Innervation of the caudal denervated ventral roots and their target muscles by the rostral spinal motoneurons after implanting a nerve autograft in spinal cord–injured adult marmosets

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Object. The authors conducted a study to determine the effects of using a nerve autograft (NAG) to promote and guide axonal regrowth from the rostral spinal cord to the caudal lumbar ventral nerve roots to restore hindlimb motor function in adult marmosets after lower thoracic cord injury.

Methods. Nine animals underwent a left-sided hemisection of the spinal cord at T-12 via left-sided T9–L3 hemilaminectomy, with section of all ipsilateral lumbar ventral nerve roots. In the experimental group (five animals), an NAG obtained from the right peroneal nerve was anastomosed with the sectioned and electrophysiologically selected lumbar ventral roots (left L-3 and L-4) controlling the left quadriceps muscle and then implanted into the left ventrolateral T-10 cord. In the control group (four animals), the sectioned/selected lumbar ventral roots were only ligated.

After surgery, all marmosets immediately suffered from complete paralysis of their left hindlimb. Five months later, some clinical signs of reinnervation such as tension and resistance began to appear in the paralyzed quadriceps of all experimental animals that received autographs. Nine months postoperatively, three of the five experimental marmosets could maintain their lesioned hindlimb in hip flexion. Muscle action potentials and motor evoked potentials were recorded from the target quadriceps in all experimental marmosets, but these potentials were absent in the control animals. Horseradish peroxidase retrograde labeling from the distal sectioned/reconnected lumbar ventral roots traced 234 ± 178 labeled neurons in the ipsilateral T8–10 ventral horn, mainly close to the NAG tip. Histological analysis showed numerous regenerating axons in this denervated/reconnected nerve root pathway, as well as newly formed motor endplates in the denervated/reinnervated quadriceps. No axonal regeneration was detected in the control animals.

Conclusions. These data indicate that the rostral spinal neurons can regrow into the caudal ventral roots through an NAG, thereby innervating the target muscle in adult marmosets after spinal cord injury.
Innervation after nerve autograft placement

vate the denervated muscles via the avulsed/reimplanted ventral nerve roots. Nevertheless, these surgically-induced reconstitutions were performed when the spinal cord was not directly injured. It is noteworthy that direct spinal cord damage often occurs clinically and is still considered beyond surgical treatment. Moreover, this kind of injury can make the treatment performance more complicated and the clinical outcome different. At present, few published works have mentioned possible surgical applications to promote axonal regrowth from CNS to PNS along the original denervated nerve root pathway after direct SCI.

Following serious SCI, both sensory and motor fibers are disrupted at the level of the lesion. The neurons situated in the ipsilateral caudal cord may or may not survive, depending on their distance from the lesion site. Although the remaining caudal motoneurons can still maintain neuromuscular connections with their peripheral targets, that with the supraspinal centers (descending fibers) is interrupted at the lesion level, thus causing the loss of normal function. Therefore, to regain the lost function in the area controlled by the neurons below the lesion level requires that the functional connection between the rostral and caudal cord be reestablished or that an alternative source of axons from the rostral cord be provided. Assuming that the tissue structure from the damaged nerve roots to the peripheral target is undamaged, the promotion of rostral spinal axonal regrowth into these nerve roots seems to be a logical method to reinnervate the denervated peripheral target via the original nerve pathway. This surgical method also allows us to reduce the length of grafted nerve so as to minimize the problems caused by nerve grafting.

Lesions can appear in any region of the spine, but they often occur in the cervical region and the thoracolumbar junction. Thoracic or lumbar spinal cord damage often results in paraplegia. For the treatment of injuries in these regions, we have developed a surgical method in adult rats by using an implanted NAG: bridging the rostral thoracic ventral horn and the caudal denervated lumbar ventral nerve roots to a hemisectioned lesion. We have shown that the rostral thoracic neurons can regrow into the caudal lumbar ventral nerve roots via the NAG, leading to innervation of the denervated hindlimb muscle. These encouraging results allowed us to develop further this repair strategy in adult marmosets. Given the close genetic and immunological relationships between marmosets and humans, the marmoset tissue probably has inherently different regenerative characteristics from adult rats. To lead to a new therapy for the treatment of spinal cord lesions in humans, we investigated whether axonal regeneration and innervation could be achieved in this nonhuman primate species after the same injury and repair procedures. The preliminary report of this work has been presented in abstract form.

