Effects of different methods of peripheral nerve repair on the number and distribution of muscle afferent neurons in rat dorsal root ganglion

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

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

The effects of three methods of peripheral nerve repair and normal controls were compared with respect to the number and distribution of muscle afferent nerve cell bodies from the extensor digitorum longus muscle in the dorsal root ganglia of the rat. Nerves were repaired using one of three methods: 1) direct epineurial suture; 2) a three-strand cable graft; or 3) a coaxially aligned freeze-thawed autologous skeletal muscle graft. In all cases the number and distribution of muscle afferent nerve cells were estimated 300 days after nerve repair.

Retrograde transport of horseradish peroxidase, injected into the extensor digitorum longus muscle, was used to identify muscle afferent nerve cell bodies in dorsal root ganglia exposed at laminectomy after fixation. It was found that all methods of nerve repair were associated with a change in both the number and distribution of labeled muscle afferent cell bodies. The number of labeled muscle afferent cells was significantly different from normal controls in all methods of repair, but was not significantly different among the three methods compared. On the other hand, cell size and cell distribution departed significantly more from normal after either of the grafting procedures than after direct repair. There was no significant difference between the two grafting techniques. These results are discussed with respect to their mechanistic and clinical interpretation.

KEY WORDS - peripheral nerve - muscle autograft - dorsal root ganglion - muscle receptor afferent - nerve regeneration - nerve repair - rat

In any consideration of peripheral nerve repair and regeneration, the importance of muscle sensory reinnervation must not be overlooked. There is an obligatory linkage between normal motor function, its control, and its coordination. Any degree of recovered motor power is useless if it cannot be controlled and coordinated appropriately. Patients who have undergone peripheral nerve repair complain more often about loss of fine control and coordination (especially in the hand) and of sensory disturbance than about loss of power after nerve repair.32,33

Several different groups of sensory nerve fibers can be found to innervate skeletal muscle; these findings are summarized in most comprehensive textbooks of anatomy or neurophysiology. Sensory nerve fibers have their cell bodies within dorsal root ganglia. When a peripheral nerve is severed, these nerve cell bodies undergo a series of reactive changes known as chromatolysis,8,34 which represents a nonspecific manifestation of cell injury. Sensory neurons appeared to be affected more severely.13,36 A return toward normal cell morphology was characteristic of the recovery and maturation phase of peripheral nerve regeneration and occurred only if axonal growth was successful. However, not all cells in repaired nerves regained normal cell morphology after regeneration; some remained atrophied while others appeared to have died.24,30,31,37,38

The extent of cell loss produced by peripheral neurotomy in various species has been reported by several authors and is by no means consistent, with cell losses varying from 7% to 53%. Differences occur with respect to both the species and anatomical site of the lesion. The precise factors that determine whether a cell will survive, atrophy, or die after distal neurotomy remain uncertain. Factors known to influence the process include the level of injury, since lesions nearer to the cell body produce more cell loss than lesions further away.5,31 Age appears also to be a contributing factor, since neonatal rats experience greater cell loss than do older rats for a given nerve lesion.31 The severity of the lesion is also important, with crush injuries producing loss of fewer cells than neurotomy.24 Ranson7 noted that cells were lost whether or not regeneration took place. However, Schmalbruch11 stated that cells were lost whether or not regeneration occurred. However, Schmalbruch11 stated that cells were lost whether or not regeneration took place. However, Schmalbruch11 stated that cells were lost whether or not regeneration took place. However, Schmalbruch11 stated that cells were lost whether or not regeneration took place. However, Schmalbruch11 stated that cells were lost whether or not regeneration took place. However, Schmalbruch11 stated that cells were lost whether or not regeneration took place. However, Schmalbruch11 stated that cells were lost whether or not regeneration took place.
epineurial suture in cat common peroneal nerve resulted in a reduction of muscle afferent cells to 24% \(^2\) or 45% \(^3\) of normal.

It can be seen from the above that many aspects of sensory nerve regeneration require clarification. Much of the work has been accomplished on varying species at different ages, and the nature of the nerve lesion is not always clear. The experiments detailed here were designed to answer the questions: "What degree of muscle sensory cell loss and atrophy is associated with various methods of nerve repair, and what effect does nerve repair have on the muscle afferent organization of the dorsal root ganglion?" In addition to a consideration of conventional methods of nerve repair, the method of grafting using freeze-thawed skeletal muscle autografts is included in this comparison.

