The fate of motoneurons in the spinal cord after peripheral nerve repair: a quantitative study using the neural tracer horseradish peroxidase

JOYCE A. GILMOUR, B.SC., LYNN M. MYLES, M.D., F.R.C.S.(ED), AND MICHAEL A. GLASBY, F.R.C.S.

Department of Anatomy, University of Edinburgh, Medical School, Edinburgh, Scotland

This study assessed the changes that occurred in the spinal motoneuron pool after the repair of a specific peripheral nerve by means of several clinically appropriate surgical techniques: nerve graft, muscle graft, and epineurial suture. The motoneuron pool relating to a single muscle was assessed at 50, 100, 200, and 300 days after repair via retrograde axonal transport of the neural tracer horseradish peroxidase. The results indicate that although a small portion of the motoneuron population dies following peripheral nerve surgery, this is not a significant number. The majority of the anterior horn cells appear to have the ability to both survive nerve transection and form new functional connections with the regenerated nerve after repair. The degree of cell loss is influenced by the nature of the injury and the method of repair implemented. Injuries involving neurotmesis result in the loss of a greater proportion of the cell population than less severe injuries involving axonotmesis. A greater proportion of the motoneuron population is preserved when the severed nerve has been repaired using a direct epineurial suture than when repair is achieved by means of a graft. The two methods of grafting produced comparable results, although the muscle graft tended to result in the preservation of a greater number of cells than the nerve graft, making it an acceptable alternative method for the surgical repair of short gaps in peripheral nerves.

KEY WORDS • peripheral nerve • muscle autograft • nerve regeneration • nerve repair • motoneuron • horseradish peroxidase • rat
Materials and Methods

The experimental and control groups each consisted of five male Sprague-Dawley rats weighing between 300 and 400 g. The animals were anesthetized and their left sciatic nerves were exposed. As far as possible, injuries were created approximating a model for the simple injuries seen in clinical practice. To create the crush injury (axonotmesis), the nerve was placed in a pair of smooth-jawed micromanipule holders, which were then closed to the second ratchet and held for 10 seconds; no formal repair was undertaken. Direct epineurial suture, using four to six 10-0 polyamide interrupted sutures, was undertaken to repair nerves that had been transected by means of a Meyer neurotome. Where grafts were inserted, a 1-cm length of nerve was excised using the Meyer neurotome. For the nerve graft groups, this excised segment was reimplanted using four to six 10-0 polyamide interrupted sutures. Coaxial muscle grafts were prepared using the technique described by Glasby and coworkers,12–15 in which autograft muscle, with parallel aligned fibers, was obtained from biceps femoris. The 1-cm muscle grafts were implanted using four to six 10-0 polyamide interrupted sutures. Standard microsurgical techniques were used throughout. The groups of rats were allowed to recover for 50, 100, 200, and 300 days.

Assessment of the Motoneuron Pool

The living motoneuron pool relating to a single muscle was assessed at 50, 100, 200, and 300 days after operation by means of retrograde axonal transport of the neural tracer horseradish peroxidase (HRP). The rats were anesthetized and 50 μl of 20% HRP solution was injected along the length of the left extensor digitorum longus muscle. Great care was taken to ensure that all areas of the muscle underwent a color change as this implied that adequate and complete infiltration by the HRP solution had occurred. The tibial and sural nerves were transected and cauterized to ensure that the only remaining route from the extensor compartment of the lower limb to the spinal cord was the extensor digitorum longus branch of the peroneal component of the repaired sciatic nerve; there could then be no unwanted uptake of HRP from surrounding tissues.

The rats were returned to normal conditions for 48 to 72 hours to allow transport and accumulation of HRP in the spinal motoneurons. Mesulam26 showed that sacrifice and assessment within this time period will give reproducible results. A transcardial perfusion was then performed, using procedure II as described by Rosene and coworkers,12–15 in which autograft muscle, with parallel aligned fibers, was obtained from biceps femoris. The 1-cm muscle grafts were implanted using four to six 10-0 polyamide interrupted sutures. Standard microsurgical techniques were used throughout. The sections were subjected to the tetramethyl benzidine procedure described by Mesulam26 and underwent an enzymatic reaction. The suggested incubation time resulted in the formation of an unacceptable level of artefact; therefore the timing was modified accordingly. The sections were mounted on chrome alum-coated slides and counterstained with 1% neutral red prior to being dehydrated, cleared, and mounted. All sections and slides were stored at 4°C, because enzyme denaturation is greatly reduced at lower temperatures.

