目标肌肉神经分布重建对大鼠幻肢痛的影响及神经分布研究

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摘　要　目标肌肉神经分布重建技术（Targeted Muscle Reinnervation，TMR）通过对假肢的实时直觉控制提高截肢者的运动功能。然而对于 TMR 技术是否能减轻幻肢痛、移植神经在肌肉内如何分布等问题，目前知之甚少。本文旨在探讨 TMR 手术对大鼠幻肢痛的影响和术后目标肌肉内神经的再分布情况。我们利用坐骨神经横断组（SNT）作为大鼠的疼痛模型，将神经近端移植到目标肌肉中作为 TMR 模型，并通过大鼠行为学来评价疼痛程度。实验发现，对照组大鼠不出现自残行为，而 SNT 组和 TMR 组从手术后第二天开始出现自残行为并逐渐加重，但 TMR 组的自残情况明显轻于 SNT 组。利用 Sihler's 肌内神经染色法可以在目标肌肉内观察到移植神经末端再生的细小分支。实验结果初步证明 TMR 技术对幻肢痛有一定的缓解作用，并且术后的神经可以在目标肌肉内重新分支分布。

关键词　目标肌肉神经分布重建；幻肢痛；大鼠；Sihler's 肌内神经染色法

Influence of Targeted Muscle Reinnervation on Phantom Limb Pain and Distribution of Reinnervated Nerve in Rats

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Abstract　Targeted muscle reinnervation (TMR) can improve amputees' movement ability by providing simultaneous and intuitive control of artificial limbs. However, little is known about whether TMR can relieve the phantom limb pain and how intramuscular nerve branches are distributed in the targeted muscle after the operation. The purpose of this study is to investigate the effects of TMR on phantom limb pain and to explore the possibility of post-operative neuranagenesis in rats. The rat model was established by sciatic nerves transaction (SNT group) and the proximal ends of these nerves were grafted into targeted muscles (TMR group). The degree of the phantom limb pain was measured by observing the autotomy behaviors of rats. The pilot results show that rats in the sham group have no autotomy behaviors, while rats in SNT group and TMR group show typical autotomy behaviors two days after the operations and the behaviors become worse gradually. However, the TMR group show obviously less pain than the SNT group. Meanwhile, some small new branches rising from the transferred nerves could be observed in targeted muscle with the aid of Sihler's nerve staining technique. The experimental results suggest that TMR can possibly alleviate the phantom limb pain and the transferred nerve can regenerate to innervate with the targeted muscle.

Keywords　targeted muscle reinnervation; phantom limb pain; rat; Sihler's intramuscle nerve staining

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1 Introduction

Due to the prevalence of various physical injuries by traffic accidents and natural disasters and so on, the population of amputees is growing year by year. After these injuries, the limb amputation is a major consequence[1,2]. The limb amputation impedes basic activities of daily life, including dressing, feeding, personal hygiene, and more advanced skills required by employment and leisure activities. Up to now, the targeted muscle reinnervation (TMR) has provided an appealing concept to use "EMG signals" in which electrodes are directly connected with the residual nerves of the high-level amputees. TMR has an efferent and an afferent component, and the targeted muscle can be reinnervated with residual nerves (branch of brachial plexus) for the amputated limb. The resultant EMG signals of the targeted muscle now serve as the motor commands of the missing limb to drive a motorized prosthetic device[3-5].

It is reported that after amputation or limb denervation, about 50% to 85% patients suffer from phantom limb pain, which includes a wide variety of symptoms ranging from tingling and itching to burning and aching[6,7]. The mechanism of phantom limb pain still remains unclear. After peripheral nerve injury, the primary afferent into the spinal cord is blocked and neural plasticity occurs, such as central sensitization and cortical reorganization, which are the most commonly cited reasons for the existence or development of phantom pain[6,7]. Besides rebuilding a neural circle between targeted muscle andnervous system, TMR can also effect neuroplasticity[8-10]. However, little is known about whether TMR can relieve the phantom limb pain.

In the fourth week after TMR, we found that redirected nerve could be regenerated into the targeted muscle using Sihler's nerve staining method, and the observation of autotomy behavior revealed that the autotomy scores decreased obviously. The results suggest that TMR could alleviate neuropathic pain caused by denervation.

