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Research Review

Progress in Spinal Cord Research

A refined strategy for the International Spinal Research Trust

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Achieving regeneration in the central nervous system represents one of the greatest intellectual and practical challenges in neurobiology, and yet it is an absolute requirement if spinal cord injury patients are to have any hope of recovery. The mission of the International Spinal Research Trust (ISRT), established in 1980, is to raise money specifically for spinal research, with a view to ending the permanence of paralysis caused by spinal cord injury. This review summarises some of the major steps forward made in recent years in understanding the mechanisms involved in spinal cord injury and where these discoveries fit in with the ISRT's overall objectives. We review approaches aimed at (1) preventing immediate adverse reactions to injury such as neuronal death and scar formation; (2) minimising inhibitory properties of the CNS environment and maximising the growth potential of damaged neurons; (3) understanding axonal guidance systems that will be required for directed outgrowth and functional reconnection; and (4) optimising the function of surviving systems. We also discuss 'infrastructural' prerequisites for applying knowledge gained through spinal research to the clinical condition, including basic scientific issues such as developing representative animal models of spinal cord injury and sensitive quantitative methods for assessing growth and functional restoration. In addition, we point out the importance of communication. The need to share knowledge between research groups is vital for advancing our understanding of injury and repair mechanisms. Equally important is the need for communication between basic scientists and clinicians which will be essential for what is the ultimate goal of the ISRT, the development of relevant treatment strategies that will prove beneficial to the spinal injured patient.
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Introduction

Understanding the problem of spinal cord injury

Sadly, spinal cord injury (SCI) in humans remains incurable. This is not to say that we should be without hope, for in the past few years basic research discoveries have made great strides in SCI, both into the underlying biology of the problem as well as into possible repair strategies, some of which are nearly ready for application in the clinical setting. Nor is it to say that there has been no impact of basic research on treatment of patients with SCI, as the National Acute Spinal Cord Injury Studies (NASCIS), based initially on laboratory investigations, have resulted in the regular use of the anti-inflammatory corticosteroid methylprednisolone to prevent early damage secondary to trauma in acute SCI.[1] However, while this worthwhile therapy may prevent further damage as a result of post-traumatic cellular events, it does nothing to promote repair of previously damaged tissue or to instigate recovery of function.

Curing SCI in humans, while a multi-faceted question, is most heavily reliant upon two central issues. The first is coaxing axons to grow within the central nervous system (CNS), a problem that in the first instance will be addressed in the laboratory. Encouragingly (and perhaps surprisingly), this hurdle, thought to be insurmountable a few decades ago, is shrinking at a swift pace, with various growth-promoting and/or anti-inhibitory approaches resulting in centimetres of axonal growth, at least in the rat. The second problem, which is perhaps now requiring

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greater focus as the number of basic research discoveries builds up, is applying the knowledge acquired in experimental animals to human SCI. Addressing these problems of solving the basic biological problems of SCI and transferring the knowledge to the clinic comprises the primary goals of the International Spinal Research Trust (ISRT).

The ISRT research strategy

The ISRT first published a research strategy in 1996.[2] This document described at length the reasons for establishing a coherent research strategy, identified areas in spinal cord research that would require particular attention if the ISRT's overall objectives (to repair the damaged spinal cord and restore useful function) were to be achieved, and it defined priority areas for funding and support. The aim of that publication was to (i) help basic and clinical researchers focus funding proposals, and (ii) to increase the number and quality of applications made to the ISRT for funding. The adoption and publication of a research strategy by the ISRT has been successful on both of these fronts. However, as there have been significant advances over the past few years in the understanding of the basic biology of the spinal cord, the processes operating following spinal cord disease and trauma, and the requirements for repairing damage, the ISRT has refined its research strategy in the hopes of bringing some of these advances, which have occurred primarily in basic research, toward application in human SCI.

The aim of this review is twofold: it is in part meant to describe the revised framework of the ISRT's refined research strategy, which combines 'vertical targets' addressing key biological problems with 'horizontal capabilities', aimed at developing tools, skills and facilities required to underpin and optimally approach the biological problem. Since steps forward in the laboratory bring the ISRT closer to the realisation of its goals, this review is equally intended to outline some of the more important advances in spinal cord research made in the past few years. The strategy's vertical targets consist of (i) minimising the deleterious effects of early trauma, inflammation and scar deposition; (ii) combining trophic support with blockade of inhibitory influences in the damaged cord to promote regeneration; (iii) sustaining directed outgrowth, resulting in appropriate re-connection of damaged axons with targets; and (iv) in cases of partial cord damage, exploiting the function of surviving, intact fibres (Figure 1). The 'horizontal capabilities' required to support these goals consist of (i) developing representative animal models of SCI; (ii) developing sensitive, quantitative methods for assessing axonal regrowth and functional recovery in the