**Materials and Methods**

Twenty-six adult Sprague-Dawley rats with a mean weight of 350 gm were studied. A 1-cm gap in the left sciatic nerve was repaired either by direct epineurial suture (five rats), an autologous freeze-thawed muscle graft (five rats), or the interposition of a three-strand cable graft (five rats). An operating microscope and standard microsurgical technique were used throughout. Five rats that did not undergo operation were studied for comparison ("normals"). Six rats with nerve section and ligation but no repair were used as controls.

**Repair with Epineurial Suture**

The sciatic nerve was exposed by reflecting the biceps femoris muscle from its origin in five rats. The nerve was divided 1 cm from the sciatic notch and a piece was excised to leave a gap of 1 cm. Careful mobilization of the ends allowed apposition without significant tension. An epineurial repair was carried out using four to six 10-0 polyamide (Ethilon) sutures. The peroneal and tibial fascicles were aligned as closely as possible. The overlying muscle, subcutaneous tissues, and skin were closed in layers using interrupted 6-0 Vicryl sutures.

**Repair Using Cable Graft**

It proved impossible to harvest enough autologous nerve from a single rat since this would have meant paralyzing two limbs, which was not acceptable to the Home Office Inspector. This problem was solved by using inbred littermates, where one rat was sacrificed to provide enough isogenous nerve to graft four other rats. Rejection was not a problem in these rats, which are bred specifically to minimize genetic differences. There was no significant difference between the regenerative response to an isogenous nerve graft versus an autologous nerve graft in these rats at 100 days after repair.

A cable graft was fashioned from three 1-cm strands of median, ulnar, and radial nerves. Two strands of cable graft were aligned with the tibial fascicle and one with the peroneal fascicle. The three strands were held together in a bundle by a drop of fibrin glue (Tisseel\(^\text{a}\)), thus making them easier to handle. One or two 10-0 polyamide epineurial sutures were inserted into each strand to hold the graft in position. Care was taken not to allow fibrin glue to coat the cut surfaces of the nerve. Although in this situation fibrin glue is a xenograft, it has been shown by others not to influence the regenerative response (unpublished data). The wound was closed as described above.

**Repair Using Muscle Graft**

In five rats the nerves were repaired using a freeze-thawed method of muscle grafting described elsewhere.\(^{10-12}\) A strip of biceps femoris muscle measuring approximately 2 x 0.75 cm was removed, making sure that the muscle fibers ran parallel to the long axis. Enough fresh muscle must be removed to allow for up to 50% shrinkage during freezing and thawing. The muscle was dipped into liquid nitrogen until frozen (1 to 2 minutes), then quickly thawed in distilled water causing disruption of the muscle cells and trimmed to size. It was sutured between the cut ends of the nerve using four 10-0 polyamide sutures at each end. The sutures passed through the epineurial sheath of the nerve and involved a large portion of the muscle graft. The wound was closed as described above.

**Control Group**

Six rats were used as controls to measure horseradish peroxidase (HRP) leakage. In three control rats, HRP solution was injected into the left extensor digitorum longus muscle. The muscle was then totally denervated by cutting all branches to it from the peroneal nerve. The tibial and sural nerves were cut and ligated with silk. No reaction product was found in the dorsal root ganglia of L2-6 after 72 hours in these rats. In the remaining three rats, HRP solution was injected into the extensor digitorum longus muscle and the peroneal nerve was ligated at the knee with 3-0 silk sutures. The tibial and sural nerves were cut. No reaction product was found in the dorsal root ganglia of L2-6 after 72 hours in these three rats. This was interpreted as showing that there was no significant leakage of HRP into surrounding muscles and that denervation of the tibial and sural territories was complete.

