J. Anat. (1997), **190**, pp. 51–56 Printed in Great Britain

Review*

Nerve fibre regeneration across the peripheral–central transitional zone

THOMAS CARLSTEDT

Department of Orthopaedics, Karolinska Hospital, Stockholm, Sweden

(*Accepted 20 June 1996*)

ABSTRACT

Neurons cannot negotiate an elongation across the peripheral (PNS)–central nervous system (CNS) transitional zone and grow into or out of the spinal cord in the mature mammal. The astrocytic rich CNS part of the spinal nerve root is most effective in preventing regeneration even of nerve fibres from transplanted embryonic ganglion cells. Regeneration of severed nerve fibres into the spinal cord occurs when the transition zone is absent as in the immature animal. Before the establishment of a transition zone there is also new growth of neuronal processes from dorsal horn neurons distally to the injured dorsal root. Thus the experimental strategy to reestablish spinal cord to peripheral nerve connectivity has been to delete the transitional region and implant severed ventral or dorsal roots into the spinal cord. Dorsal root implantation resulted in reestablished afferent connectivity by new neuronal processes from secondary sensory neurons in the dorsal horn of the spinal cord extending into the PNS. The ability for plasticity in these cells allowed for a concurrent retention of their original rostral projection. Ventral root implantation into the spinal cord corrected deficit motor function. In a long series of experiments performed in different species, the functional restitution was demonstrated to depend on an initial regrowth of motor neuron axons through spinal cord tissue (CNS). These findings have led to the design of a new surgical strategy in cases of traumatic spinal nerve root injuries.

Key words: Spinal cord; astrocytes.

INTRODUCTION

Recently a surgical method was described that resulted in the reestablishment of functional connectivity between the spinal cord and denervated muscles after spinal nerve root avulsion (Carlstedt et al. 1986). When this technique was applied in man, deficits in motor function were corrected (Carlstedt et al. 1995). The outcome depended on regeneration within the spinal cord and through a peripheral (PNS)–central nervous system (CNS) transition zone (Cullheim et al. 1989).

Although generally abortive, it is evident that regeneration within the spinal cord can occur in some situations, for instance after lesions in the immature animal or by means of transplantation of immature neurons, particularly cholinergic and catecholaminergic neurons (Wiktorin & Björklund, 1992). Compensatory growth such as collateral sprouting by dorsal horn neurons after dorsal root injury (Chambers & Liu, 1958; Murray & Goldberger, 1986) and the growth of supernumerary axons from alpha motoneurons after peripheral nerve lesions (Havtorn & Kellert, 1987) are other examples of neuronal growth in the spinal cord. The demonstration that most intrinsic CNS neurons could regrow if offered a PNS conduit for elongation (David & Aguayo, 1981) indicated that the nonneuronal cells in the spinal cord were mainly responsible for the abortive regeneration. Tissue components not present in the CNS, such as

Correspondence to Dr Thomas Carlstedt, PNI Unit, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex HA7 4LD, England.

* Based on a contribution to the Anatomical Society Symposium on Glial Interactions in the Nervous System, held in Cork in September 1995.

nerve growth factors and extracellular matrix molecules, have been identified in the PNS and claimed to be necessary for regeneration (Korsching, 1993). Moreover, growth inhibitory molecules have been found in the CNS (Schnell & Schwab, 1990).

The anatomy of the spinal nerve root makes it a most attractive model for studies of injury-induced reactions of nerve fibres and glia cells in both the PNS and the CNS. It is possible to manipulate the nerve fibres distally in the PNS portion of the root without interfering with the integrity and the vascular system of the CNS tissue in the proximal part of the root (Berthold et al. 1993). A lesion in the distal part of the dorsal root will be remote from the CNS tissue but will give rise to anterograde axonal degeneration in the PNS and CNS portions of the same nerve fibres. No mechanical or indirect vascular trauma is caused to the CNS root segment. In the adult mammal, neurons cannot negotiate an elongation across the PNS–CNS interface in the root and grow into or out of the CNS, i.e. the spinal cord (Oorschot & Jones, 1990).