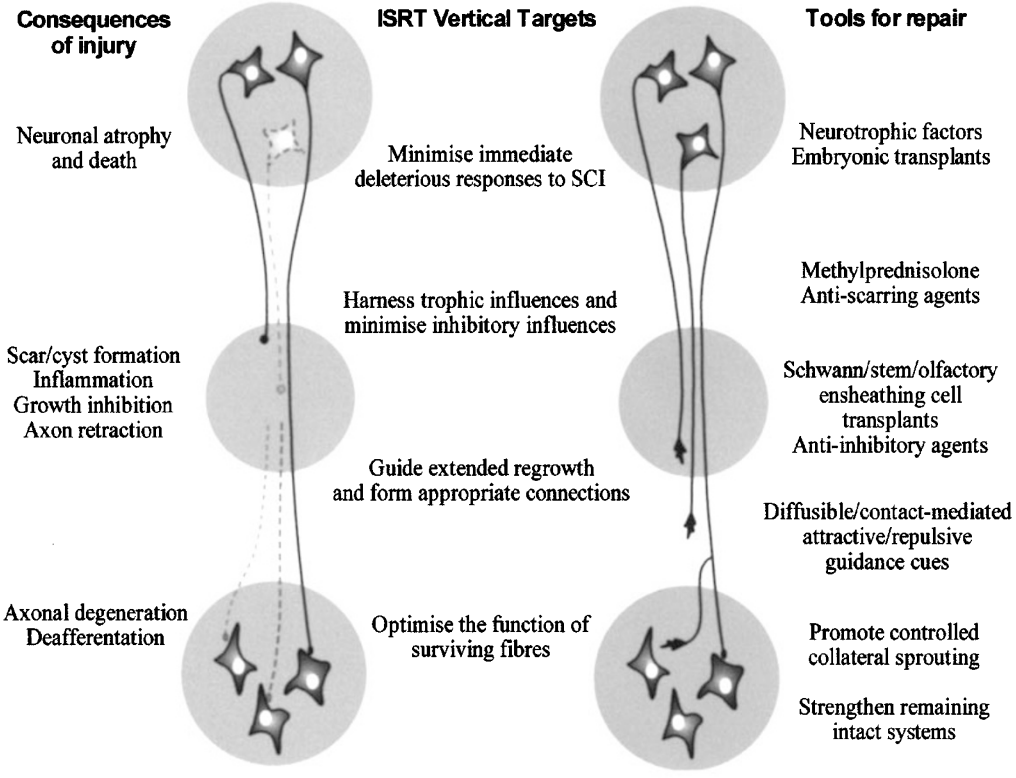

Figure 1 Summary of the events subsequent to spinal cord injury, the primary goals of the ISRT with respect to addressing these events, and the projected requirements for doing so

laboratory and in the clinic; (iii) establishing the capability to conduct clinical trials of new therapeutic interventions in SCI, and (iv) promoting cross-disciplinary education and training, and collaborative research and clinical practises focused on SCI and its treatment. The following sections will describe in turn, the vertical and horizontal targets of the ISRT strategy, as well as examples of recent work from leading laboratories in the field.

Vertical Target 1: Minimising the deleterious effects of early trauma, inflammation and scar formation (Table 1)

Although the ultimate goal for the treatment of SCI is to promote regeneration of lesioned axons so that they can re-grow and re-form functional connections with their denervated targets, an immediate and necessary goal is to minimise the secondary reactions that occur following SCI.

Much of the post-traumatic tissue damage and subsequent neurological deficits that occur with SCI are due to secondary reactive processes. The initial injury triggers a cascade of molecular and cellular changes that begin within minutes but continue for days and weeks. Many of the mechanisms which underlie these secondary processes are still not known in great detail. Thus, research into understanding the mechanisms involved in early trauma and cell death, inflammation and glial scarring will provide direction for therapeutic interventions designed to minimise secondary damage and lead to enhanced function after SCI.

As mentioned above, the current clinical treatment of SCI is to administer high doses of the steroid methylprednisolone. Methylprednisolone inhibits post-traumatic lipid peroxidation[3] and thus interrupts the biochemical cascade that occurs after injury providing neuroprotection from secondary deterioration. The results of two National Acute Spinal Cord Injury

Table 1 Vertical Target 1: Minimising the deleterious effects of early trauma, inflammation and scar formation

Action plans		*Priority*
V1.1	Promote informed debate, involving basic scientists and clinicians, on the merits, risks and scope for interventions in the aftermath of SCI which exploit existing anti-inflammatory, anti-proliferative, neuroprotective and immunosuppressive drugs, with a view to fostering well-founded clinical best practice. This should include harnessing the insights and testing capabilities of existing large study groups, world-wide (e.g. the NASCIS group).	High
V1.2	Fund the detailed molecular and cellular characterisation of post-injury trauma in acute and chronic animal models of SCI, with particular attention to the consequences of different types of injury and to the acquisition of a complete understanding of all the main mediators, their inter-dependencies and their temporal patterns.	High
V1.3	Fund work that exploits the insights generated by V1.2 to design and test experimental interventions (including new pharmacological agents) to antagonise individual and combined mediators, in order to identify those that are the dominant factors in reducing subsequent nerve regeneration. These may not be the dominant factors in causing the damage or scar *per se*, although the minimisation of cell death is likely to be a worthwhile 'permissive' influence on subsequent regenerative potential.	High
V1.4	Fund work that exploits the insights generated by V1.2 to determine the scope and effectiveness of harnessing the natural regulators of trauma, inflammation and scar formation after SCI, again assessing the outcome by its beneficial impact on subsequent nerve regeneration rather than on the tissue disruption *per se*.	High
V1.5	Fund studies that characterise the critical molecular and cellular components of an established SCI lesion in inhibiting nerve regeneration, including exploration of how these might change with time post-lesion.	High
V.16	Fund studies to examine the applicability of the results of V1.2–V1.5 to man, including attempts to adjust the insights generated from animal models to the scale and complexity of SCI in man.	High
V1.7	Fund research into accessible (if necessary, surrogate) markers of early trauma, inflammation and scar formation, and of the key molecular and cellular mediators, in man.	Medium (awaits progress in V1.6)
V1.8	Fund research, in chronic animal models and, as far as possible, in man, to understand the temporal pattern of axonal retraction and both local and distant neuronal death after different types of SCI.	Medium

Exclusions, future issues, etc.	*Proposed approach*
Work in non-spinal cord models should be explicitly justified. Funding for the acquisition of reagents from laboratories researching inflammation etc. outside the spinal cord, for use in SCI research, will be considered under this strand of the Strategy and within Horizontal Capability 4.	Proposals should include plans for testing the validity of the results and conclusions in the spinal cord.

Studies (NASCIS 1 and NASCIS 2) revealed that the dose and timing of administration were critical for neuroprotective effects and significant improvements in neurological function were observed in human patients only when they received high doses within 8 h after injury.[1,4]

Experimental studies in adult rats have shown methylprednisolone treatment to reduce lesion size,[5] increase the percentage of spared tissue and restore neurological function,[6] improve locomotor recovery[7] and increase axonal regeneration across Schwann cell grafts.[8] Methylprednisolone treatment combined with delivery of basic fibroblast growth factor was found to have additive beneficial effects on reversing neurological deficits.[9] Recently, Oudega *et al*[10] demonstrated that methylprednisolone treatment shortly after a spinal cord transection could attenuate inflammatory reactions, with long term reduction of ED-1 positive cells and a reduction in tissue loss. They also observed transient sprouting and reduced axonal retraction of vestibulospinal fibres.