Materials and Methods

Surgical Procedure

Nine adult marmosets (Callithrix jacchus, male or female, weighing 300–400 g) were used in this study. Anesthesia was induced by an intramuscular injection of a mixed solution (2.1, 0.1 ml/100 g of body weight) of ketamine (50 mg/ml) and chlorpromazine (5 mg/ml). Under a surgical microscope, a multiple left hemilaminectomy was performed from the T-9 to L-3 vertebra. At the T-9 vertebral level (T-10 cord level), the left lateral bone was removed slightly more to expose the ventrolateral spinal cord to allow the subsequent implantation procedure. After carefully opening the dura mater and exposing the left lumbarosacral nerve roots, a left hemisection of the spinal cord was performed using microscissors at T-12. All ipsilateral lumbarosacral ventral nerve roots were then cut at this T-12 level or at their exit area from the spinal cord to avoid spontaneous locomotor recovery after simple hemisection. The sectioned lumbar ventral roots (left-sided L-3 and L-4 nerve roots), which supply the ipsilateral quadriceps muscle, were selected by an electrophysiological method. Briefly, each of the cut ventral roots were stimulated by two 0.5 mm stainless steel monopolar electrodes. A bipolar electrode was implanted into the left quadriceps to detect and record the MAPs while a 0.5 mm stainless steel ground electrode was subcutaneously inserted between the lumbar spinal cord and the quadriceps. When an appropriate nerve root is stimulated, a MAP appears in the muscle site. The roots not transmitting a MAP were shortened by about 1 cm and then ligated to avoid contact with the lesioned spinal cord.

In the experimental group (five animals), an NAG obtained from the right peroneal nerve was surgically connected to the selected L-3 and L-4 ventral roots by a No. 10-0 nylon microsuture and then implanted into the left ventrolateral cord at the T-10 cord level (rostral hemisection) through a small incision. The depth of implantation was approximately 2 mm, thereby leaving the NAG tip probably in, or close to, the spinal ventral horn. After implantation, the NAG was fixed with the dura sheath by two points of No. 10-0 nylon microsutures. A collagen tube (1.5-mm inner diameter) was placed to protect the extradural part of the NAG and sectioned/reconnected lumbar ventral roots.

In the control group (four animals), no repair procedure was performed after the left L-3 and L-4 ventral roots were sectioned. The nerve roots were ligated and placed into the nearby muscle with a collagen tube. After the preceding procedures, the wound was closed in layers with No. 4-0 Nylon sutures. All surgeries were performed under sterile conditions and were well tolerated by the animals. Only conscious, the marmosets were carefully returned to their cages. Each animal lived in a 100 × 100 × 100–cm cage with a nest box at a constant temperature of 23°C and was fed twice a day. All animals were handled according to French laws concerning primate experiments.

Clinical Observation

Following surgery, all animals were regularly observed once a week and videotaped once a month to record the responses of the left paralyzed hindlimb; for example, we assessed the posture and use of the limb when the animal was moving or not in the cage. Each observation was compared with the precedent to assess whether the behavior was changed. Concomitantly, a passive mobilization of this limb was performed after being calmed by an injection of ketamine (0.05 ml/100 g of body weight) to assess if and when the clinical signs of reinnervation, such as tension, resistance, and contraction, appeared in the quadriceps. The volume of quadriceps was estimated by measuring the perimeter of the muscle at the middle point. A percentage compared between the paralyzed quadriceps volume and its contralateral counterpart was recorded.

Electrophysiological Examination

Nine months after surgery, electrophysiological examinations, including the MAP and MEP monitoring, were performed in all animals. Anesthesia was induced as in the initial surgery. The NAG and its distal sectioned/reconnected lumbar ventral roots (in experimental animals) or the only denervated lumbar ventral roots (in control animals) were carefully exposed and isolated from the surrounding tissues without affecting their integrity. A recording bipolar electrode needle was inserted into the ipsilateral quadriceps and a grounding electrode (0.5-mm stainless steel) was subcutaneously placed between the lumbar spinal cord and the quadriceps. The stimulation responses were recorded. For measuring the MAPs, two monopolar electrodes (0.5-mm stainless steel, anode and cathode) were either placed onto the ex-
posed NAG (in experimental animals) or the denervated ventral roots (in control animals). The stimulation was delivered with a pulse width of 0.1 msec and an intensity of 0.1 mA. In this examination, the two mono-epileptor electrodes were also placed into the surrounding muscles to determine whether the MAPs were being initiated.

