

## Survival, regeneration and functional recovery of motoneurons after delayed reimplantation of avulsed spinal root in adult rat

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### Abstract

We have established that extensive reinnervation and functional recovery follow immediate reimplantation of avulsed ventral roots in adult rats. In the present study, we examined the consequences of reimplantation delayed for 2 weeks after avulsion of the C6 spinal root. Twelve and 20 weeks after delayed reimplantation, 57% and 53% of the motoneurons in the injured spinal segment survived. More than 80% of surviving motoneurons regenerated axons into the reimplanted spinal root. Cholinesterase–silver staining revealed axon terminals on endplates in the denervated muscles. The biceps muscles in reimplanted animals had atrophied less than those in animals with avulsion only, as indicated by muscle wet weight and histological appearance. After electrical stimulation of the motor cortex or the C6 spinal root, typical EMG signals were recorded in biceps of reimplanted animals. The latency of the muscle potential at 20 weeks was similar to that of sham-operated controls. Behavioral recovery was demonstrated by a grooming test and ipsilateral forepaw movements were well coordinated in both voluntary and automatic activities. These results demonstrate that ventral root reimplantation can protect severed motoneurons, enable the severed motoneurons to regenerate axons, and enhance the recovery of forelimb function even when it is delayed for 2 weeks after avulsion.

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### Introduction

A common and difficult problem in severe brachial plexus injury involves avulsion from the spinal cord; that is, the roots are torn from the spinal cord at the transition between the central and peripheral nervous systems. This type of nerve injury is generally not treated because it is considered a type of central nervous system injury and thus not amenable to surgery (Narakas, 1984). Clinically, spinal root avulsion occurs when crushing and traction forces are applied to the soft tissues. Under these conditions, it is

difficult to determine the exact location of the nerve injury, so delayed nerve repair has been advocated. Typically, secondary nerve repair within 2–3 weeks is advised, or longer when a neuroma has formed and demarcation is obvious (Watchmaker et al., 1996). A short time lag between the accident and the surgery is recognized as a significant factor for a successful outcome (Carlstedt et al., 2000). Following delayed nerve repair, poor intrinsic and extrinsic muscle recovery and only protective sensation are obtained, regardless of whether the secondary repair is made early (within 2 or 3 weeks) or late (6 months or later) (Chuang et al., 2001).

One means of promoting the regeneration of damaged CNS is through the implantation of a peripheral nerve graft in the immediate environment of the injured neurons

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(Stichel and Muller, 1998) or after a short time lag (Carlstedt and Noren, 1995). However, such implantation has a number of limitations. Apart from the inevitable mechanical damage caused by the physical insertion of the nerves into the spinal cord, most favorable grafts attract only a small proportion of the host fibers (Sellin et al., 1980) and abnormal connections may be formed (Cheng et al., 1996).

Recent studies show that a new technique of immediate reimplantation of the avulsed ventral root not only improves the survival of injured motoneurons and enhances axon regeneration, but also does not cause additional injury to the spinal segment in the process (Chai et al., 2000; Gu et al., 2004). In the present study, we investigated the effects of delaying this new method of ventral root reimplantation on the regeneration and functional recovery of motoneurons.

## Materials and methods

### *Animals*

Thirty-eight adult male Sprague–Dawley rats (200–250 g) were divided into three groups: (1) sham-operated controls (6 rats); (2) C6 ventral root avulsion only (14 rats); and (3) C6 ventral root avulsion with delayed reimplantation (18 rats). In the latter group, 8 animals were sacrificed at 12 weeks and 10 animals were sacrificed 20 weeks after reimplantation. All procedures were approved by the Committee for the Use of Live Animals in Teaching and Research at the University of Hong Kong.

### *Surgical procedures*

All animals were anesthetized with ketamine (80 mg/kg, i.m.) and xylazine (8 mg/kg, i.m.) and all procedures were carried out under sterile conditions.

#### *Sham-operated controls*

The dura was left intact, and the C5 and C7 spinal nerves were cut and ligated.

#### *Avulsion only*

After removing the right vertebral lamina of C5, the dura was opened and the ventral root and dorsal root with the ganglion of C6 were selectively avulsed from the spinal cord by traction with a fine hook under a surgical microscope. Traction was exerted almost parallel to the natural course of the root. The site was checked visually to confirm complete avulsion.

#### *Delayed reimplantation*

The avulsed ventral roots of C6 were dissected free, fixed to the muscle surface and labeled with a 10-0 suture. Reimplantation of ventral root was made following the procedure described in a previous study (Chai et al., 2000).

Briefly, 2 weeks after avulsion, the avulsed roots were dissected free and the ventral root was carefully reimplanted into the ventrolateral aspect of spinal segment C6. With a fine glass probe, a small slit was made in the pia mater and care was taken not to injure the spinal white matter. The dorsal root was anchored to the inner surface of dura mater by 10-0 silk to assist attachment of the ventral root to the ventrolateral side of the spinal cord. The dura was closed and the defect covered by a small piece of gelfoam. A piece of muscle was placed over the hemilaminectomy site. The muscles, subcutaneous tissues and skin were closed in separate layers.

Next, the brachial plexus was explored through a hockey-stick incision. The pectoralis major and minor muscles were retracted laterally and the components of the brachial plexus were then dissected between the anterior and middle scalene muscles, exposing the spinal nerves; the C5 and C7 nerves were ligated and cut. To avoid direct reinnervation, the proximal and distal extremities of the cut spinal nerves were ligated and about 5 mm of spinal nerve was removed. In addition, the branch between the medial and lateral fasciculi of the brachial plexus was ligated and cut. Muscles, fascia and skin were then sutured successively in layers.

The animals were allowed to recover from the anesthetic under supervision and were taken to a recovery room where they were checked regularly for several hours. The rats resumed drinking and eating within 1 day and had an uneventful recovery without visible functional disorder other than flaccid paralysis of the right forelimb. Animals were allowed to survive for 12 or 20 weeks after surgery.

### *Grooming test*

One week following surgery, all animals were videotaped and the behavior of the right (lesioned side) forelimb was noted, especially when the animal ate or climbed. From the beginning of the 12 postoperative weeks, behavioral analysis was carried out by weekly observation of each animal's response in the Terzis grooming test, an established method of evaluating forelimb behavior in the rat (Bertelli and Mira, 1993). The test consists of pouring water over the animal's head to elicit grooming movements of the forepaws toward the head. In normal grooming, the animal raises both forelimbs, licks them, reaches up to the area behind the ears with a smooth motion and finally lowers the limbs down to the snout. The movements proceed repetitively for a few seconds. Pouring water over the snout elicits an abbreviated grooming response to wipe away the wetness; this response was recorded with a digital camera. A short time later, when the animal is more relaxed, a prolonged series of grooming movements are carried out. Because the movements are always bilateral and follow the same pattern, this test provided a reliable method to assess functional recovery of the experimental side relative to the normal side. Terzis grooming test video records were graded by an independent

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