The methylprednisolone studies have provided one example of a successful intervention strategy for spinal cord damage. However, improvements seen in the clinical situation have been at best modest and claims of its effectiveness have remained controversial.[11–14] Furthermore, only one aspect of secondary pathology is targeted with this treatment and, although encouraging, there is still a long way to go before optimum combinations of treatments can be determined that will attenuate the many contributors and variables involved in secondary pathology and leading to severe neurological deficits.

Secondary cell death

Secondary cell death is one of the reactive processes that occurs following SCI and results in the death of cells in the tissue surrounding an injury site that were not damaged or cut by the primary lesion. Extensive secondary cell death begins to occur within the first few hours following SCI and leads to both neuronal and glial cell loss and cavitation.[15–17] The mechanisms of secondary cell death are unclear but glutamate, calcium and free radicals may play key roles.[18–22]

The most appropriate strategies for reducing secondary cell death after SCI are likely to be those that provide neuroprotection and glioprotection. For example, Novikova *et al*[23] demonstrated that exogenous delivery of brain derived neurotrophic factor (BDNF) was neuroprotective when delivered to the site of a spinal cord hemisection, with an observed increase in neuronal survival and a reduced necrotic zone. Such neuroprotection does not in itself promote functional regeneration but is a necessary and intermediate goal in the treatment of spinal injuries.

As well as the occurrence of local cell death at an injury site, the axons of projection neurons are severed following a SCI, leading to retrograde degeneration in the cell bodies of projection neurons. Thus, neuroprotection may also be a useful strategy for preventing the retrograde cell death and atrophy that is often seen in axotomised neurons in the days and weeks following spinal cord lesions. Delivery of the neurotrophins neurotrophin-3 (NT3) and BDNF has been shown to rescue adult corticospinal and rubrospinal neurons from axotomy-induced death and atrophy.[24,25] Ciliary neurotrophic factor has also been reported to improve the survival of axotomised corticospinal neurons from degeneration.[26] Neuroprotective effects have also been demonstrated in ascending spinal projection systems, where NT3 can promote cell survival in different populations of ascending projection neurons after spinal cord hemisection.[27,28] Transplants of foetal spinal cord tissue can also provide a trophic environment for axotomised neurons and have been demonstrated to promote the rescue of axotomised rubrospinal neurons from retrograde cell death following spinal cord hemisection.[29] Furthermore, foetal transplants combined with neurotrophic factor treatment were found to be additive in their ability to rescue rubrospinal neurons, completely reversing the severe cell atrophy observed after hemisection.[30]

Inflammatory responses

Following SCI a pronounced inflammatory reaction occurs which is characterised by the recruitment of peripheral inflammatory cells, such as neutrophils and monocytes, and the activation of resident glial cells.

While inflammation in the PNS has been studied in much detail, it is only recently that attention has focused on the importance of the inflammatory response in a CNS injury. Following injury to the PNS, where regeneration occurs, there is a pronounced inflammatory response. High numbers of macrophages are rapidly recruited to the injury site where they help degrade the distal cut axon by secreting proteases and engulfing axonal and myelin debris.[31,32] PNS macrophages may also create positive conditions for peripheral nerve regeneration by secreting factors that can promote axonal growth and stimulate Schwann cell proliferation.[31,33–37] The inflammatory response in the CNS, however, follows a different pattern.[32,38–41] There is a lower recruitment of inflammatory cells in a CNS injury with recruited cells localised to the lesion site and, although the onset of recruitment is equally fast in the CNS and PNS, peak levels are reached later in the CNS than the PNS.[15,31,42,43] In the CNS, resident microglial cells and astrocytes are responsible for the removal of debris and the persistence of myelin and axonal debris in the CNS is believed to be a major impediment to axon regeneration.

There is much debate over the significance of inflammation following SCI, with both detrimental and beneficial effects being attributed to the inflammatory response. The inflammatory reaction may result in the subjection of nearby neurons and glia to

deleterious secondary injury cascades or, conversely, may provide mechanisms of beneficial trophic support.

Inflammation as a detrimental response to SCI

Inflammatory responses to SCI have been strongly linked to the processes of secondary damage. The secondary injury that develops in the days and weeks following the initial SCI leads to an increase in the lesion cavity to many times the size of the initial wound. Fitch *et al*[44] linked this increased cavitation to processes of inflammation and demonstrated *in vivo* that inflammatory processes could initiate a cascade of secondary tissue damage, progressive cavitation and glial scarring. They further showed in a culture model that progressive cavitation could be prevented by treatment with anti-inflammatory agents which inhibited macrophage inflammatory gene transcription.[44]

Further evidence for the detrimental role of neutrophils and macrophages is that reactive oxygen species are produced during the process of phagocytosis by these cells.[45] Thus, neutrophil influx in a SCI has been associated with oxidative damage of surrounding healthy tissue.[46] Activated neutrophils have also been said to contribute to the secondary pathology in SCI by inducing endothelial cell damage and methods of inhibiting neutrophil activation have resulted in reduced intramedullary haemorrhaging and functional improvements in motor performance following compression injury.[47,48] Similarly, interventions which reduce the mononuclear phagocyte population after a SCI can lead to reduced secondary tissue damage.[49,50] Furthermore, Carlson *et al*[46] found a significant correlation between the amount of tissue damage observed after a contusion injury and the number of macrophages/microglia present, with fewer cells apparent in areas where there was greater intact tissue.

Inflammation as a beneficial response to SCI

Dusart and Schwab[15] have argued that secondary damage occurring in SCI is not primarily due to destructive effects of neutrophils and macrophages. They suggested that the inflammatory response to SCI is qualitatively similar to that of the PNS and that inflammatory cells that release cytokines and growth factors could be important for neuroprotection and glial scar formation. Other studies have also proposed a beneficial role for inflammation after SCI due to the presence of chemoattractant cytokines in the inflammatory response.[51,52]

Studies of macrophages after CNS injury have led to further conflicting evidence for the role of inflammation. It has been demonstrated that increasing the number of macrophages in a CNS injury can promote neurite outgrowth,[53,54] implicating a role for macrophages in promoting neuronal sprouting and regeneration.