**Experimental Procedure**

At 300 days after the operation, the rats were anesthetized, and the extensor digitorum longus muscle on the surgical side was exposed through a skin incision on the lateral aspect of the lower limb from the knee to the dorsum of the foot, thus exposing the tibialis anterior muscle and its tendon. This tendon was cut and pulled proximally to expose the tendinous expansion of the extensor digitorum longus muscle running under the extensor muscle retinaculum. This was followed upward to the muscle belly, taking care not to disturb the neurovascular bundles entering the muscle. The muscle belly was injected with 50 \(\mu\)l of a 20% HRP solution using a Hamilton microliter syringe and ensuring that all areas of the muscle became infiltrated with solution. The tibial and sural nerves were divided.

| L. M. Myles, J. A. Gilmour, and M. A. Glasby |
| J. Neurosurg. / Volume 77 / September, 1992 |

458
Peripheral nerve repair in rat dorsal root ganglion

as they left the main sciatic trunk. A piece of each of these nerves was excised to ensure no regeneration or leakage across the gap. The wound was closed with 6-0 Vicryl sutures. The rats recovered and were allowed free access to food and water. Between 40 and 48 hours after this procedure, the rats were again anesthetized and killed by perfusion through the heart. In each rat the chest was opened by a median sternotomy, and 0.1% sodium nitrite was injected into the left ventricle of the heart to produce vasodilatation. Normal saline (250 ml) was then infused to flush out red blood cells, and the left ventricle was perfused with 500 ml of 1.25% glutaraldehyde with 1% paraformaldehyde fixative at room temperature, followed by 100 ml of sucrose buffer solution.

The dorsal root ganglia of L2-6 were removed by performing a thoracolumbar laminectomy under a dissecting microscope. The T-13 spinal nerve was identified running under the last rib, and the T-13 dorsal root could be followed backward to the spinal cord. The lumbar dorsal roots could be identified by counting downward from T-13. The dorsal root ganglia could be seen as fusiform swellings of the dorsal roots, lying in the spinal foramina. They were removed, labeled, and stored individually in sucrose buffer at 4°C. The ganglia were frozen in embedding medium, oriented longitudinally, and cut serially at 40 μm in a Reichart-Jung freezing microtome. Sections were stained using the TMB method of Mesulam.13 They were examined under a compound microscope and only stained cells that showed a nucleolar profile were counted. Since the nucleolus is single, central, and easily identified,14 noting this feature ensured that no cell was counted twice in successive sections unless the nucleolus had been split with the microtome knife. A correction factor for split nucleoli was applied, taking into account the size of the nucleoli and the thickness of the section.15 The corrected count was calculated as follows:

\[ \text{corrected count} = \frac{\text{measured count} \times t}{t + 2r} \]

where \( t \) = section thickness (in μm) and \( r \) = the mean radius of the unit counted (in μm).

Cell diameters were measured using the Magiscan computerized morphometric analysis system and data entered into Lotus "Symphony" spreadsheet software for statistical analysis and graph plotting on a Tandon AT computer system.

Each section was also examined under a light microscope with a drawing attachment. Scale drawings were made of serial sections, and the positions of stained cells were carefully marked. Each section was copied onto transparent acetate sheets which could then be superimposed to give a three-dimensional image of each dorsal root ganglion. In this way the distribution of stained cells within a given ganglion could be estimated.

Results

All rats made a good recovery by 300 days although, as expected, function never quite returned to normal.23 The toe-spreading reflex could be elicited in all animals and was thought to reflect good functional sciatic nerve regeneration.

Number and Distribution of Labeled Cells

Figure 1 shows the mean number of labeled cells in each dorsal root ganglion from L2-6 in the normal rats and following the three methods of repair. No labeled cells were found in the L-2 ganglion. In the normal rats, labeled cells were found predominantly at two levels, L-4 and L-5, with few cells counted outside this range. In any one normal rat, labeled cells were found in two distributions, each restricted to three consecutive levels, L-3 or L-4-6. After direct nerve suture, this pattern could still be seen but with fewer cells at L-4 and L-5 and with slightly more cells at L-3 and L-6. After cable grafting or muscle grafting, the spread of cells was much greater, with significant numbers found beyond the L-4-5 band. In any individual rat, labeled cells could be found at all levels. This was presumably due to misdirection of fibers within the graft leading to disorganization of sensory reinnervation.

The mean total numbers of labeled cells over levels L-3-6 in normal rats and in rats following repair by direct epineurial suture and by cable and muscle grafting can be seen in Fig. 2. After repair by direct neurotomy, the mean cell loss from control values was 29% (range 24% to 36%). After cable grafting or muscle grafting, the mean cell loss was 36% (cable graft range 26% to 45%, muscle graft range 32% to 42%). Cell counts after all three methods of repair were significantly different from normal (p < 0.0005). However, cell counts following all types of repair were not significantly different from each other in any combination, although absolute counts after direct suture were higher than after grafting procedures.

