching the representation of two forepaws in the rat. The cross in the midline indicates the position of Bregma which is located at Horsley-Clarke coordinates A9, L0.

The rats in different periods after the above operations were anesthetized with a single injection of sodium pentobarbital in an initial dose of 40～50 mg/kg, *i. p.* and then maintained in a dose of 2.5～4.0 mg/kg·h *i. v.* for continuous sedation. The animal's body temperature was maintained at 37℃ using an animal body temperature controller (SS20-2, Dajiang Electronic Company, Anhui) adjusting the current of an electric heating pad according to rectal temperature. The rat was placed in a stereotaxic apparatus and then a portion of the left parietal and frontal bones of the skull was removed between 0 mm and 2 mm to Bregma which corresponds to Horsley-Clarke coordinates A9.0- A11.0, and from L0 to L4.5 mm lateral to the midline. The dura was cut and retracted, and 37℃ mineral oil was poured over the cortex to prevent it from dehydration.

1.2 Stimulation and recording

The location of the primary somatosensory cortex was defined according to stereotaxic co-ordinates, 0.5～1.5 mm anterior to Bregma and 3～4 mm lateral to the midline [7]. Somatosensory evoked potentials (SEPs) were recorded from cortical surface of the rats in different periods after the above operations with an Ag-AgCl electrode with a diameter of 0.5 mm. The electrical stimuli were delivered through a pair of bare hypodermic stainless-steel needles (gauge 27) with a interval of 5 mm. These needles were inserted into the volar skin of the forepaw. The electrical stimulus was a 45 ms train of biphasic pulses with duration of 0.25 ms and intensity ranged from 0.8 mA to 4 mA at 200 Hz. It was repeated once a second for 15 seconds. The SEP signals were amplified and stored in an electrophysiological system (Model: U-ML, MacLab, Powerlab, Australia) for analysis. The latency of SEPs was defined as a peak latency of the SEP trace, which is the time in ms from the onset of the electrical stimulus to the peak of each SEP response. The peak-to-peak response amplitude of SEP of the primary somatosensory cortex was measured. The positive peak was measured from the pre-existing negative peak.

At the end of a experiment, the experimental rats was deeply anaesthetized, and the success of the operations of the nerve root avulsion of the brachial plexus and the contralateral C7 nerve transfer were checked. Data of the experimental rat failed in the operation were not included in results.

1.3 Map construction

The latency of the SEP response evoked by a stimulus at healthy forepaw usually was from 11ms to 12 ms, while it was from 19 ms to 21 ms for the injured forepaw. Like previously reported [8], we defined an area of somatosensory representation as a cortical region in which SEP peak-to-peak amplitudes reached a certain response amplitude of 10～30 μV depending on the noise level of the cortex. The mean values of the amplitude and peak latency were expressed as $\bar{x} \pm s$. Changes in the amplitude and latency of the SEP of the injured forepaw at different times after operation were tested by Independent-Samples T-Test. The statistical software is SPSS11. The representation maps of the injured forepaw and the healthy forepaw were described using the Origin 6.0 software.

2 Results

2.1 Recovery process

The animals' pain reflex to nipping the forepaw was tested weekly for monitoring functional recovery process with a hemostat after the operation. In rats of Group Ⅰ, pain reflex evoked in either limb was observed repeatedly when it was nipped. In contrast, in the rats Group Ⅱ with nerve root avulsion of brachial plexus, no pain reflex was observed at all when the injured forepaw was stimulated.

In rats of Group Ⅲ, the signs of recovery appeared initially on an average of about 5 months after the operation. Mechanical stimulation of the injured forepaw evoked the withdrawal reflex of both the injured forepaw and healthy forepaw. It suggested that the sensory nerve of the injured forepaw had rebuilt and both of forepaw might share the same motosensory cortical area. However, if the stimulation was applied to the healthy forepaw, the rat only withdrew the healthy forepaw. The other interesting sign of recovery appeared on about 10 months after the operation. The reflex elicited by stimulation of one forepaw was completely separated from the other. When the injured forepaw was stimulated mechanically, the rat withdrew the injured forepaw without a withdrawal of the healthy forepaw. As the healthy forepaw was stimulated the rat only withdrew the healthy forepaw.