Further understanding of the mechanisms involved in inflammation following SCI is necessary before therapeutic interventions can be targeted towards inflammatory responses. The roles played by the various types of inflammatory cells are still not clear and the goal is to determine positive *versus* negative mechanisms in order to inhibit aspects that result in cell injury and death while preserving aspects that may contribute to regeneration and repair.

Glial scarring

Whenever the CNS is damaged it undergoes an injury response known as glial scarring whereby astrocytes become activated and proliferate to form a solid network of interweaving processes encapsulating the lesion. The glial scar has long been considered to be a barrier to the regrowth of injured CNS axons.[55–58] The inhibitory nature of the glial scar may be due to either a physical barrier blocking the advancement of the growth cone and/or the release of chemical factors which actively inhibit axon outgrowth.

The extracellular matrix produced by reactive astrocytes and other scar-associated cells at the lesion site contains molecules known for their potent growth inhibitory properties *in vitro* and therefore may be an important contributor to the nonpermissive nature of CNS scars. For example, *in vitro* models of glial scarring have demonstrated that cells or tissue taken from the glial scar of adult animals and used as a culture substrate are inhibitory to neurite outgrowth, whereas neonatal scar tissue can support neurite outgrowth.[59,60] Inhibition was correlated with the expression of chondroitin sulphate proteoglycans (CS-PGs) and tenascin in the glial scar tissue.[60] During development, proteoglycans are one of several groups of ECM molecules that are involved in mediating the restrictive boundary-like properties ascribed to glial cells.[61] Thus, such molecules are likely to play an important role in the inhibitory glial environment of an adult CNS injury. Astrocyte cell lines that are inhibitory or permissive to growth have been compared in order to identify molecules associated with inhibition and the nature of inhibition in the non-permissive cells has been largely attributed to the production of CS-PGs.[62–64] The proteoglycans NG2, versican and phosphacan were found to be produced by the inhibitory astrocyte cell line Neu7 but a function blocking antibody against NG2 neutralised the majority of inhibition, permitting neurite outgrowth of DRG cells on the normally non-permissive cell line.[64] This is in accordance with a study by Dou and Levine[65] which found NG2 to be a potent inhibitor of axon growth. In the normal CNS NG2 is present on a population of adult oligodendrocyte precursor cells[66] and these are cells which are rapidly recruited to a CNS lesion site.[67] Thus, oligodendrocyte precursors, and other cells that produce NG2, may be an important cell type to target when designing therapies to reduce inhibitory ECM molecules.

Recent *in vivo* studies have highlighted the importance of molecular barriers presented by glial scarring at the lesion site. Davies *et al*[68] used a microtransplantation technique that minimises scarring to inject dissociated adult rat DRG tissue directly into an adult rat white matter tract. They found that in the absence of a glial scar axons rapidly extended for long distances in white matter. Abortive regeneration only occurred in those rats where the transplant procedure was more traumatic and which led to increased levels of proteoglycans being present at the transplant site. The failure of axon regrowth was thus said to be directly associated with the reactive glial extracellular matrix which forms at a lesion site. Davies *et al*[69] further demonstrated that transplanted adult DRG neurons could grow in white matter tracts that were undergoing Wallerian degeneration. They transplanted adult DRG neurons directly into the degenerating dorsal columns, rostral to a spinal cord transection lesion and robust regeneration of the transplanted cells was observed. Axons extended through degenerating white matter until they reached the lesion site where axon regeneration stopped abruptly. Thus, growth arrest was again associated with molecular repulsion from extracellular matrix molecules associated with the glial scar.

These experiments highlight the importance of understanding the ECM molecules present in a lesion environment and associated with the inhibitory glial scar. Further understanding and characterisation of the cell types and secreted molecules involved in creating these physical and molecular barriers to regeneration will lead to therapeutic possibilities based on the neutralisation or enzymatic digestion of such molecules in order to permit regeneration.

In summary, two major events occur following SCI. First, massive enlargement of the lesion area occurs due to a process called secondary cell death. Second, a cascade of cellular reactions occur in the surrounding CNS tissue which includes invasion by inflammatory cells, reactivity of astrocytes and the formation of cavities and glial scars. Therapeutic interventions for SCI should be directed at reducing or alleviating the secondary progressive injury process. Interfering with the damaging series of cellular events that follow injury to the spinal cord will make it possible to preserve damaged neurons, thus making them accessible to regeneration promoting strategies.

During the initial period of cell death and damage the main therapeutic aim must be to provide neuroprotective and glioprotective treatments in order to reduce tissue destruction. After this period, any form of treatment designed to repair CNS damage will inevitably have to take place in a glial scar environment. The promotion of axon regeneration through or over a glial scar may require multiple strategies that not only replace the growth factors and surface molecules that stimulate axon elongation but also remove chemical inhibitors that may be present in the scar environment. The ISRT is committed to funding research that (i) delineates the significance of early trauma, tissue disruption and damage, and scar formation as causes of neuronal death and as obstacles to successful nerve regeneration and repair in the CNS; and (ii) identifies ways of minimising cell death and the generation of such obstacles in the aftermath of SCI, and of reversing or by-passing their deleterious effects during a later reparative intervention. The ISRT's specific action plans in this respect are outlined in Table 1.

Vertical Target 2: Integrated exploitation of trophic and inhibitory influences on axonal regeneration (Table 2)

The inhibitory nature of the CNS environment has been the subject of intensive study since the '80s when Aguayo and collaborators first demonstrated that CNS axons grow readily when offered a segment of peripheral nerve as a growth substrate.[70–74] While it had been known since Ramon y Cajal's time that CNS tissue is inhibitory to regeneration of cut axons, these experiments offered the first real hope of long-distance regeneration in the CNS, and thus recovery following SCI. Variations on this theme have included optic nerve-to-superior colliculus grafts,[75] brainstem-to-spinal cord grafts,[76] bridging grafts within the damaged cord,[77] and dorsal root-to-spinal cord grafts.[78] All of these result in growth of axons into and within the grafts, and in some cases (when performed in combination with other procedures such as neurotrophic factor treatment), exit from the graft and re-entry into the distal CNS tissue. There are two possible reasons for the ability of these peripheral nerve grafts to support regeneration. The first is that the inhibitory cues present within the CNS are absent from PNS. The second is that peripheral nerve grafts contain cells which secrete neurotrophic factors required by CNS axons in order to overcome inhibition.