Several attempts with different types of neurons, some of which have demonstrated regenerative capacity within the CNS, have been performed to show regrowth from the periphery into the spinal cord after dorsal root injury. The astrocytic barrier which is found between the PNS and the CNS is, however, most effective in preventing regeneration (Cajal, 1928). Several axons are arrested, forming terminal enlargements or synaptoid endings indicating contact inhibition (Carlstedt, 1985). The phenotype of the astrocytes at the PNS–CNS interface in the root is mainly a fibrous GFAP-positive cell (Kozlova et al. 1995). After dorsal root crush a 'fibrous astrocytic scar' forms (Reier et al. 1983) of about the same extent as that of the original CNS root part, but depending on the characteristics of the regrowing neuron, the astrocyte can feature different phenotypes as well as disintegrating if regeneration to the CNS root segment is prevented (Carlstedt et al. 1989). Extension of neuronal processes from spinal cord neurons out into the dorsal root does not occur after a dorsal root lesion. This might have been expected due to the proximity between the growth promoting components of the PNS segment in the dorsal root and the dorsal horn neurons.

Although nerve fibre regrowth does not take place across the PNS–CNS transition in the mature animal, regeneration of severed nerve fibres has been demonstrated in the immature animal before the transition zone has been established (Carlstedt et al. 1987). Elongation of dorsal root axons into the spinal cord as well as extensions of new neuronal processes from dorsal horn neurons distally to the injured dorsal root occurred (Carlstedt, 1988). Orthotopically allogenic as well as xenogenic transplantations of embryonic dorsal root ganglion neurons to adult hosts demonstrated the nonpermissive nature of the astrocytes of the established PNS–CNS transition zone (Rosario et al. 1993). The regenerative ability of the immature neurons could not negotiate elongation across the PNS–CNS transitional zone, but instead circumvented it and entered the spinal cord through the pia mater by following blood vessels (Kozlova et al. 1995).

As nerve fibre regeneration is possible before an astrocyte rich PNS–CNS transitional zone has developed in the root, the current strategy to reestablish connectivity between the root and the spinal cord is by deleting the PNS–CNS transition zone. This review summarises these experiments and describes the pursuit from the original laboratory finding of reestablished functional connectivity between the spinal cord and the periphery to the application of a new surgical strategy in man.

DORSAL ROOT–SPINAL CORD TRANSITION

Implantation of a cut dorsal root into the superficial layers of the dorsal horn did not result in any substantial ingrowth of dorsal root nerve fibres in spite of deletion of the transitional zone. This indicated that although the transitional zone had been deleted, the regenerative ability of the adult dorsal root ganglion (DRG) cells was not sufficient to extend axons among the glia cells in the injury zone in the dorsal horn. Instead there was an outgrowth of dorsal horn neurons along the implanted root. It is well known that intrinsic spinal cord neurons, i.e. within the dorsal horn, can elongate along a PNS conduit (Fernandez et al. 1990). Instead of further investigating the possibilities for the DRG neurons to regrow into the spinal cord the possibility of reconstructing the sensory pathway by replacing the damaged primary sensory neurons with a peripheral outgrowth from the dorsal horn or secondary sensory neurons was attempted (Carlstedt et al. 1991). The primary sensory neurons, i.e. the dorsal root ganglion as well as the transition zone were deleted in order to offer the secondary sensory neurons the possibility of extending into the PNS and replacing the DRG neurons. The distal end of the dorsal root was joined to a peripheral nerve. A considerable number of dorsal horn neurons had regenerated new processes into the implanted ganglionectomised dorsal root as