For the MEPs, a parietal craniotomy was performed (5 mm to the midline and 2 mm posterior to the coronal suture) over the right sensorimotor cortex by using a drill. An insulated 0.5 mm stainless steel monopolar electrode needle was placed onto the pial surface over the exposed sensorimotor cortex, and a reference electrode (8 mm) was submucosally placed into the hard palate. The stimulation was delivered in a similar manner as during the MAP examination, except the intensity was 5.0 mA. The amplitudes of MAPs and MEPs were recorded and measured between the largest positive and negative peaks.

After these examinations, the denervated/reconnected lumbar ventral roots were cut 1 cm distal to the graft–root junction. Identical procedures measuring the MAPs and MEPs were then performed to determine whether conduction activity was broken down subsequent to the cutting of the reconnected ventral roots distal to the stimulation site. In addition, the distal portion of the cut denervated/reconnected ventral roots was restimulated to verify whether the same MAPs reappeared.

Histological Examination

Horseradish Peroxidase Retrograde Labeling Study. When the electrophysiological examinations were completed, the proximal portions of the cut denervated/reconnected lumbar ventral roots (1 cm distal to the graft–root junction) were isolated from the surrounding tissues by using parafilm coated with Vaseline. A similar portion of the only denervated lumbar ventral roots was isolated from control animals. They were then immersed in a gelatin foam soaked with 30% HRP. One hour after immersion, the parafilm and gelatin were removed. The wound was irrigated with saline and closed in layers by using No. 4-0 Nylon sutures. Forty-eight hours later, the animals were reanesthetized and underwent intracardiac perfusion with heparin (1000 units) in 400 ml phosphate-buffered saline (0.1 M, pH 7.2) followed by 500 ml 3.6% phosphate-buffered glutaraldehyde. The thoracic and lumbar spinal cord segments were removed and immersed in 30% phosphate-buffered saccharose at 4°C for 3 days. A cryomicrotome was used to cut the isolated spinal cord into longitudinal 30 μm sections. According to the Mesu- lami’s method, the sections were incubated with tetramethylbenzidine and hydrogen peroxide. After counterstaining with neutral red, dehydration, and being placed on coverslips, the sections were analyzed under a light microscope. The HRP-labeled neurons were counted only when the nucleolus was visualized in the cell body to avoid counting the same neuron more than once. The thickness of all sections was also calculated in order to determine the location of the HRP-labeled neurons in relation to the horns of spinal cord.

Histological Analysis of the NAG and Denervated/Reinnervated Lumbar Ventral Roots. After killing the animals, the NAG and its distal denervated/reconnected lumbar ventral roots were removed from the spinal cord and placed in 3.6% glutaraldehyde for 3 hours and then stored in phosphate-buffered saline (0.1 M, pH 7.2) at 4°C. After fixation in osmium tetroxide and embedding in epon, the sections were cut using the cryostat and then mounted on slides. Based on the method described by Karnovsky and Roots, the sections were stained with acetylthiocholine iodide. The motor endplates were identified and localized with light microscopy.

Results

Clinical Observations

In the postoperative period, all animals immediately suffered complete paralysis and progressive atrophy of the left hindlimb muscles. Five months later, some clinical signs of reinnervation began to appear in the paralyzed limb in all experimental animals that received autografts; the signs included tension and resistance of the paralyzed quadriceps recorded during passive mobilization of the lesioned limb, and atrophy reached a plateau. However, the paralyzed limb was still dropping when the animals were climbing in the cage. Nine months postoperatively, the most evident clinical improvement was established in three of the five experimental animals. In contrast to behavior observed during the early stages of paralysis, the three marmosets could maintain the lesioned limb in hip flexion, especially when they were climbing in the cage. Furthermore, the most powerful resistance was recorded while passively stretching their lesioned limb (change of posture). Concomitantly, some reflex contractions of the quadriceps in these three animals were also noted when the limb was passively mobilized. This contraction was not intermittent and was not found when the animals lived in their cages. Because the animals were calmed by receiving ketamine before the examination, this phenomenon did not seem to be related to the strong respiration. For the remaining two experimental animals, although the posture of their left hindlimb was not obviously changed, the limb became more resistant to the passive mobilization than in the earlier postoperative period. Nevertheless, during the 9-month follow-up period, no voluntary use of the paralyzed limb was observed in any animal.