Inhibition within the CNS

The notion that factors within CNS myelin are inhibitory has been best popularised by Schwab and collaborators who, with a neutralizing antibody IN-1, have blocked the effect of NI-250 (now known as Nogo), one of the major inhibitory proteins expressed by CNS myelin.[79] The result has been that in several CNS injury paradigms including spinal cord injuries, infusion of this antibody has produced regeneration of some axons.[80] In addition, it appears as though it can induce the collateral sprouting of spared corticospinal tract axons following unilateral pyramidal tract lesions[81,82] (see also Vertical Target 4). Of course, IN-1 is only one of a large number of possible inhibitory molecules. Other candidates include myelin-associated glycoprotein (MAG), also derived from oligodendrocytes,[83,84] proteoglycans such as versican, aggrecan and

Table 2 Vertical Target 2: Integrated exploitation of trophic and inhibitory influences on axonal regeneration

Action plans		*Priority*
V2.1	Fund research to identify, quantify and profile over time, all the significant trophic and inhibitory factors (defined at the molecular level) that influence the regenerative capability of the injured spinal cord.	High
V2.2	Fund research that complements the detailed pursuit of individual molecular factors with broader 'cataloguing' approaches designed to identify all the molecular changes in an injured and regenerating spinal cord (e.g. by differential genomics or proteomics approaches).	High
V2.3	Fund experimental interventions (including tests of new pharmacological agents, see V2.6) that probe the relative importance of individual factors in influencing regenerative capacity in the spinal cord, recognising that this may vary over time post-injury and between different injury types.	High
V2.4	Fund research into the cellular sources (including glia) and regulatory mechanisms (including the influence of cytokines, other growth factors and injury) governing the biosynthesis of the key trophic and, especially, inhibitory factors, and their recruitment into sites of CNS injury.	High
V2.5	Develop approaches/agents for enhancing trophic factors and neutralising inhibitory factors, exploiting biosynthesis regulators, agonists/antagonists and agents selectively modulating their post-receptor signal transduction pathways, for stimulating and maintaining regenerative axonal outgrowth in a controlled and tract/target organ-specific way (see also Vertical Target 3). This may include applying the trophic factors themselves to the injured spinal cord.	
V2.6	HighPromote the construction of a truly integrated picture of the roles and interplays between trophic and inhibitory factors in the injured and regenerating spinal cord, including through the organisation of structured workshops and the commissioning of integrative reviews of the literature.	Medium: requires further progress in V2.1 – 2.4
V2.7	Fund research that tests the applicability, to the human spinal cord *in vivo*, of the integrated picture of trophic and inhibitory molecules and their pharmacological manipulation, as generated from studies *in vitro* and in animal models in V2.1 – 2.6.	Medium: largely dependent on V2.5
V2.8	Fund research that creates the assays and markers that might be required to allow clinical interventions based on these factors to be assessed and tailored to the needs of individual patients.	Medium: requires evidence on the diversity of molecular patterns seen in human SCI
V2.9	Promote the sharing of novel reagents pertinent to this field (including neutralising antibodies and assay reagents) between laboratories, including efforts to gain access to materials available from commercial organisations.	Medium
V2.10	Develop novel protein delivery systems for the infusion of macromolecular trophic agents and inhibitory factor antagonists into the injured human spinal cord, if work in V2.6 has not (yet) identified suitable low molecular weight agonists/antagonists.	Medium
V2.11	Ensure that all experimental systems for exploiting trophic and inhibitory systems consider their feasibility for application in humans (with regard to cost, antigenicity, chronic delivery, specificity, etc.), and develop strategies that address whichever of these issues risks becoming an obstacle to translating basic research understanding into a realistic basis for clinical intervention.	Medium

Exclusions, future issues, etc.	*Proposed approach*
Work in non-spinal cord models should be explicitly justified.	Proposals should include plans for testing the validity of the results and conclusions in the spinal cord.
Funding for the production of reagents as a service to others will only be considered if there are no alternative means of supply for critical materials. Commercial production will not be subsidised by ISRT.	Some modest provision for this, e.g. through enhanced consumables expenditure or technical support, might be considered within an ISRT grant.
The mathematical modelling of multi-factorial influences on nerve regeneration, incorporating changes over time, may be useful or even essential but the Trust would seek to harness more generic research in this field, in preference to funding new, SCI-specific algorithms.	Exploration of this field might usefully be conducted within the integrative workshop/review format described in V2.5.

phosphacan from astrocytes and NG2 from oligodendrocyte precursors (see Vertical Target 1).

The nerve graft experiments have also led to purification and transplantation of Schwann cells into the spinal cord, either by injection of purified cells following microlesions,[85] or in bridges across gaps following complete lesions.[86] While these procedures have also led to some axonal regeneration across the transplanted Schwann cells, a major problem is getting the axons to grow out of the transplant and into the

CNS tissue on the other side. The picture has improved somewhat with the recent discovery of a new type of glial cell derived from the olfactory bulb (the olfactory bulb ensheathing cell or OBEC), and which is permissive to axonal growth and migratory when injected into the spinal cord.[87] These cells not only have the ability to support growth at the transplant site, but by virtue of the fact that they migrate into undamaged adjacent cord tissue, they serve to bridge the inhibitory scar and allow the axons to penetrate into the undamaged regions where they can continue to elongate and/or make synaptic connections.[88]

Other approaches to lessen the inhibition proffered by the CNS have included eliminating cells that make inhibitory molecules by X-irradiation of the cord,[89,90] or by immunolesioning CNS glia with antibodies and complement.[91] Embryonic spinal cord transplants are also an attractive alternative, as this tissue is neither inhibitory to adult CNS axonal growth, nor is its own outgrowth affected by the adult CNS environment, and thus embryonic cord transplants may act as 'relays', receiving inputs from regenerating axons, and transmitting information to the other side of the lesion.[92]