2.2 Latency and amplitude of SEPs

In normal rats, the SEP responses to electrical stimulation on a forepaw could be recorded only in the contralateral somatosensory cortex. As the control, the mean latency of SEPs was (11.5 ± 0.3) ms and the mean amplitude of SEPs was (278 ± 38) μV when a constant stimulating of 0.8 mA was used in 6 rats tested.

No SEP response to stimulation on the injured forepaw was recorded from both sides of the somatosensory cortex either in Group Ⅱ rats with the nerve root avulsion of brachial plexus rats during the period of 16 months' study or in those Group Ⅲ rats with the nerve root avulsion and C7 transfer operation in 2.5～4 months after the operation.

In 6 rats of Group Ⅲ, 5～9 months after the C7 transfer operation, the cortical representations of both the healthy forepaw and injured forepaw were recorded only from the ipsilateral somatosensory cortex to the injured forepaw rather than the contralateral cortex (Figure 2). The mean latency of SEPs evoked by 0.8 mA current stimulating the healthy forepaw was (12 ± 1.1) ms and the mean amplitude of SEPs was (333 ± 14) μV, while the mean latency of SEPs was (21.0 ± 0.9) ms and the mean amplitude of SEPs was (36 ± 3) μV when the injured forepaw was evoked. The mean latency of SEPs of the injured forepaw was 9 ms longer than that of the healthy forepaw and the mean amplitude of SEPs of the injured forepaw was 9 times less than that of the healthy forepaw (both $P < 0.001$). It indicates that since the 5th month after the C7 transfer, the somatosensory function of the injured forepaw appeared to recover partially and the cortical representation of the injured forepaw exclusively lied in the ipsilateral cortex.

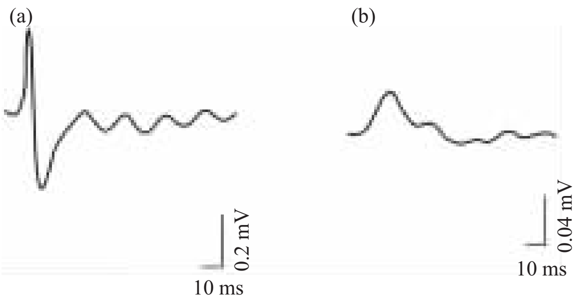

Fig. 2 The SEP waveform recorded from the left cortex by using electrical stimuli on the contralateral healthy forepaw (a) and the injured forepaw (b) of a rat 5~9 months after the C7 transfer operation

The intensity of stimulating electrical current was constant of 0.8 mA.

In 6 rats of Group Ⅲ, 10～16 months after the C7 operation, the cortical representations of the healthy forepaw and injured forepaw were recorded only from the ipsilateral somatosensory cortex to the injured forepaw rather than the contralateral side (Figure 3). The mean latency of SEPs was (12 ± 0.2) ms and the mean amplitude of SEPs was (306 ± 21) μV when the healthy forepaw was stimulated, while the mean latency of SEPs was (19.5±1.2) ms and the mean amplitude of SEPs was (113 ±10) μV when stimulating in the injured forepaw. The mean latency of SEPs of the injured forepaw was 7.5 ms longer than that of the healthy forepaw and the mean amplitude of SEPs of the injured forepaw was 2.7 times less than that of the healthy forepaw significantly (both $P < 0.001$).

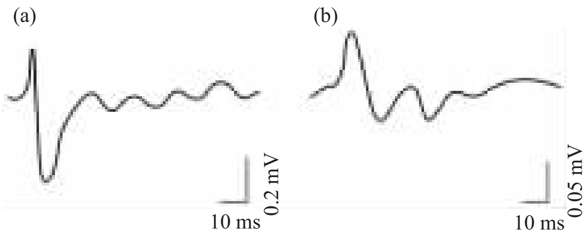

Fig. 3 The SEP waveform recorded inform the left cortex using electrical stimulation on the contralateral healthy forepaw (a) and the injured forepaw (b) of a rat 10~16 months after the C7 operation

The intensity of stimulating electrical current was constant of 0.8 mA.