The volume of the denervated/reinnervated quadriceps in experimental animals was generally 40 to 60% of that of its contralateral counterpart, clearly larger than only 10 to 20% observed in its contralateral counterpart in control animals. There was no evident difference in the quadriceps volume among the five experimental animals. No tension and resistance of the paralyzed hindlimb muscles were recorded in the control animals during passive mobilization of the lesioned limb during evaluation.

Macroscopic Observation of the NAG and Denervated/Reinnervated Lumbar Ventral Roots

When the spinal cord, NAG, and denervated/reconnect- ed lumbar ventral nerve roots were reexposed, a perfect, well-vascularized, nervous tissue–like continuity was seen from the thoracic cord to the reconnected lumbar ventral roots in all experimental animals (Fig. 1). In this continuity, the graft–root junction completely disappeared. No atrophy was observed in the NAG and dener-
Innervation after nerve autograft placement

![Image](image_url)

**Fig. 1.** Photograph showing the tissue continuity among the thoracic spinal cord (SC), implanted nerve autograft (thick black arrow), and denervated/reconnected lumbar ventral roots (thick hollow arrow) in an experimental animal 9 months after surgery. This continuity is similar to nervous tissue and is well vascularized. The graft–root junction completely disappeared (arrow) and the collagen tube was nearly degraded. The head direction (H) is indicated.

vated/reconnected lumbar ventral roots. The collagen tube, which was covering the extradural portion of NAG and reconnected lumbar ventral roots, was nearly degraded. In control animals, no tissue continuity was found; in fact, marked atrophy of the untrimmed ventral roots (left L-3 and L-4) was noted.

**Electrophysiological Monitoring**

When the left L-3 and L-4 ventral nerve roots were selected in the initial surgery, normal MAPs were recorded from the left quadriceps muscle in all marmosets. The mean amplitudes of the MAPs transmitted by L-3 and L-4 ventral roots were 14,941 ± 6429 μV and 4937 ± 1268 μV, respectively (means ± standard deviation).

Nine months postoperatively, no MAP activity was recorded in the paralyzed quadriceps of control animals when the denervated L-3 and L-4 ventral roots were stimulated. In contrast, MAPs were recorded in the denervated/reinnervated quadriceps of all experimental animals while stimulating the exposed NAG (Table 1; Fig. 2). The mean amplitude of the MAPs was 1023 ± 361 μV (range 520–1460 μV). After cutting the denervated/reconnected lumbar ventral roots distal to the stimulation site, this potential activity disappeared in the quadriceps. No MAP was observed when the surrounding muscles were stimulated. When the distal portion of the cut denervated/reconnected lumbar ventral roots (caudal to the cut area) were stimulated, the MAPs reappeared nearly with the same latency and amplitude.

Additionally, MEPs were recorded in the denervated/reinnervated quadriceps of all experimental animals, with a mean amplitude of 256 ± 137 μV (range 91–496 μV; Table 1; Fig. 2). After cutting the denervated/reconnected lumbar ventral roots, the MEPs disappeared in the muscle site. In control animals, no MEP was recorded in the paralyzed quadriceps.

**TABLE 1**

<table>
<thead>
<tr>
<th>Experimental Animal</th>
<th>Behavior Improved*</th>
<th>MAP (μV)</th>
<th>MEP (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL1</td>
<td>+</td>
<td>1460</td>
<td>90.62</td>
</tr>
<tr>
<td>RL2</td>
<td>–</td>
<td>792.9</td>
<td>193.3</td>
</tr>
<tr>
<td>RL3</td>
<td>+</td>
<td>1406</td>
<td>197.2</td>
</tr>
<tr>
<td>RL4</td>
<td>–</td>
<td>937.5</td>
<td>302.7</td>
</tr>
<tr>
<td>RL5</td>
<td>+</td>
<td>519.5</td>
<td>496</td>
</tr>
</tbody>
</table>

*+ = the animal could maintain the lesioned hindlimb in hip flexion; − = the posture of the lesioned hindlimb was not changed.