Even though many studies have demonstrated that molecules in adult CNS white matter can prevent regeneration of CNS axons, other recent experiments have called the inhibitory nature of the CNS environment into question. Cultured adult mouse dorsal root ganglion neurons, when micro-injected into intact or degenerating rat white matter tracts, can grow for long distances and form synapse-like structures, despite the presence of many of the previously defined inhibitory molecules.[68,69] In the few cases where regeneration failed, it was found that CSPGs associated with CNS scar tissue were expressed, envelope-like, around the injected material. These studies suggest that perhaps the CNS scar, and not white matter *per se* is the critical inhibitory feature in failure of axonal regeneration. Indeed, neurons seeded onto cryosections of white matter in culture can extend neurites for long distances, provided that the neurite and tract orientation are parallel.[93]

Trophic influences

In addition to a lack of inhibitory molecules, it may be that peripheral nerve grafts support CNS axonal elongation due to secretion of neurotrophic factors. The most astonishing example of the neurotrophic potential of peripheral nerve derives from rat experiments in which small nerve segments were implanted into the vitreous humour of the eye following optic nerve lesions. The result was a dramatic regeneration of retinal ganglion cells beyond the lesion, whereas there is normally no regeneration following the same lesions without nerve implants.[94] Most studies to date have investigated the enhancing effects of neurotrophic factors when administered in conjunction with other manipulations. For example, nerve growth factor (NGF) can enhance axonal growth through foetal spinal cord transplants or peripheral nerve grafts.[78,95,96] Additionally, BDNF and NT3 enhanced supra- and propriospinal axons into Schwann cell-seeded guidance channels *in vivo*,[97] and boosted growth of descending axons into foetal transplants.[98] Few studies have shown that treatment with neurotrophic factors on their own can induce regeneration. Some notable exceptions include two studies in which neurotrophic factors promoted robust regrowth following lesions to the dorsal columns or to the dorsal roots.[99,100] NT3 promoted extensive growth beyond the dorsal column crush lesion epicentre, and NGF, NT3, and glial cell line-derived neurotrophic factor (GDNF), but not BDNF promoted re-entry of damaged dorsal root axons back into the spinal cord where they made functional connections with dorsal horn neurons, and resulted in functionally relevant restitution of sensation.

The objectives of the ISRT, then, include supporting research which (i) delineates the full spectrum of trophic and inhibitory molecules involved in regulating the ability of the injured human spinal cord to regenerate; (ii) defines the manipulations of trophic and inhibitory molecules that will yield the greatest impact on reparative regeneration within the human spinal cord; (iii) determines the temporal pattern of trophic and inhibitory influences, both with regard to their time course(s) after injury as well as with respect to differential staging that might be required of any therapeutic manipulation of endogenous factors or with exogenous factors; (iv) to define the differences between the sensory, motor and autonomic systems with respect to their responses to trophic and inhibitory molecules; and (v) to integrate influences on axonal growth with those on axonal guidance (see Vertical Target 3). See also Table 2.

Vertical Target 3: Guiding extended regrowth and establishing appropriate connectivities (Table 3)

Of course while formidable, overcoming inhibitory barriers to growth forms only part of the problem faced in designing a solution to SCI. Once axonal elongation is initiated, there must be some way to maintain axonal ougrowth in a guided fashion such that appropriate targets can be reached and innervated, as it is probably unreasonable to assume that once growing, axons can find their own way (although there is now evidence that appropriate synaptogenesis can be achieved in some cases).[100,101] Axon guidance mechanisms have been studied mainly in development (and then most often in invertebrate organisms such as *C. Elegans* and *Drosophila*), and we are still very far from realising potential for known guidance molecules following SCI even in simple vertebrates. Nevertheless, the importance of studying guidance cues is obvious for SCI, where random and disorganised

Table 3 Vertical Target 3: Guiding extended regrowth and establishing appropriate connectivities

Action plans		*Priority*
V3.1	Fund research to identify, quantify and profile over time all the significant trophic influences on axonal outgrowth pertinent to the regenerating spinal cord.	High
V3.2	Fund experimental interventions that delineate the relative importance of these trophic factors over time, status of regeneration and according to different patterns/sites of spinal cord injury, and to integrate them into a unified understanding of axonal guidance mechanisms.	High
V3.3	Fund research into the cellular sources and regulatory mechanisms governing the biosynthesis of the key trophic influences on spinal cord regeneration.	High
V3.4	Develop approaches/agents for pharmacologically manipulating trophic factors, exploiting biosynthesis regulators, agonists/antagonists and agents selectively modulating their post-receptor signal transduction pathways, for guiding regenerative axonal outgrowth in a controlled and tract/target organ-specific way (see also Vertical Target 2). This may include applying the trophic factors themselves to the injured spinal cord.	Medium: requires progress in V3.1–3.3
V3.5	If/while the exploitation of trophic mechanisms necessitates the application of the macromolecular factors themselves, support the study of the appropriate protein delivery systems in the spinal cord, as in V2.10	Medium
V3.6	Investigate the capabilities of different transplanted cell types (Schwann cells, ensheathing cells, etc.) in promoting and/or guiding axonal outgrowth across and beyond the lesion site, including their ability to bridge over intrinsically growth-inhibitory endogenous environments. This work includes the *ex vivo* manipulation of such cell populations to expand their numbers or trophic capacity, and the generation of genetically modified cells.	High
V3.7	Develop methods for the ethical production and standardisation of transplantable cell populations to allow these to be used routinely (and preferably without the need to use patient-specific source materials) in the treatment of SCI.	Medium: requires further progress in V3.6
V3.8	Ensure that experimental systems that use transplantable cells as bridges/guidance systems consider their realistic applicability in the clinic and devise strategies to overcome any obstacles to such widespread applicability. This work should include the exploration of ways in which to implant cells in a reproducible and controlled manner, such as incorporation in gels, tubes and other matrices.	Medium
V3.9	Promote the sharing of novel reagents and cell preparations pertinent to this field (including neutralising antibodies and assay reagents) between laboratories, including efforts to gain access to materials available from commercial organisations.	Medium

Some other Action Plans that directly mirror those in Vertical Target 2 may also be pertinent to trophic factor biology.