Distribution of Cell Size Following Repair

The frequency distributions of dorsal root ganglion

---

J. Neurosurg. / Volume 77 / September, 1992

459
cell diameters in normal rats and after sciatic nerve repair by direct suture or muscle or cable grafting can be seen in Fig. 3. Repair by any of these methods was followed by a reduction in mean cell size. The mean cell diameter was 42.29 ± 14.64 μm (± standard deviation) in normal rats (652 cells), 38.28 ± 12.48 μm after epineural repair (422 cells), 25.12 ± 12.24 μm after repair by muscle grafting (385 cells), and 35.00 ± 11.74 μm after repair by cable grafting (314 cells). Large cells (diameter > 60 μm) were seen less often after nerve repair. After all three methods of repair, the cell diameter was significantly different from that in normal rats. There was no significant difference in cell size between muscle- and cable-grafted rats, but both of these groups had distributions that were different from rats that had undergone repair by direct suture at a significance level of p < 0.01 and from normal rat sciatic nerve at a significance level of p < 0.0005.

Arrangement of Cells Within the Dorsal Root Ganglion

In a normal animal the dorsal root ganglion cells lie in irregular-shaped cords within the ganglion, intermingled with axons, and there is a definite clustering of afferents from the extensor digitorum longus muscle toward the superior pole of the ganglion, suggesting that there is a degree of somatotopic arrangement of muscle afferents. This pattern is lost after all three methods of nerve repair, with labeled cells being scattered throughout the ganglion.

It is apparent, therefore, that nerve repair by any means is associated with a change in both the number and distribution of muscle afferent nerve cell bodies in the appropriate dorsal root ganglia. The departure from normal values with respect to cell number was significantly different, although there was no significant difference among the three methods of repair. Direct suture appeared to offer marginally better preservation of cell size than the more invasive methods using grafts, but these did not significantly differ when compared to each other. Cell redistribution was greater where grafting procedures replaced simple neurorrhaphy, although the specific nature of the grafting process did not appear to affect the final outcome.

Discussion

Cell Numbers

After each of the three methods of repair undertaken in this study, the total labeled dorsal root ganglion cell counts were significantly reduced from normal levels. Mean normal cell counts (113 ± 4) compare well with those (95 ± 10) made by Peyronnard, et al.25 The mean cell loss after muscle-graft and cable-graft repair was 36%, but it is important to note that the difference between this value and that obtained for direct epineurial suture repair (29%) did not reach statistical significance. It would therefore appear that the extent of cell loss is related to the process of neurotomy and degeneration rather than to whether successful regeneration takes place. This confirms Ranson's original statement.26,27

There has been some debate in the literature as to whether the HRP method gives accurate cell counts. It has been stated that the HRP method underestimates the motoneuron pool,22 and figures of 70% have been found compared to axon counts in the nerve trunk.20 After neurotomy, injured cells do not take up or transport protein tracers (such as HRP) as well as normal nerves.12,23 This failure of uptake and transportation of HRP was found to continue as long as regeneration was
Peripheral nerve repair in rat dorsal root ganglion

prevented and was particularly severe in primary sensory neurons of large diameter. It has been shown that reattaching transected nerves to their own distal segments delayed the decrease in HRP labeling of dorsal root ganglion neurons and sustained the uptake of HRP by many large cells for a long period of time (up to 80 weeks). It would seem reasonable therefore to assume that HRP labeling 300 days after repair with the direct nerve suture method or other grafting method is of a sufficient degree to allow accurate cell counts to be made. It would have been interesting to look at transected nerves without repair for comparison, but unfortunately the use of HRP to assess cell loss after nerve section without repair is not entirely satisfactory and is subject to large errors for the reasons explained above.