Control Group

Five same-aged rats that were not treated surgically were used as normal controls for comparison. Six additional rats were used as controls to assess possible HRP leakage. The six animals were injected with HRP as described previously, however in three animals the extensor digitorum longus muscle was denervated by cutting all supplying branches from the peroneal nerve; in the other three animals, the peroneal nerve was ligated with 3-0 silk sutures at the knee. The tibial and sural nerves were then transected and cauterized as before. No reaction product was subsequently found in the motoneurons of the spinal cord. The lack of reaction product indicates that there was no significant leakage of HRP from the extensor digitorum longus muscle into the surrounding tissue and that denervation of the tibial and sural territories was complete.

J. A. Gilmour, L. M. Myles, and M. A. Glasby

The sections were examined microscopically and a morphometric analysis system* was used to obtain uncorrected counts of all the HRP-labeled cell bodies within the ventral horn of the spinal cord. The Student t-test was used to test the significance of differences found in the number of labeled cells in the spinal cord.

Results

Assessment of the Grafts

All of the rats showed progressive signs of recovery up to 300 days after surgery, by which time the toe-spreading reflex could be elicited from 92% of animals.43 The return of this reflex is believed to reflect good reinnervation by the sciatic nerve.28 The remaining 8% of animals who failed to regain their toe-spreading reflex on the operated side were found to have acquired fixed deformities of the ankle joint that prevented any movement of the foot. This occurred in five animals whose nerves had been transected and then repaired by means of the muscle graft and in one that had received a nerve graft. The histological methods employed by Glasby10 were used to assess the grafts and the distal nerves. The histological appearance in the present experiments was similar to that described in a variety of studies by Glasby and colleagues12 and others on the use of nerve and muscle grafts and was thus assumed to be normal.

The labeled motoneuron pool associated with the extensor digitorum longus muscle was located within the L3–5 levels of the spinal cord. In control animals the cells formed a discrete population concentrated primarily in lower L-4 and L-5 levels, whereas in experimental animals the labeled cells were more widely distributed and extended from the upper L-3 level through L4–5. Figure 1 (upper left) shows the mean ± standard error of the mean number of labeled motoneurons in the spinal cords of control animals at 50 days after injury and repair broken out by each of the three methods. The standardized crush of the nerve resulted in a loss of only 14% of the normal cell population compared to the greater loss of 43.79%, 55.49%, and 68.92% of cells following a direct epineurial suture, freeze-thawed muscle autograft, and full-thickness nerve autograft, respectively. The latter three groups were significantly different from normal values but not from one another (p < 0.05).

One hundred days after the nerve injury and repair (Fig. 1 upper right), the nerve crush was still associated with a greater number of labeled cells (loss of 72 cells, 19.41%). This difference was only significant (p < 0.05) when compared to the nerve graft result. The three injuries involving transection were associated with values significantly less (p < 0.05) than normal values. By 200 days the trend had changed somewhat with the muscle graft associated with many more labeled cells than either the nerve graft or the direct epineurial suture and producing a very similar result to the nerve crush. There was no significant difference (p > 0.05) among the four repaired groups, although values for the nerve graft and direct epineurial suture were significantly less (p < 0.05) than normal control values (Fig. 1 lower left). At 300 days (Fig. 1 lower

---

* Magiscan system obtained from Joyce-Loebl, Ltd., United Kingdom.
Spinal cord motoneurons after peripheral nerve repair

**FIG. 1.** Graphs showing the mean number ($\pm$ standard error of the mean) of horseradish peroxidase–labeled motoneurons in the L3–5 levels of the spinal cords of control rats at 50, 100, 200, and 300 days after injury and repair by muscle graft, nerve graft, or epineurial suture (N-N). **Upper Left:** At 50 days, the nerve crush results in less normal-cell loss than in any of the three repairs. **Upper Right:** At 100 days, the nerve crush is still associated with less nerve loss. **Lower Left:** At 200 days, the nerve crush and muscle graft are similar. **Lower Right:** At 300 days, there is no significant difference between the four groups.
right there was no significant difference ($p > 0.05$) among any of the experimental groups when compared to each other, although the nerve graft and direct epineurial suture results were significantly less ($p < 0.05$) than control values.

Table 1 and Figure 2 show the changes in the mean number of labeled motoneurons in the spinal cord after each method of injury and repair and at each time period after operation. N-N suture = epineurial suture.