2 Materials and Method

2.1 Animal Preparation

Twenty-four Sprague-Dawley rats (200～250 g, SPF Level, provided by the Guangdong Medical Laboratory Animal Center) were housed in a climate controlled room under 12h day/night cycle and had random access to food and water. This study was approved by the Ethics Committee for Animal Research, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.

2.2 Targeted Muscle Reinnervation Operation

Before the surgical procedure, the rats were anesthetized with 10% chloral hydrate (3 mL/kg) through intraperitoneal injection. The fur on the operation region was shaved and treated with a depilatory cream and then the skin was sterilized with povidone iodine and 75% alcohol.

In the lower limb experiments, all rats were divided into three groups randomly, sham-operation group (sham, n＝8), sciatic nerve transection group (SNT, n＝8) and targeted muscle reinnervation group (TMR, n＝8). For the sham group, after the rat was anesthetized, the right sciatic nerve was exposed and then the incision was sutured. For the SNT group, the sciatic nerve was exposed the same way and a 1 cm of sciatic nerve segment was resected and the skin incisions were then closed. For the TMR group, the nerve was transected the same way as that of the SNT group, and the proximal ending of the nerve was sutured into biceps femoris with 10－0 silk sutures (Fig. 1A). All procedures were performed with a dissection microscope.

In the upper limb experiments, only the right side of the animal's chest was processed targeted muscle reinnervation operation. During the procedure, the pectoralis major muscle was selected as targeted muscle. A continuous "┐" shaped incision was made from the middle of the right forelimb cubital fossa to axillary fossa

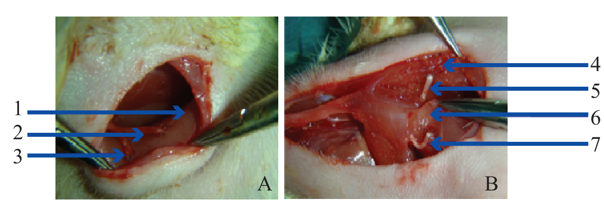

Figure 1. The targeted muscle reinnervation (TMR) operation in rats

A: the tibial nerve and the common peroneal nerve were sutured into biceps femoris; B: the median nerve and the ulnar nerve were sutured into pectoralis major

1: biceps femoris; 2: common peroneal nerve; 3: tibial nerve; 4: pectoralis major (superficial layer); 5: median nerve; 6: pectoralis major (deep layer); 7: ulnar nerve and then along the anterior axillary fold to the front chest. Careful dissection was conducted to identify and expose the median nerve (lateral side) and ulnar nerve (medial side) structures as they arised from the brachial plexus. The median nerve was separated and cut inside the cubital fossa. The right side pectoralis major muscle was denervated by transecting the original nerves that innervate it. Then, the remnant median nerve was transferred into the pectoralis major muscle with 10—0 nylon sutures (see Fig.1B). Finally, after the surgery procedure was finished, the skin incision was closed using 4—0 suture needles layer by layer.

2.3 Post-Operative Care

After surgery, the rats were allowed sufficient recovery from anesthesia and put back to their cages. The rats were injected with penicillin once a day (20 UI/day) for three days to prevent infection.

2.4 Behavioral Analysis

From the day after surgery, the rats were scored every day using a modified scale devised by Wall et al[10]. Briefly, one point was given for the removal of one or more nails, and an additional point for injury or removal of each half toe, to a maximum of 11.

2.5 Tissue Harvest and Preparation

Four weeks after the operation, the rats were anesthetized with chloral hydrate through intraperitoneal injection. The original surgical incision was reopened, and the bilateral sides of the whole pectoralis major were dissected carefully for histomorphometric analysis.

The dissected pectoralis majors were fixed in 10% formalin fixative, according to the Sihler's intramuscular nerve staining procedure[11]. The pectoralis major specimen were stained with Sihler's hematoxylin step by step for 12 weeks. The distribution of the transferred nerves within the pectoralis major region was recorded.