It is noticed that although there was no statistical difference in the mean latency of the SEPs evoked by stimulating the injured forepaw between those of 5～9 months after the operation (21 ± 0.9) ms and 10～16 months after the operation (19.5 ±1.2) ms ($P = 0.49 > 0.05$), however, the mean amplitude of SEPs of the injured forepaw during 10 ～16 months after the operation (113 ± 10) μV was 3.1 times larger than that

Table 1 Latency and amplitude of SEPs in the left somatosensory cortex evoked by stimulating the healthy forepaw (HF) and injured forepaw (IF)

Time (Mon) after the operation	Latency (ms) of the HF	Latency (ms) of the IF	*P* value	Amplitude (μV) of the HF	Amplitude (μV) of the IF	*P* value
5	12 ± 1.1	21.0 ± 0.9	0.001	333 ± 13	36 ± 3	0.001
10	12 ± 0.2	19.5 ± 1.2	0.001	306 ± 21	113 ± 10	0.001
P value		0.49			0.005	

of SEPs in 5～9 months after the operation (36 ± 3) μV significantly ($P = 0.005 < 0.01$) . The results indicate that the peripheral nerve fibers and the function of somatosensary cortex were recovering continuously during the period of 10～16 months after the operation (Table 1) and the cortical representation of the injured forepaw exclusively lied in the ipsilateral cortex.

2.3 Cortical mapping of SEPs

To understanding the mechanism of somatosensory cortex reorganization after the operation of the contralateral healthy C7 nerve root transfer, the cortical representations of the two forepaws were mapped by measuring SEP responses.

In the normal rats, SEP responses were always evoked in the contralateral somtosensory cortex when stimulating unilateral limb, as shown in Figure 4a. In the rats of Group Ⅱ, no SEP response could be evoked

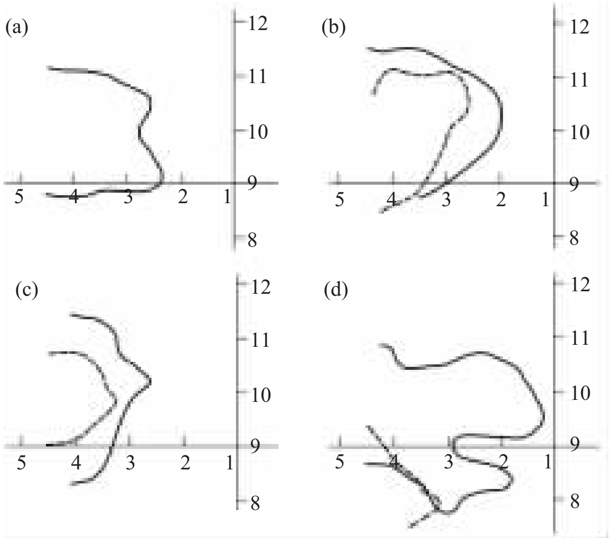

Fig. 4 The representations of the injured forepaw (left) and the healthy forepaw (right) in the somatosensory cortex ipsilateral to the left injured limb

(A) The SEP responding area in the left somatosensory cortex evoked by electrical stimuli in the right forepaw of a normal rat. (b) and (c) The overlapped representation of both the injured forepaw and the healthy forepaw was exhibited in two rats 6.5 months and 10 months after the C7 transfer operation respectively. (d) The representation of the injured forepaw and the healthy forepaw were separated in the left hemispheres of somatosensory cortex in a rat 10 months after the operation. Solid lines indicate cortical representation of the healthy forepaw and dashed lines indicate that of the injured forepaw. The contours on the left somatosensory cortex indicate the SEP iso-value lines of 40 μV (a), 20 μV (b), 10 μV (c), and 10 μV (d), respectively. Due to the difference in neuropaths, threshold and individual animals, the intensities of stimulating electrical current were 0.8 mA in (a), 0.8 mA for the two forepaws (b), 1.4 and 2.8 mA for the right and left forepaw stimuli (c), and 0.8 mA and 4 mA for the right and left forepaw stimuli (d), respectively.