well as further into the periphery and in some cases connected with pacinian receptors (unpublished observations). Analysis of the topographic distribution as well as soma size of these cells indicated that these neurons could be secondary sensory neurons, projection neurons or interneurons (Brown, 1981). In double-labelling experiments, it was shown that some of these neurons when extending processes into the implanted dorsal root also retained their rostral projections (Carlstedt et al. 1991). This is in line with previous observations that after a dorsal root injury in the immature animal, before the establishment of a transitional zone, there is a growth of additional processes into the dorsal root from the dorsal horn neurons. These had the characteristics of dendrites within the CNS but appeared as myelinated PNS axons in the dorsal root (Carlstedt, 1988). Thus it seems possible, by implanting the avulsed dorsal root, to reestablish connectivity between the periphery and the spinal cord that is not dependent on regeneration from the primary sensory neuron but by extension from secondary sensory neurons in the dorsal horn of the spinal cord. The injured primary sensory neuron is replaced by extensions from the secondary sensory neuron growing to the periphery, thereby reestablishing the segmental spinal cord afferent circuit. Moreover, in some cases the central trajectory is also retained and the sensory pathway completed by the de novo formation of new peripheral processes.

VENTRAL ROOT-SPINAL CORD TRANSITION

In pioneering studies Cajal (1928) found that spinal cord neurons after injury would regrow into neighbouring spinal roots. With respect to ventral roots it has been demonstrated that axonal regrowth occurs from spinal cord motoneurons after a spinal cord lesion (Risling et al. 1983). This regenerative capacity of motoneurons was further explored in rats where lumbar ventral roots were avulsed from the spinal cord and implanted into the ventrolateral part of the cord (Carlstedt et al. 1986). These results were later confirmed (Horvat et al. 1987; Hoffman et al. 1990; Bertelli & Mira, 1994). Restored connectivity between the injured motoneuron and the muscles could be demonstrated, but the interpretation of these initial results was hampered by the limitations of the initial experiments, i.e. retrograde labelling procedures as well as isometric muscle twitch responses to implanted root stimulation. Intraneuronal recording and staining with horseradish peroxidase demonstrated that alpha and probably also gamma motoneurons were able to reinnervate ventral root implants and that their axons could conduct nerve impulses (Cullheim et al. 1989). These neurons could be excited or inhibited by impulses in afferent fibres and their contribution to elicit reflex activity was normal, such that muscle twitch responses were induced by electrical stimulation of the implanted root. These experiments unequivocally demonstrated axonal regrowth from the motoneuron pool through CNS tissue in the spinal cord to the implanted ventral root. Other routes of regrowth could be excluded in these experiments, since the experimental procedure involved extensive excision of adjacent roots and intracellular labelling of the regenerated neurons (Cullheim et al. 1989).

By means of the intracellular staining technique (Cullheim et al. 1989), it was demonstrated that the reestablished anatomical links between the motoneuron pool and the implanted root had occurred as a true spinal cord regeneration within a CNS environment. The regenerated axons had regrown in CNS tissue for a considerable distance before reaching the PNS tissue of the implanted root. These axons, which could be followed from the neuron soma to the implanted root exhibited all the characteristics of CNS fibres, i.e. oligodendroglial-derived myelin sheaths interrupted by nodes of Ranvier filled with tufts of microvilli-like extensions from surrounding astrocytic processes (Cullheim et al. 1989). Regrowth of the motoneuron axons can therefore not be explained by a tentative invasion of the CNS tissue by Schwann cells. In contrast, it has been demonstrated that the glial cells migrated into the implanted root, thereby extending the zone of CNS axon regeneration into the replanted root (Cullheim et al. 1989). Thus the CNS environment at the site of root implantation does not seem to impede elongation of motor axons after this type of injury. With electron microscopy and indirect immunohistochemical methods the occurrence of tissue elements with potential beneficial effects on axonal regeneration was assessed in the region of the spinal cord where the avulsed roots had been implanted. These studies revealed that the traumatic cicatrix at the lesion site is mainly composed of a loose trabecular web of astrocytic processes which surrounds the regrowing axons, invading leptomeningeal cells and blood vessels with expanding perivascular spaces (Risling et al. 1993). Both high and low affinity nerve growth factor receptors such as trkA, trkB and p75 were demonstrated in the scar tissue. Thus it can be suggested that the scar tissue can bind and present neurotrophins to the injured and regrowing motor axons. The extracellular matrix of the scar contained collagen and laminin. These immunoreactivities were distributed around the regen-

erated motor axons and formed strands that interconnected the ventral horn with the implanted ventral root. These findings indicate that the regenerative growth takes place in an environment that is markedly different from the normal CNS.