In this examination, we found that the greater MAP was not always recorded in the three experimental animals that were able to maintain their lesioned limb in hip flexion. There were also some variations between MAP and MEP recordings. The greater MAP was not usually accompanied by the greater MEP in the same animal (Table 1).

**Histological Examination**

**Horseradish Peroxidase Retrograde Labeling Study.** The retrogradely labeled neurons were found in all experimental animals. The mean number of HRP-labeled neurons was 234 ± 178 (range 89–581). After analyzing the spinal sections, the labeled neurons were restricted to the ipsilateral T8–10 ventral horn, mainly close to the implanted NAG tip (Fig. 3). These neurons generally ranged in size from 25 to 30 μm. Judging by their location and size, most of the labeled neurons were probably thoracic motoneurons. In control animals, no labeled neuron was found in the spinal cord.

There was an obvious relation between the HRP labeling and what was observed clinically. The number of HRP-labeled neurons (581, 200, and 168, respectively) found in the three experimental animals able to maintain their lesioned limb in hip flexion were superior to those (131 and 89, respectively) in the two others.

**Histological Analysis of the NAG and Denervated/Reconnected Lumbar Ventral Roots.** Light microscopic analysis of the thionin-stained semithin sections demonstrated numerous clusters of myelinated axons in the NAG and the denervated/reconnected lumbar ventral roots (Fig. 4) in all experimental animals that received autografts. The majority (50–70%) of the regenerating fibers in the NAG successfully entered to the distal denervated/reconnected ventral roots. For example, in one experimental marmoset we found 10,174.5 regenerating myelinated axons/mm² in the NAG and 5426 fibers/mm² in the denervated/reconnected lumbar ventral roots; in another of the experimental animals we found 11,602 myelinated fibers/mm² in the NAG and 8,680 axons/mm² in the reconnected roots.

Electron microscopic examination of the ultrathin sections confirmed the presence of microfasciculi and regenerating axons in this denervated/reconnected root pathway (Fig. 5).

These results correspond with the HRP retrograde labeling study. In the animals with the most HRP-labeled neurons in the spinal cord the greatest number of regenerating fibers were shown in the reconnected root pathway.
Histological Analysis of the Denervated/Reinnervated Quadriceps. Neuromuscular junctions in the denervated/reinnervated quadriceps showed typical cholinesterase activity similar to that found in the contralateral side. The newly formed motor endplates in the denervated/reinnervated quadriceps were smaller and irregularly grouped unlike that regularly and horizontally located in the contralateral counterpart (Fig. 6). The muscle fibers of denervated/reinnervated quadriceps were thinner than normal.

Discussion

In this study we showed that an NAG can be used to treat lower thoracic SCI in adult marmosets. This was accomplished not by bridging the spinal cord rostral and caudal to the area of lesion but by establishing a bypass between the rostral ventral horn and the caudal ventral roots. The results obtained from clinical, electrophysiological, and histological examinations supported that the rostral spinal motoneurons regrew into the caudal denervated/reconnected lumbar ventral nerve roots through the NAG, thereby innervating the denervated target muscle.

Many studies conducted in humans, nonhuman primates, and other mammals have shown that sectioned peripheral axons can regenerate through foreign nerves and innervate different motor or sensory fields. Because one possibility for regaining the lost function following serious SCI (that is, transection or hemisection) is to offer an alternate source of axons, we designed this study to promote and guide axonal regrowth from the thoracic motoneurons (rostral to the hemisection site) to the caudal sectioned lumbar ventral roots to innervate the paralyzed hindlimb muscles. Regarding this type of innervation, some authors have demonstrated that functional connectivity can be reestablished between the intercostal nerves and the transected brachial or lumbar plexus. In previous work, we also showed that thoracic spinal neurons are able to regrow into the sectioned lumbar ventral roots via an implanted NAG, where they innervate the paralyzed quadriceps in adult rats after the same surgically-induced injury and repair procedures. Although the overall results are inconclusive and a convincing functional recovery is lacking, the neuroanatomical connections between these neurons and the target muscles were established. Although thoracic motoneurons do not ordinarily innervate hindlimb muscles, it is still questionable that innervation by these ectopic neurons can actually bring habitual functional recovery. However, the CNS has the capacity of adapting to severe changes in the arrangement of its outputs and inputs. Based on these results, we believe that additional treatments, including reeduca-