Exclusions, future issues, etc.	*Proposed approach*
Work in non-spinal cord models and non-mammalian systems should be explicitly justified.	Proposals should include plans for testing the validity of the results and conclusions in the mammalian spinal cord.
Basic work delineating trophic mechanisms in embryogenesis will not normally be funded by ISRT.	Work addressing the relevance of such developmental mechanisms in the adult or regenerating nervous system is potentially of interest to the Trust.
Funding for the production of reagents as a service to others will only be considered if there are no alternative means of supply for critical materials. Commercial production will not be subsidised by ISRT.	Some modest provision for this, e.g. through enhanced consumables expenditure or technical support, might be considered within an ISRT grant.
The mathematical modelling of multi-factorial trophic influences on nerve regeneration, incorporating changes over time, may be useful or even essential but the Trust would seek to harness more generic research in this field, in preference to funding new, SCI-specific algorithms.	Exploration of this field might usefully be conducted within the integrative approach described in V3.2.

growth may be without function or may even be deleterious.

Trophic influences

The most complete story in terms of axon guidance during development involves the floor plate, a ventral midline structure made up of specialised glial cells, which is crucial to the appropriate targeting of (among others) commisural axons – those which connect one side of the nervous system with the other. The floor plate exerts a trophic influence on commisural axons by releasing netrin-1, a diffusible chemoattractant originally purified from chick brain,[102] which then guides axons expressing the netrin-1 receptor DCC (deleted in colorectal cancer[103], toward the midline (for more extensive reviews see[104–107]). Netrin 1-may also act as a diffusible repulsive cue, as *in vitro* studies have shown that both floor plate and netrin-1-expressing COS cells can repel trochlear axons, which grow

ventro-dorsally *in vivo*.[108] Such trophic cues are of obvious importance in SCI, where appropriate guidance of regenerating axons will be required.

The most intensively-studied repulsive guidance system in the developing CNS involves a class of molecules known as semaphorins, and their receptors, the neuropilins (reviewed in[105,109,110]). In particular, semaphorin III (now known as Sema3A) has been shown to repel or collapse growth cones of primary sensory, sympathetic, and motoneurons,[111–114] and these actions occur via neuropilin-1, the Sema3A receptor.[115–117] The importance of semaphorins and their receptors in SCI is becoming more apparent as Sema3A has been found in fibroblast-like cells in CNS scar tissue, while neuropilin-1 expression has been found on neurons projecting to the areas immediately around the scar.[118]

Most actions of netrins and semaphorins are thought to be at-a-distance. That is, they are diffusible attractive or repulsive cues. Such diffusible factors are likely to be important for guiding long-range regrowth following SCI, but contact-mediated guidance will probably also be important for directional decision-making at critical choice-points. Short-range guidance cues are provided by another set of proteins known as ephrins, and their receptor molecules, the Eph receptors (reviewed in[119–121]) Little is known about ephrin and Eph receptor expression and function in the spinal cord. However, during development, complementary expression of Eph A5 in the ventral cord and its ligand ephrin A5 in the dorsal cord is thought to guide ventralward growth of spinal cord axons.[122] Following SCI, the expression of ephrin B3 increases, and may contribute to contact-mediated inhibition of axonal regeneration.[123]

Axonal branching

As with growth cone guidance, axonal branching patterns are unlikely to be revealed as intrinsic properties of damaged axons, and therefore the ISRT is interested in funding the cataloguing of all agents whch contribute to this process. Again, in just the past year, significant advances have been made in this area.

Axonal branching takes on two forms. One is the simultaneous ramification of two or more daughter axons from a parent fibre as it elongates. It has been known for some time that neurotrophins regulate the terminal arborisation of some types of axons: in the PNS, NGF regulates the complexity of primary afferent terminations in the skin, as well as sympathetic axonal and dendritic morphology.[124,125] In the CNS, NGF and GDNF can prevent aberrant terminal arborisation associated with peripheral nerve injury,[126,127] and BDNF promotes the terminal branching of retinal ganglion axons in the optic tectum.[128]

A second form of axon branching occurs long after a parent axon has navigated its course, and a daughter axon sprouts as a collateral from somewhere along its length. This is referred to as 'delayed interstitial branching', and examples include cortical layer 5 neurons, which after having grown into the spinal cord during development, send a collateral into the basilar pons (see[129]), and primary afferent neurons which, following bifurcation upon entry into the spinal cord forming a longitudinal bundle, send collaterals ventrally into the grey matter of the dorsal horn.[130] This latter process is now thought to be mediated by Slit-2,[131] which promotes delayed interstitial branching *in vitro*, and which is expressed at the appropriate time for collateral formation in the embryonic rat spinal cord.

The above findings are encouraging and have obvious relevance to SCI, as even though we are far away from manipulating these guidance molecules in animal models, growth promoting and growth guiding factors will ultimately be required for effective treatment strategies. The objectives of the ISRT, with respect to guiding extended regrowth and establishing appropriate connectivities include (i) delineating all trophic factors that guide axonal outgrowth in specific directions, and the regulation of neuronal responsiveness; (ii) exploiting and controlling the potential of axonal sprouting and synaptic plasticity for regeneration and the expansion of functional innervation patterns (see also Vertical Target 4); and (iii) as in Vertical Target 2, combining growth with guidance. See also Table 3.

Vertical Target 4: Optimising the function of surviving CNS axonal fibres in partial spinal cord lesions (Table 4)

While a great research effort is rightly being directed at strategies to promote regeneration of lesioned axons after SCI, fewer studies have directly addressed the issue of what happens to surviving axons. The majority of SCIs are incomplete and result in some tissue sparing and some axon pathways remaining intact. Thus, an important strategy for SCI research has to be directed towards optimising the function of surviving CNS axons following a partial SCI. A research effort directed at enhancing the capacity of uninjured neural connections to undergo sprouting and plasticity could lead to enhanced functional recovery after SCI.