An alternative pathway for reestablished connectivity between the motoneuron pool and the avulsed roots was demonstrated to occur along the surface of the spinal cord (Risling et al. 1991; Smith & Kodama, 1991). An avulsion of the roots usually leaves tufts of the most proximal parts of the roots attached to the spinal cord (Livesey & Fraher, 1992). Regrowth of axons was found to be promoted by the pia mater, which has a reported competence for sustaining nerve fibre growth (Risling et al. 1985). Thus abundant myelinated fibres could be followed from the avulsion site to the implanted ventral roots. This type of reinnervation takes place entirely in the peripheral nervous system.

In primates, intraspinal replantation of avulsed roots to the brachial plexus significantly promoted motor recovery in the muscles supplied by the lesioned spinal cord segments (Carlstedt et al. 1993). After total denervation of muscles in the arm following root avulsions, reinnervation was documented 2–3 mo after corrective surgery. Shortly afterwards there was evidence of clinical recovery. A gradual improvement in the function of the affected arm eventually led to normalised motor behaviour with a full range of motion (Carlstedt et al. 1993).

Of ultimate importance for regeneration and recovery of function is the survival of motoneurons after injury. Proximity as well as type of injury and the time period during which the neurons are disconnected from the periphery seems to be of importance for the amount of induced cell death. Thus avulsion has been found to be particularly hazardous for the spinal motoneurons (Hoffman et al. 1993; Wu, 1993). About 50–80% of the motoneuron population has disappeared 1 mo after the injury (Lindå et al. 1993). In these primate experiments where the avulsed roots were implanted in the spinal cord directly after avulsion there was no loss of neurons. This indicates that a rapidly reestablished connection between the spinal cord and the peripheral nerves is of great importance for motoneuron survival.

The primate experiments also demonstrated muscle reinnervation from unspecific neurons. It might be anticipated that lack of neurotrophism could create functional chaos, where attempted voluntary contraction would only result in mass muscle contractions without purpose and coordination. Simultaneous electromyographic recordings from agonistic and antagonistic muscle groups in the arm also demonstrated cocontractions. It was not possible to provoke isolated voluntary activation of either group of muscles. However, a sufficient dexterity in gross arm movement could be observed in these animals. It is therefore possible that the alien neurons after established connectivity might not participate in functionally purposeful movements.

Motor recovery was attributed to spinal cord implantation of the avulsed ventral roots. It therefore seems possible to correct motor deficits after root avulsion in severe brachial plexus injury in man by surgery. Repair of spinal nerve root injury has been performed in man with partial upper or lower as well as total brachial plexus lesions. Surgery has been performed within 10 d–10 mo after the accident. Restitution of connectivity with muscles regularly innervated from the implanted spinal cord segments has been noted on electromyography in all operated patients. Clinical restitution of activity has occurred only in those cases operated within a month after the injury. Proximal arm muscles such as biceps recovered to grade 4 on the Medical Research Council scale of 1–5, but there were cocontractions in deltoid and triceps.

COMMENTS

Both neuronal as well as nonneuronal factors have an influence on regeneration in the PNS–CNS transitional zone. The outcome from a series of experiments is that (1) reestablished connectivity between the spinal cord and injured roots can occur across a PNS–CNS interface or transitional zone after the ventral or dorsal root has been implanted into the spinal cord; (2) both sensory and motor neurons have a remarkable ability for plasticity and regeneration in the spinal cord; and (3) spinal cord scar tissue can be supportive for axonal elongation.