![Figure 2](image_url)

**Table 1**

<table>
<thead>
<tr>
<th>Repair</th>
<th>Days After Operation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>muscle graft</td>
<td>39.98 ± 16.79</td>
</tr>
<tr>
<td>N-N suture</td>
<td>50.49 ± 25.75</td>
</tr>
<tr>
<td>nerve graft</td>
<td>27.91 ± 20.55</td>
</tr>
<tr>
<td>nerve crush</td>
<td>77.25 ± 21.35</td>
</tr>
<tr>
<td>normal</td>
<td>89.82 ± 2.61</td>
</tr>
</tbody>
</table>

* Numbers are means ± standard error of the mean.

Figure 1 indicates the different influences each of the methods of repair has on the motoneuron population and, as expected, the nerve crush regularly resulted in the loss of fewer motoneurons than any of the three injuries involving transection of the nerve.

Direct epineurial suture initially preserved a greater number of labeled cells than either of the grafting methods. The most significant result arose from the comparison of the nerve graft and muscle graft data because these involved the same nerve gap to be bridged and same number of suture lines in the repair. There was some qualitative variation in the results, with a consistent finding that more motoneurons became labeled when muscle grafts were used; however, this finding was not statistically significant ($p > 0.05$) and should not be taken to imply a different potential clinical outcome.

**Discussion**

Researchers have found that, following the surgical repair of peripheral nerves, there is a slow but incomplete return toward normality in terms of function, anatomical features, and physiological characteristics. This is in keeping with the present study in which rats showed progressive signs of recovery up to 300 days after operation, by which time 92% of all animals exhibited the toe-spread reflex. Those animals that failed to regain their toe-spread reflex were found to have fixed ankle flexion, which prevented any movement. Brushart obtained a similar result after the regeneration of the sciatic nerve across an 8-mm gap, which was encased in mesothelial tubes. In each of the recorded cases of fixed ankle flexion, the method of repair used has been one that tends to result in a prolonged period for regeneration (two suture lines in the case of the grafts and regeneration over a gap in Brushart’s report). Although this may appear to be a major disadvantage to the use of such methods of repair, it must be remembered that this result is unlikely in humans, because routine physiotherapy following such a surgical procedure would tend to ensure its prevention.

**Postoperative Changes in the Number of Spinal Motoneurons Associated With the Extensor Digitorum Longus Muscle**

The chromatolytic changes that occur in the cell body after peripheral nerve section have been well documented in the literature. There is general agreement that such changes are most pronounced between 1 and 3 weeks af-
Spinal cord motoneurons after peripheral nerve repair

ter nerve section and that any peripheral neurons that are going to die and degenerate will do so within the first 5 weeks after injury.2134 The neurons that have successfully regenerated axons subsequently recover from chromatolysis. The degree of chromatolytic change and cell loss that occurs following peripheral nerve section is dependent on the species of animal,19,20 distance of the injury from the spinal cord,7,19 whether repair was undertaken,21,31,42 and the age at which the injury occurs.3,17,19,21,31 The loss of motoneurons is particularly marked in neonatal and developing animals, because the immature cells do not have the same ability to resist trauma to their axons and separation from their muscle.33 The degree of cell loss is much less marked in cases in which the nerve has been repaired and allowed to regenerate;21,42 however, even in such cases muscle reinnervation is found to be abnormal not only in degree but in specificity.35

The reports of other authors have indicated that the number of functional motoneurons present in the spinal cord following peripheral nerve repair is significantly less than normal values.5,25

These findings also hold true for this study. Indeed, the findings of Brushart, et al.,4 that the motoneuron population was 69% of its normal value 3 months after repair by direct epineural suture, compare very well with our result of 68.53% at 100 days after the same repair. The failure of many anterior horn cells to regain peripheral connections must reflect inherent limitations in response to peripheral nerve injury; however, this may be affected to some extent by the surgical technique applied.5 Brushart, et al.,5 compared two different methods of repair: direct epineural suture and individual fascicular suture. The use of individual fascicular suture produced significantly better results than the direct epineural suture in accuracy of reinnervation; however, there was no significant difference in numbers of labeled motoneurons between the two methods of repair. Again, these findings hold true for the present study in which there was no significant difference between the methods of repair that involved suturing, although this was not the case when compared with the nerve crush. Brushart explained his result by the fact that the epineural suture allows the fascicles to change position within the epineural sheath, which leads to subsequent misalignment.

In this study, the better results obtained following a nerve crush also can be attributed to the fact that neural alignment was maintained throughout and would therefore lead to a greater degree of reinnervation. This may be an additional contributory factor to the consistent, although not significant, differences found among the different methods of repair. The direct epineural suture consistently produced better results than either of the repair techniques that involved the use of a graft because the use of a graft results in the regenerating nerves having to negotiate two sets of suture lines, as opposed to the one involved in a direct epineural suture. This negotiation increases the probability of mismatch between axons and endoneurial tubes and ultimately with target organs.