Fig. 2. A: Schematic drawing showing the electrophysiological examination procedures in this study. The numbers represent the area and order of the stimulation. The denervated/reconnected lumbar ventral nerve roots were cut after the MEPs (3) recording. The stimulation of 4 to 6 was performed after cutting the denervated/reconnected root pathway. B: Electromyographic recordings in the denervated/reinnervated quadriceps showing either typical evoked potentials (1, 3, and 5) or negative findings (2, 4, and 6) by each corresponding stimulation in an experimental animal 9 months after surgery. The mean amplitudes of the MAPs of 1 and MEPs of 3 in the five experimental animals were 1023 ± 361 μV and 256 ± 137 μV, respectively.
Innervation after nerve autograft placement

Both extrinsic and intrinsic factors can influence axonal regeneration from the spinal ventral horn to the ventral root. Because mainly or only axotomized neurons in adult mammalian CNS are receptive to the growth-stimulating conditions and can regrow their axons into the grafts, the limited size of a spinal cord lesion should be an important extrinsic factor. In addition to the axotomy itself, the distance between the cell body and the site of axotomy is also an important factor. It has been shown that the grafts were usually innervated by neurons within a few millimeters of the graft tip when they were inserted into small lesions in the brain or spinal cord. Considering such two important factors and the fact that spinal cord white matter plays a primary inhibitory role in axonal regeneration, implantation of a nerve graft into the spinal cord close to or in contact with the ventral horn (gray matter) is therefore prerequisite to allow spinal axonal regeneration and elongation into the graft. Transection or hemisection of the spinal cord can seriously impair the local blood supply, with consequent secondary tissue necrosis and cavity formation, which can rostrally diffuse to approximately two spinal cord segments (preliminary unpublished data) and may disturb axonal regeneration; therefore, the NAG in our studies was not implanted into the spinal cord through the hemisection (T-12) but through a small incision at the T-10 cord level. Bertelli, et al., have shown that useful motor function can be achieved after median nerve neurotization by peripheral nerve grafts implanted directly into the spinal cord. It is noteworthy that the peripheral nerve often contains both motor and sensory fibers. When the motor axons successfully regrew into the peripheral nerve stump, they could enter sensory branches and be directed to sensory end organs. Similarly, sensory axons could be led to motor end plates. Not only did these axons fail to establish functional communication, they also excluded appropriate axons from the pathways they occupied. Therefore, selective repair of motor or sensory branches is likely important for the peripheral target to be correctly innervated. Because such selectivity can occur at the spinal root and nerve trunk lev-

Fig. 3. Upper: Schematic drawing showing the location of HRP-labeled neurons in the thoracic spinal cord. These neurons were found in the ipsilateral T8–10 ventral horn, mainly close to the implanted NAG tip. The mean number of HRP-labeled neurons in the five experimental animals 9 months after surgery was 234 ± 178. Lower: Photomicrograph of a spinal longitudinal histological section (30 μm) showing the typical HRP-labeled neurons (arrows) in the left T9–10 ventral horn of an experimental animal. The head direction (H) and the site of central canal (stars) are indicated. Scale bar = 40 μm.

Fig. 4. Photomicrographs showing thionin-stained semithin cross-sections (1 μm) of the NAG and the distal denervated/reconnected lumbar ventral roots in an experimental animal. Numerous regenerating fiber clusters and myelinated axons (arrows) can be seen in the NAG (upper: scale bar = 50 μm) and denervated/reconnected lumbar ventral roots (lower: scale bar = 20 μm).
the lumbar ventral roots can be easily selected and reconnected.