in either side of the cortex by stimulating the injured limb because of neural pathway cutting. However, in the rats of Group Ⅲ, from the 5th month to 16th month after the operation, the overlap of representative areas of SEPs evoked by stimulating the injured forepaw and the healthy forepaw was always found in the left cortex ipsilateral to the injured limb. This overlap was found in 12 rats of 13 rats with successful SEPs by the left stimulation (12/13, 93%) like the two samples of rats of Group Ⅲ in Figure 4 b and c. The only exception exhibited a significant separation of responding areas emerged the 10th month after the operation (Figure 4d).

It should be noted that in a remaining rat of Group Ⅲ, we failed to evoke a SEP response in either side of hemispheres when the injured forepaw was stimulated 5 months after the operation. However, the electrical stimuli on the motor cortex ipsilateral to the injured limb did drive both the left and the right forepaws of the rat to move suggesting that the recovery process of motor function may be faster than that of the somatosensory function in these cases.

3 Discussion

It has known for long time that injury on one side of limb usually causes functional reorganization in the contralateral sensory cortex. Many experiments have shown that following peripheral nerve lesion, the representation of deafferented body parts in the contralateral side became responsive to inputs from neighboring parts of the body[9～13]. However, our study here for the first time showed a dynamically functional reorganization in the ipsilateral somatosensory cortex based on a rat model of clinical treatment of nerve root avulsion of brachial plexus with the contralateral C7 nerve root transfer. It is demonstrated that change of the target innerved by the peripheral nerve resulted in a remarkable change of functional reorganization in the somatosensory cortex in the adult rats. The extensive plasticity of the brain induced by an alteration of sensory input from the other side of the body suggests a new strategy for potential application in clinical treatments of the leg disabled.

3.1 Ipsilateral cortical reorganization of the injured forepaw

For rats during less than 5 months after the C7 transfer operation, there was no SEP evoked in either of both somatosensory cortices by stimulating the injured forepaw. This corresponds to the behavioral observation that the animal showed none of pain reflex and implies that the transferred C7 had not regenerated to reach the median nerve enough in the injured limb.

In the early period of 5～9 months after the transfer, the somatosensory representation of the injured forepaw and the healthy forepaw overlapped on the same area in the ipsilateral somatosensory cortex to the injured forepaw in most rats studied. This indicates that the left somatosensory cortex received the sensory inputs from the right healthy forepaw and the left injured forepaw only when the healthy C7 nerve root has transferred and grown to connect with the skin and muscles of the left injured forepaw. This explains why rats quivered their injured forepaw and the healthy forepaw simultaneously when the injured forepaw was stimulated by pain stimuli during the same period. It is also in agreement with many cases, in which patients have feeling of the healthy hand when the injured hand was touched.

The case shown in Figure 4d is interesting because of the separation of somatosensory representation of the two limbs. This may correspond to the behavior we observed that rats exhibited separate pain reflex responses to stimulation in their two limbs independently at the later stage. This is also similar to observation in some cases, in which patients who can eventually feel the stimuli on both hands independently and move their arms freely. The finding indicates that the cortical representation can be reorganized remarkably following the change of peripheral input sources, even from another side of the body. A most close sample is a study on two adults with webbed fingers (syndactyly) who were tested before and after a surgical separation. After the separation of the two fingers, the magnetoencephalography (MEG) measure revealed that the representation of the two fingers became separately in the contralateral somatosensory cortex[14]. It is clear that cortical reorganization is closely related to changes of peripheral information input.