Postoperative Changes in the Position of the Spinal Motoneuron Pool Associated With the Extensor Digitorum Longus Muscle

The normal topography of specific groups of motoneurons in the spinal cord is well established.4–6,30,33,35,36,40,41 and there is general agreement that the motoneuron pool associated with a specific muscle lies in a longitudinally oriented column in the lateral ventral horn, extending for up to three adjacent ventral roots, with α and γ motoneurons intermingled. The results of our study are consistent with the finding that the labeled motoneuron pool associated with the extensor digitorum longus muscle is located within the L3–5 regions of the spinal cord. However, there appears to be a shift in the peak concentration of labeled cells between the control and experimental populations. In control animals, the cells formed a compact population concentrated primarily in lower L-4 and L-5, whereas in experimental animals the cells regularly extended from upper L-3 through L-4–5. Brushart and Mesulam7 found a similar shift in the topographical position of the motoneuron population following the repair of a peripheral nerve by means of a direct epineurial suture. These postoperative anatomical alterations are being studied in greater detail both to quantify the degree of topographical shift and assess to what extent the shift is influenced by the method of repair.

Results of Peripheral Nerve Repair by Two Methods of Grafting

It was of considerable interest to find that repair of a nerve gap with a freeze-thawed muscle graft was associated with the consistent labeling of greater numbers of anterior horn cells than when a similar repair was performed with a full-thickness nerve graft. The relatively few clinical studies that have been conducted using muscle grafts have demonstrated that the grafts perform poorly if used to repair gaps of more than 5 cm. Muscle grafts seem to have considerably less usefulness in mixed nerves than in nerves that are entirely sensory or entirely motor. Pereira, et al.,32 suggested that a short muscle graft produces a better level of regeneration in digital nerves than direct suture. This is probably because the latter is almost always associated with a degree of tension at the suture line.

There can be little doubt that in human clinical practice, interfascicular repair27 is preferable to any other grafting technique, especially when long gaps are involved. Previous studies29 have suggested that for short nerve gaps in the rat there is no difference between muscle and nerve grafts with respect to recovery of function or electrophysiological and morphometric indices of recovery in the peripheral nerve. The small number of animals used in this study show a greater labeling of anterior horn cells from the extensor digitorum longus when muscle grafts are used, but this does not reach statistical significance (p > 0.05). A larger study would reveal whether this phenomenon is real or is a product of the statistical methods.

These experiments indicate that, within the constraints of the experimental model, the preservation of motoneuron cytoarchitecture falls off with the progression in the method of repair from direct suture to grafting. Within the latter group and over short distances, the method of grafting has little or no effect. Previous studies10–16 have attempted to assess functional recovery after different methods of nerve repair. Although both morphological and electrophysiological indices of recovery have invariably been different from those on the normal side, this is
expected and does not imply an inability of reinnervated muscle to generate normal levels of isometric tension. With this in mind, it may be concluded from the present study that there is also little correlation between the changes in morphology seen at a spinal level after reinnervation and the commonly measured indices of function. It therefore would appear that the failings of any given method of nerve repair are more likely to be defined by the events at the periphery than those occurring centrally, where a fairly uniform response is to be expected. This study has involved immediate repair only. Despite an increasing amount of information that delayed repair is inferior to immediate repair, the former is still widely practiced. It would be of considerable interest to compare the results obtained here with a similar study in which delayed repair of the peripheral nerve was used. It seems likely, from a speculative point of view, that this avenue will prove fruitful in distinguishing the relative value of immediate and late repair of peripheral nerves.

Acknowledgments
The authors would like to thank Mrs. J. S. Wood and Mr. R. Shields for their skilled technical assistance.

References

J. Neurosurg. / Volume 82 / April, 1995
Spinal cord motoneurons after peripheral nerve repair


40. Sherrington CS: Notes on the arrangement of some motor fibres in the lumbrosacral plexus. *J Physiol (Lond)* 13:621–772, 1892


Manuscript received February 5, 1994.
Accepted in final form July 6, 1994.
This work was supported by the Sir Jules Thorn Charitable Trust, with additional financial assistance from the Ian Karten Charitable Trust, the Smithkline Foundation, and Tenovus Scotland.

*Address reprint requests to:* Joyce A. Gilmour, B.Sc., Department of Anatomy, University of Edinburgh, Medical School, Teviot Place, Edinburgh, EH8 9AG, Scotland.