In the adult mammal, there is no regeneration from the nerve root across the transitional zone and into the spinal cord. There is, however, regrowth both from ventral and dorsal horn neurons across a spinal cord lesion site and a newly established transitional zone into the roots. Thus, after ventral root implantation in the spinal cord, functionally different neurons in different motor neuron populations in the spinal cord can contribute to the reinnervation of muscles. The motor neurons, which have a remarkable regenerative ability, would respond to the avulsion injury not only be regrowing a new axon but also by collateral sprouting, new aberrant or supernumerary axons (Havtorn & Kellert, 1987) or 'dendraxons'

(Lindå et al. 1985). As these new processes would be expected to grow nonspecifically, the biceps muscle, for instance, might after reinnervation following nerve root implantation in the spinal cord have connections with several different kinds of functionally alien neurons, some of which could have simultaneous connection with agonists as well as antagonists. This might have consequences for the functional outcome in terms of cocontractions, as was observed in man after ventral root implantation.

After implantation of ganglionectomised dorsal roots into the spinal cord, neurons in the dorsal horn show a capacity to transform from intrinsic spinal cord neurons to neurons with extensions in both the PNS and CNS. Some of these neurons retained their original rostral projections and extended new processes along the implanted dorsal root. These secondary sensory neurons could serve as bipolar neurons with both peripheral and central extensions and connections which would replace the injured primary sensory neurons. Any functional consequences of these structural findings has as yet not been established.

Although the primary sensory neuron cannot regrow across the transitional zone and into the spinal cord after a lesion, it has an ability to regrow inside the spinal cord (Murray & Goldberger, 1986). This discrepancy in regrowth in different regions of the spinal cord might depend both on intrinsic neuronal regenerative propensities as well as an inability to support growth or even an inhibition by a glial scar. Thus the growth related protein GAP-43, which is expressed by regenerating neurons, is produced by the primary sensory neuron only after an injury to its peripheral branch. The neuron is unable to express this protein, however, after dorsal root injury, i.e. a lesion of the central branch (Chong et al. 1994). There might, therefore, be a defect in intrinsic growth capacity of the dorsal root neurons that is of significance for the abortive regenerative ability into the spinal cord after dorsal root or central branch injury. Moreover, the scar formed by the astrocytes in the transition zone acts as a 'barrier' to regrowing axons (Reier et al. 1983; Sims & Gilmore, 1994). However, after a direct injury, i.e. when implanting a root into the spinal cord, astrocytes form a scar which, as was already recognised by Cajal (1928), is able to support growth by the release of trophic factors. Thus the scar tissue consequent on the degeneration of axons distant from the zone of injury as compared with the scar at the site of injury appears to have quite different propensities for sustaining regeneration in the spinal cord (cf. Bovolenta et al. 1994). In this series of experiments growth support by the scar after root implantation in the spinal cord has been demonstrated. The high content of extracellular matrix molecules of importance both for neurite elongation as well as polarisation, together with the nonneuronal cells expressing neurotrophin receptors and the high vascular permeability seen in the scar after root implantation in the spinal cord, indicate that the CNS scar tissue has features in common with the PNS. The local injury sustained by the spinal cord on root implantation thus appears to transform the CNS tissue so that it develops features in common with PNS tissue. This might be of crucial importance for the reestablishment of spinal cord–peripheral nerve connectivity. The related reactions and abilities shown by motor and sensory neurons as well as glial cells will be considered further in attempts to produce regeneration in the transitional zone.

In conclusion, spinal root avulsion is a shallow spinal cord injury. In a long series of experiments it has been demonstrated that the motor deficits due to this spinal cord lesion can be corrected by a novel surgical strategy. The outcome in man after ventral root implantation in terms of restored function depends on regeneration within spinal cord tissue. The present experimental findings as well as their clinical application may hopefully lead further in the search for means to repair more extensive transverse spinal cord lesions.

REFERENCES

Bertelli JA, Mira JC (1994) Brachial plexus repair by peripheral nerve grafts directly into the spinal cord in rats. *Journal of Neurosurgery* **81**, 107–114.

Berthold C-H, Carlstedt T, Corneliusson O (1993) Anatomy of the nerve root at the central-peripheral transitional region. In *Peripheral Neuropathy*, 3rd edn. (ed. Dyck PJ, Thomas PK, Griffin JG, Low PA, Poduslo J), pp. 73–91. Philadelphia: W. B. Saunders.