3.2 SEP latency of the injured forepaw

Somatosensory information from the periphery to the cortex is through pathways of polysynaptic connections between neurons at different levels，including the spinal cord, brain stem，thalamus and cortex. The SEP peak latency or the nerve conduction time from the periphery to cortex depends on the length of the neural path, the total number of involved synapses, and the number and proportion of large axons along the somatosensory pathway. Due to the contralateral C7 transfer, the length of the C7 transferred peripheral nerve injured is significantly longer than that of the healthy one, and the number of regenerated large size nerves to the injured limb, which depends on the completeness of the C7 regeneration, should be less than that in the normal pathway from the healthy forepaw, while the total number of involved synapses is comparable. As a result, the mean latency of the injured forepaw was significantly longer than that of the healthy forepaw.

On the other hand, the latency of SEP evoked by stimulation on the injured forepaw tends to be getting somehow shorter with time from 5～9 months to 6～16 months though this is not statistically significant. This might reflect increasing number of the regenerated large nerve fibers to the injured limb. It should be noted that even if the C7 nerve grows completely to the injured limb the latency will never recover to that of normal limb because of length difference of the two pathways and the multiple sources (C5～C8) of the median nerve.

3.3 SEP amplitude of the injured forepaw

The SEP response amplitude depends on how many cortical neurons are synchronously elicited by ascending impulses through the somatosensory pathway when the stimulating current is constant. The mean SEP response amplitude evoked by stimulating the injured forepaw was much larger in rats in 10～16 months than that in 5～9 months after the C7 transfer operation, indicating that more C7 nerve fibers regenerated significantly with time after the operation and less scatter of arriving impulses to the cortex.

The mean SEP amplitude evoked on the ipsilateral hemisphere by stimulating the injured forepaw we saw in these rats 10～16 months after the operation was significantly lower than that of the healthy forepaw. This may reflect the fact that the median nerve innervating the forepaw is originated from not only the C7, but also the C5, C6 and C8 nerves of the brachial plexus in normal animals while only the C7 serves for the injured forepaw. As a result, the median nerve in the injured limb is impossible to recover as complete as normal even if the transferred C7 nerve regenerated fully to form a new median nerve. Thus，the total number of the median nerve fibers regenerated in these rats had to be much less than those of normal rats. On the other hand, we could not rule out the possibility that the C7 transferred nerves were regenerated incompletely to the forepaw of the injured limb at that period.

Because the transhemispheral reorganization has been found in the motor cortex of the same animal model as described here in our laboratory (Lou L, Shou T, Li Z, et al. Transhemispheric functional reorganization of the motor cortex induced by the peripheral contralateral nerve transfer to the injured arm. Neurosci, in press), it is interesting to see whether there is a complete functional reorganization that also shows contralateral representation of the two hemispheres of somatosensary cortex in a longer period than 16 months after the operation.

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对侧外周神经移位到损伤手臂引起的体感皮层功能动态重组 *

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摘要 单侧肢体的外周神经损伤通常导致对侧体感皮层的功能重组. 然而，接受了对侧颈 7 (C7) 外周神经移位手术治疗单侧手臂臂丛全撕脱的病人，在术后早期当其患手被触摸时，只在其健手产生感觉. 在术后晚期，病人才逐渐恢复其患手和健手的正常、独立的功能. 我们在模拟对侧颈 7 (C7) 外周神经移位手术病例的大鼠模型上，用记录体感诱发电位的方法研究了患手和健手的体感代表区. 患手的体感和运动功能由于 C7 神经的再生而逐渐恢复. 术后第 5 个月始， 13 只大鼠患手的体感代表区只出现在其同侧的皮层，同时患手和健手的代表区在该皮层内是高度重叠的 (除掉一个例外)，虽然刺激它们产生的体感诱发电位的潜伏期和反应幅度有很大的不同. 结果表明，移位到患手的对侧外周神经能够导致同侧体感皮层动态的功能重组，提示身体另侧感觉输入的介入激发了大脑显著的可塑性.

关键词 功能重组，神经可塑性，感觉皮层，外周神经，神经移位

学科分类号 Q421

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