Results of our present HRP study and histological analysis showed that the rostral thoracic motoneurons successfully regrew into the NAG and denervated/reconnected ventral roots. In addition to the facts that 1) MAPs and MEPs were elicited in the denervated/reinnervated quadriceps and eliminated upon cutting the reconnected ventral roots distal to the stimulation site, 2) newly formed motor endplates were found in the target muscle, and 3) negative results were obtained in all control animals, we underscore an additional observation: the reestablished neuroanatomical pathways in experimental marmosets should have occurred between the rostral ventral horn and the quadriceps muscle via the NAG. On the other hand, the behavioral recovery of the target muscle was very limited. No evidence of recovery of knee extension in their lesioned hindlimb was shown in the five experimental animals. This might be the result of inadequate regeneration, marked atrophy of the target muscle, and limitation of central plasticity. Nine months after surgery, in three of the five experimental marmosets we found that their paralyzed hindlimb was shown in the five experimental animals. This might be the result of inadequate regeneration, marked atrophy of the target muscle, and limitation of central plasticity. Nine months after surgery, in three of the five experimental marmosets we found that their paralyzed hindlimb could be maintained in hip flexion. Because the quadriceps muscle is a powerful extensor of the knee and because several other muscles can flex in the hip joint, this result might suggest that some hip flexors such as iliopsoas also received innervation from the denervated/reconnected root pathways. Generally, the iliopsoas muscle receives its innervation from L-1 (psosas portion) and downwards. However, the variability in the lumbar plexus, referred to as prefix/postfix, could occur and offer additional nerve pathways, thus directing some regenerating fibers to the iliopsoas. If it is true, the distribution of the regenerating axons was probably random, thereby causing a variable innervation of the quadriceps in the experimental animals and consequently causing the discrepancy between the electrophysiological recording from the quadriceps and the clinical manifestation of the lesioned limb. We also noted that the quality of the MEPs did not correlate with the quality of the MAPs. This result was probably due to the fact that the MEP monitoring assesses CNS–graft connectivity whereas the MAP monitoring assesses graft–peripheral nervous system connectivity. Surely, other elements also influence the MAP and MEP recording, such as the stimulation site and the number of reinnervated muscle fibers with which the bipolar recording needle makes contact. Interestingly, some reflex contraction of the denervated/reinnervated quadriceps was also recorded in these animals during passive mobilization of the lesioned hindlimb. Because the mobilization was cautiously performed in all animals after a state of ketamine-induced calm was initiated, there is little chance that traction of the abdominal wall muscles was involved. As behavioral performances generally reflect complex integration of sensorimotor processing, such reflex contractions probably indicated that some sensory receptors functioned in the denervated/reinnervated muscles. These stretch receptors might have survived or regenerated after

Fig. 5. Electron micrograph showing an ultrathin cross-section of the denervated/reconnected lumbar ventral roots in the same animal as shown in Fig. 4. Note the presence of microfasciculi and regenerating axons (arrows). Scale bar = 2 μm.

Fig. 6. Photomicrographs of longitudinal histological sections (30 μm) displaying cholinesterase activity in the denervated/reinnervated quadriceps and its contralateral counterpart. A: The typical endplates (arrows) in the contralateral quadriceps muscle. B: The newly formed endplates (arrows) in the denervated/reinnervated quadriceps. These new endplates were usually smaller and irregularly grouped, unlike those horizontally present in normal muscles. The muscle fibers of the denervated/reinnervated quadriceps were thinner than its contralateral counterpart. Scale bar = 200 μm for A and B.
the hemisection and then provided the afferent link to the thoracic motoneuron pools.

Taken together, the clinical and histological results range widely in the experimental animals. Such a discrepancy might be due to the surgical repair technique. For example, the site of implantation and the location of the autograft tip could play important roles in axonal regeneration. Because it is clear that the number of regenerating fibers appearing in the reconnected nerve pathway, the better the clinical recovery, improvement of such surgical treatment should be achieved with technical modifications, neurostimulation, and other possible beneficial methods such as application of neurotrophic factors.

Conclusions

Analysis of our results indicates that an implanted NAG bridging the rostral spinal ventral horn and the caudal ventral roots can promote axonal reinnervation of the peripheral denervated target, thereby restoring partial function in spinal cord–injured adult marmosets. In light of these findings, further studies should be undertaken to determine precisely the quality of such innervation and to assess the efficacy of the same repair technique in larger species.

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References


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