Bovolenta P, Wandosell F, Nieto-Sampedro M (1992) CNS glial scar tissue: a source of molecules which inhibit central neurite outgrowth. In *Neuronal-astrocytic Interactions* (ed. Yu ACH, Hertz L, Norenberg MD, Sykova E, Waxman SG), pp. 367–379. New York: Elsevier Science B.V.

Brown AG (1981) *Organization in the Spinal Cord. The Anatomy and Physiology of Identified Neurons*. New York: Springer.

Cajal SRy (1928, reprinted 1959) *Degeneration and Regeneration of the Nervous System*. New York: Hafner.

Carlstedt T (1985) Regenerating axons form nerve terminals at astrocytes. *Brain Research* **347**, 188–191.

Carlstedt T (1988) Reinnervation of the mammalian spinal cord after neonatal dorsal root crush. *Journal of Neurocytology* **17**, 335–350.

Carlstedt T, Lindå H, Cullheim S, Risling M (1986) Reinnervation of hindlimb muscles after ventral root avulsion and implantation in the lumbar spinal cord of the adult rat. *Acta Physiologica Scandinavica* **128**, 645–646.

Carlstedt T, Dalsgaard C-J, Molander C (1987) Regrowth of

lesioned dorsal root nerve fibres into the spinal cord of neonatal rats. *Neuroscience Letters* **74**, 14–18.

Carlstedt T, Cullheim S, Risling M, Ulfhake B (1989) Nerve fibre regeneration across the PNS–CNS interface at the root–spinal cord junction. *Brain Research Bulletin* **22**, 93–102.

Carlstedt T, Aldskogius H, Rosario C (1991) Extension of dorsal horn neurons into the severed and implanted dorsal root. *Restorative Neurology and Neuroscience* **3**, 205–209.

Carlstedt T, Hallin RG, Hedström KG, Nilsson-Remahl IAM (1993) Functional recovery in primates with brachial plexus injury after spinal cord implantation of avulsed ventral roots. *Journal of Neurology, Neurosurgery and Psychiatry* **56**, 649–654.

Carlstedt T, Grane P, Hallin R, Norén G (1995) Functional return due to spinal cord implantation of ruptured spinal nerve roots. Human application of a new surgical treatment based on experimental findings. *Lancet* **346**, 1323–1325.

Cullheim S, Carlstedt T, Lindå H, Risling M, Ulfhake B (1989) Motoneurons reinnervate skeletal muscle after ventral root implantation into the spinal cord of the cat. *Neuroscience* **29**, 725–733.

Chong MS, Reynolds ML, Irwin N, Coggeshall RE, Emson PC, Benowitz LI et al. (1994) GAP-43 Expression in primary sensory neurons following central axotomy. *Journal of Neuroscience* **14**, 4375–4384.

David S, Aguayo AJ (1981) Axonal elongation into peripheral nervous system 'bridges' after central nervous system injury in adult rats. *Science* **214**, 931–933.

Fernandez E, Pallini R, Mercanti D (1990) Effects of topically administrated nerve growth factor on axonal regeneration in peripheral nerve autografts implanted in the spinal cord of rats. *Neurosurgery* **26**, 37–43.

Havtorn L, Kellert J-O (1987) Regeneration by supernumerary axons with synaptic terminals in spinal motoneurons of cats. *Nature* **225**, 1507–1516.

Hoffman CFE, Thomeer RTWM, Marani E (1990) Ventral root avulsions of the cat spinal cord at the brachial plexus level (cervical 7). *European Journal of Morphology* **28**, 418–429.

Hoffman CFE, Marani E, Oestreicher AB, Thomeer RTWM (1993) Ventral root avulsion versus transection at the cervical seven level of the cat spinal cord. *Clinical Neurology and Neurosurgery*, Suppl. **5**, 291–302.

Horvat JC, Pécot-Dechavassine M, Mira JC (1987) Réinnervation fonctionelle d'un muscle squelettique du rat adulte au moyen d'un greffon de nerf périphérique introduit dans lamoelle épinère par voi dorsal. *Compte Rendu de l'Académie de Science* **III**, 304, 143–148.