3 Results

3.1 Autotomy Behavior Observation

Sham rats showed no autotomy behavior all the time, but rats in SNT group and TMR group developed typical autotomy behaviours from 2 days after the operation and the autotomy got progressively worse. At the end of the fourth weeks, the average autotomy score of TMR rats was 2.5±1.25, which is significantly lower than that of the SNT group (5.0±0.6) (Fig. 2)

Figure 2.　The observation of autotomy behavior in rats

3.2 Histomorphometric Analysis

The whole pectoralis major muscle became transparent or semi-transparent and remained intact after Sihler's nerve staining. The intramscular nerve branches became hyacinthine after the staining and could be clearly identified within the muscle.

For the left pectoralis major muscle (control side), the pectoralis major muscle fibers formed a "flabellate" shape and were innervated by the lateral and medial pectoral nerve, which run transversely and longitudinally. It also formed a "U" nerve-loop in the middle of muscle and the branches formed a neural network clearly (Fig. 3B).

By observing the right side of the pectoralis major (TMR side) of the rats, we found that the pectoralis major intramuscular nerve staining effect was obviously less than the left side and the neural network of the

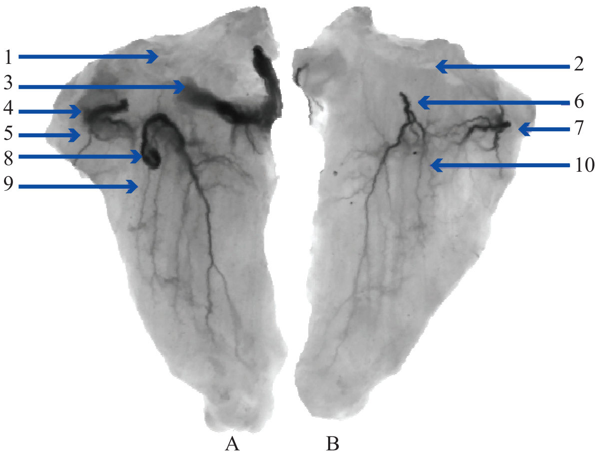

Figure 3.　The intramuscular nerve distribution by Sihler's nerve staining in rats spectoralis major muscle
A: the right side (control); B: the left side (TMR)
1, 2: pectoralis major; 3: median nerve(reinnervated); 4: ulnar nerve (reinnervated); 5: branches of ulnar nerve (the regenerated never); 6, 8: lateral pectoral nerve; 7: medial pectoral nerve; 9, 10: branches of lateral pectoral nerve

pectoralis major original innervation was not seen with all intramscular nerve branches presenting a crenation phenomenon. The median nerve and ulnar nerve were implanted and were dyed hyacinthine above the lateral region of pectoralis major and the small shape of regenerated and extremely intramuscular nerve was seen and dyed hyacinthine, with the neural network not formed (Fig. 3A).

4 Discussion

4.1 Phantom Limb Pain with TMR

The major finding of the present study is that TMR can alleviate the neuropathic pain caused by denervation. Autotomy refers to a self-mutilatory behaviour in rats after limb denervation, and this phenomenon is generally regarded as an animal model for post-denervation pain syndrome, such as phantom limb pain[11-13]. So the autotomy behavior is scored by the scale developed by Wall et al. to rate the extent of phantom limb pain[11]. After peripheral nerve transaction, changes occurred at several levels of the neural axis, including neuroma formation, spinal cord sensitization, rewiring of thalamus, reorganization of primary somatosensory and motor cortex (S1&M1), which were considered to be the mechanisms of phantom limb pain[6,7,14,15]. It is reported that TMR alters the histomorphometric characteristics of the neuroma by decreasing myelinated fiber counts and increasing fascicle diameter in the transferred nerves [15]. This may be one of the reasons of our results. Whether TMR reduces the reorganization of the central nervous system needs further investigation.

4.2 Distribution of Intramuscular Nerve with TMR

Sihler's staining is a whole mount nerve staining technique that renders other soft tissue translucent or transparent while staining the nerves[16-18]. Using the Sihler's nerve staining technique to divide the compartment of skeletal muscle is more directly and reliably. The pectoralis major of the rats can be divided into the clavicular part, the sternal part and the abdominal part with fixed distribution of intramuscular nerve branches. The lateral and medial pectoral nerve had two or three first-class branches entering into pectoralis major and then gave off tree-like branches. A large number of nerve branches form a dense area with unknown neural anastomosis. It is speculated that nerve regeneration and reinnervation was relevant to blood supply or the same feedback path among the intramuscular motor units or muscle parts.

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