**Cell Size**

Normal dorsal root ganglion cells in our study showed a unimodal size distribution on labeling (Fig. 3), in keeping with the findings of other authors. Repair by each of the three methods was followed in the present study by a reduction in cell size that was significantly different from normal. This has been noted after direct nerve suture by many authors and is thought to result from both loss of large cells and atrophy of the remaining cells. Cell diameters of muscle- and cable-grafted muscle afferents in the dorsal root ganglion were not significantly different from each other, but both were significantly different from those measured after repair by direct suture. The degree of atrophy of any neuron is said to be dependent on whether appropriate peripheral connections have been made by its axons. This would explain why the ganglion cells in which nerves have been repaired by direct suture experience less atrophy than those repaired by grafting, since the presence of two suture lines in grafted nerves increases the probability of mismatch between axons and appropriate endoneurial tubes. It is encouraging for the clinician that there is no difference in this respect between cable grafts that are technically difficult to insert and muscle grafts in which insertion is quicker and simpler. When the cable graft was inserted, every effort was made to align peroneal fascicles with the peroneal nerve and tibial fascicles with the tibial nerve. There is no opportunity to do this with a muscle graft, but it would appear from the evidence presented here to make no difference in the degree of mismatch. Presumably, infrafascicular plexus disorganization was unaffected by aligning major fascicles and remained a substantial cause of mismatch of axons with end organs.

Loss and atrophy of the largest-diameter dorsal root ganglion cells after nerve repair is presumably related to loss of large-diameter fibers associated with spindles and tendon organs. This may explain the lack of coordination seen after nerve injury.

**Somatotopic Organization**

Somatotopic organization is well recognized in the sensory cortex of the mammalian brain. This somatotopic representation of sensory modalities is also found in the dorsal horn of the spinal cord. In the normal rat, afferents from muscles innervated by the peroneal nerve tend to project to a sharply defined area of the ipsilateral dorsal horn (Rexed's lamina 2 and 3) at the L-3 segment level. Nerve section and repair caused the muscle afferent projection to expand dramatically within the substantia gelatinosa and to extend caudally into the L-4 segment. A somatotopic arrangement of muscle afferent cell bodies within the dorsal root ganglion has not been shown. Peyronnard, et al. commented on a degree of clustering of muscle afferents in the dorsal root ganglion of the rat, but found this to be inconsistent.

In this study, afferents from the extensor digitorum longus muscle in the normal rat were found to be clustered together in the dorsal root ganglion. This arrangement was found in all five rats in the normal group, suggesting that there is a degree of sensory organization to be found. The arrangement of other normal hindlimb muscle afferents, such as from the tibialis anterior and soleus muscles, should be examined to see if there is a true somatosensory distribution across the ganglion as a whole. The localized arrangement of cells was lost after all three methods of nerve repair. This was thought to reflect axonal mismatch during regeneration.

With regard to the spinal segment level, normal extensor digitorum longus afferents were found predominantly in L-4 and L-5 ganglia with occasional cells in L-3 and L-6. Stained cells were only found at three levels in any one rat (L-3-5 or L-4-6). This finding agrees with the report of Peyronnard, et al. After nerve repair, cells were found throughout L-3-6 dorsal root ganglia in all rats. The distribution of cells was widest in nerves repaired by cable and muscle grafting, with no apparent difference between them. Thus, it would seem that there are two levels of disorganization seen after peripheral nerve repair, one at the spinal segment level and one at the level of the dorsal root ganglion itself. Disorganization at a segment level could be assumed to lead to uncoordinated and inappropriate spinal reflex responses, while disorganization within the dorsal root ganglion could lead to loss of any subtle facilitatory or pattern-forming responses generated in cells at that level. This may explain why proprioceptive function remains poor after nerve repair.

It should be noted that no difference in cell size could be found between cable-grafted and muscle-grafted rats, despite the cable graft having a theoretical advantage over the muscle graft, in that more precise alignment of nerve fascicles is possible. In experimental models, the muscle grafting method has been shown to be at least as good as cable grafting and nerve grafting. There is also evidence that muscle grafting is useful in some clinical situations. These findings underline the importance of infrafascicular disorganization in determining the degree of neuron-receptor mismatch after nerve repair.
Acknowledgments

The authors thank Mrs. J. S. Wood, Mr. J. Cable, and Mr. 1. Lennox for skilled technical assistance.

References

28. Ranson SW: The structure of the spinal ganglia and of the spinal nerves. J Comp Neurol 22:159–175, 1912

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

Manuscript received July 17, 1991. Accepted in final form February 3, 1992.

Dr. Myles was Lister Professor of the Royal College of Surgeons of Edinburgh from 1990–1991, and was the holder of an Action Research Training Fellowship during this work, which was supported by the Sir Jules Thorn Charitable Trust.

Address reprint requests to: Michael A. Glasby, F.R.C.S.,
Department of Anatomy, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland.

J. Neurosurg. / Volume 77 / September, 1992