Korsching S (1993) The neurotrophic factor concept: a re-examination. *Journal of Neurosciences* **13**, 2739–2748.

Kozlova EN, Strömberg I, Bygdeman M, Aldskogius H (1995) Peripherally grafted human foetal dorsal root ganglion cell extend axons into the spinal cord of adult host rats by circumventing dorsal root entry zone astrocytes. *Neuroreport* **6**. 269–272.

Lindå H, Risling M, Cullheim S (1985) 'Dendraxons' in regenerating motoneurons in the cat; do dendrites generate new axons after central axotomy? *Brain Research* **358**, 329–333.

Lindå H, Risling M, Shupliakov O, Cullheim S (1993) *Changes in the synaptic input to lumbar motoneurons after intramedullary axotomy in the adult cat*. Thesis, Karolinska Institutet.

Liu CN, Chambers WW (1958) Intraspinal sprouting of dorsal root axons. *Archives of Neurology and Psychiatry* **79**, 46–61.

Livesey FJ, Fraher JP (1992) Experimental traction injury of cervical spinal nerve roots; a scanning EM study of rupture patterns in fresh tissue. *Neuropathology and Applied Neurobiology* **18**, 376–386.

Murray M, Goldberger M (1986) Replacement of synaptic terminals in lamina II and Clark's nucleus after unilateral lumbosacral dorsal rhizotomy in adult cats. *Journal of Neuroscience* **6**, 3205–3217.

Oorschot DE, Jones DG (1990) Axonal regeneration in the mammalian central nervous system: a critique of hypotheses. *Advances in Anatomy, Embryology and Cell Biology* **199**, 1–121.

Reier PJ, Stensaas LJ, Guth L (1983) The astocytic scar as an impediment to regeneration in the central nervous system. In *Spinal Cord Reconstruction* (ed. Kao CC) pp. 163–195. New York: Raven Press.

Risling M, Cullheim S, Hildebrand C (1983) Reinnervation of the ventral root L7 from ventral horn neurons following intramedullary axotomy in adult cats. *Brain Research* **280**, 15–23.

Risling M, Dalsgaard CJ, Terenius L (1985) Neuropeptide Y-like immunoreactivity in the lumbosacral pia mater in normal cats after sciatic neuroma formation. *Brain Research* **358**, 372–375.

Risling M, Carlstedt T, Lindå H, Cullheim S (1991) The pia mater, a conduit for regenerating axons after ventral root replantation. *Restorative Neurology and Neuroscience* **3**, 157–160.

Risling M, Fried K, Lindå H, Carlstedt T, Cullheim S (1993) Regrowth of motor axons following spinal cord lesions: distribution of laminin and collagen in the CNS scar tissue. *Brain Research Bulletin* **30**, 405–414.

Rosario CM, Aldskogius H, Carlstedt T, Sidman RL (1993) Neurons of dorsal root ganglia allografted into adult rats differentiate and regenerate axons peripherally into the sciatic nerve and centrally into the dorsal horn of the spinal cord. *Experimental Neurology* **120**, 16–31.

Sims TJ, Gilmore SA (1994) Regrowth of dorsal root axons into radiation-induced glia deficient environment in the spinal cord. *Brain Research* **634**, 113–126.

Smith KJ, Kodama RT (1991) Reinnervation of denervated skeletal muscle by central neurons regenerating via ventral roots implanted into the spinal cord. *Brain Research* **551**, 221–229.

Snell L, Schwab ME (1990) Axonal regeneration in the rat spinal cord produced by an antibody against myelin associated neurite growth inhibitors. *Nature* **343**, 269–272.

Wictorin K, Björklund A (1992) Axon outgrowth from grafts of human embryonic spinal cord in the lesioned adult rat spinal cord. *Neuroreport* **3**, 1045–1048.

We W (1993) Expression of nitric-oxid synthase (NOS) in injured CNS neurones as shown by NADPH diaphorase histochemistry. *Experimental Neurology* **120**, 153–159.