Neuroanatomical remodelling, or plasticity, can occur following deafferentation lesions, where the axonal projections to an area are destroyed or following target removal, where a lesion destroys neuronal populations. Remodelling after deafferentation refers to the apparent reinnervation of denervated neurons and is generally explained in terms of the increased availability of terminal space as a result of the deafferentation.[132–134] In comparison, remodelling after neuronal target ablation refers to the anomalous growth of axons which innervate other neurons in the absence of their normal target neurons.[133,135]

Following adult CNS injury varying degrees of spontaneous functional recovery are known to occur and it is thought that compensatory sprouting

Table 4 Vertical Target 4: Optimising the function of surviving CNS axonal fibres

Action plans		*Priority*
V4.1	Promote collaborative work with clinicians and the associated healthcare professions to integrate prospective reparative interventions with current best clinical practice and to identify any changes in post-injury treatment that will enhance the efficacy of future reparative interventions, e.g. by maintaining residual innervation patterns and function (see also V1).	High
V4.2	Fund the experimental and clinical study (collaboratively, as necessary) of agents and other interventions able to increase the function of residual innervation, e.g. through an improved sensitivity to sub-optimal numbers of synaptic contacts or through the improved control of deleterious phenomena such as spasticity.	High
V4.3	Fund research into the promotion of the re-myelination of patent but damaged nerve fibres in the spinal cord as a means of restoring a more competent innervation, e.g. by the introduction of pro-myelinating factors or of remyelinating cells.	High
V4.4	Fund research into the promotion of nerve sprouting to re-innervate disconnected effector systems, including studies of the capacity of the SCI and other CNS centres to re-create sophisticated functional innervation by re-patterning the central connectivities, even of peripherally inappropriate synapses.	High
V4.5	Harness work using extrinsic nerve stimulation as a means of maintaining residual function (of nerves and effector/sensory systems) and as a means of regaining functional control, as an approach to gaining greater insight into the biological mechanisms involved in SCI or repair. (The development of prostheses *per se* is regarded primarily as beyond the Trust's funding focus.)	Medium
V4.6	Explore the value of extrinsic nerve stimulation as an adjunct to other regenerative interventions, e.g. through the promotion of tropic guidance of axonal outgrowth by intact nerve tracts.	Medium: awaits progress in V2, V3.
V4.7	Fund research into the value of surgical intervention in cases of established SCI with scarring or other deleterious post-injury developments, as a means of restoring partial function or of creating a more supportive regenerative environment (e.g. creation of a cleaner/less obstructive lesion site).	Medium

Exclusions, future issues, etc.	*Proposed approach*
Many activities in this area are primarily the responsibility of others, but the Trust will seek to harness the benefits of collaboration and mutual awareness.	ISRT's Clinical Initiative will include these aspects of SCI treatment

mechanisms may play a role in this process ([136]; reviewed in [137]). Plasticity of dorsal root projections have been demonstrated following a spinal cord hemisection lesion and it was suggested that the sprouting of primary afferents within the spinal cord mediated the recovery of different facets of motor behaviour.[138]

Injury-induced sprouting is often more extensive in developing animals than in mature animals.[132,139–141] The capacity for greater structural neuroplasticity in young rats may account for their remarkable ability to recover from trauma compared to the case in the adult. It is well known that neonatal rats have a far greater capacity for recovery after CNS lesions than the adult,[140,142–144] although this does not apply to all anatomical pathways and aspects of motor function.[145–147]

Castro and Mihailoff[148] compared the plasticity responses of two corticopontine projections in newborn rats. Rats received either deafferentation or neuronal target ablation lesions or a combined lesion. They also compared responses when the deafferent lesion was in the sensorimotor cortex or the occipital cortex. They found extensive remodelling of sensorimotor corticopontine fibres in response to either deafferentation of the sensorimotor cortex or neuronal ablation. The degree of sprouting was increased further with the combined lesion, suggesting that separate mechanisms of plasticity operate for different lesions and that these mechanisms may be additive. Deafferentation of the occipital cortex induced no remodelling of sensorimotor projections. Thus, they demonstrated topographic specificity after deafferentation lesions and that interactions can occur between associated pathways such that corresponding pathways were capable of plasticity but pathways which differed anatomically and functionally were not. Finally, the occipital corticopontine projection showed no remodelling after any of the lesions, showing that different CNS projections can in themselves differ in their inherent capacity for plasticity. Thus, these results imply that neonatal anatomical remodelling mechanisms involve complex rules of specificity.

Bernstein-Goral *et al*[149] used foetal tissue transplants in the lesioned neonatal rat spinal cord to elucidate whether regenerating axons and sprouting axons had different growth requirements. When non-target tissue was placed in a hemisection lesion innervation of the transplant tissue was observed only by the axon collaterals of intact sprouting

neurons. However, when target-specific transplants were placed in the hemisected cord the majority of axons innervating the transplant tissue were the regenerating axons of axotomised neurons. Thus, regenerating axons were regulated by target-specific cues whereas axonal sprouting appeared to be directed by a different set of signals. Separate mechanisms for axonal sprouting and axonal regeneration are also likely to exist in the adult. In agreement with the findings in the neonate, a study looking at gene expression after adult injury demonstrated that axonal sprouting in the adult nervous system is mediated by a molecular mechanism that is distinct from that involved in regenerative axonal growth.[150]

Wang *et al*[151] looked at the plasticity of three different spinal cord systems following a dorsal rhizotomy injury in the adult rat. The descending serotonergic and noradrenergic pathways responded differently to deafferentation, with an observed increase in serotonin levels but no change in levels of dopamine β-hydroxylase, the synthesizing enzyme for noradrenaline. Plasticity of the intrinsic tachykinin system was apparent from an initial decrease in substance P (SP) expression followed by a restoration of SP levels. As the majority of SP in the dorsal horn is derived from primary afferent terminals, which are removed by the rhizotomy injury, the subsequent SP upregulation most likely came from intrinsic spinal cord interneurons. Thus, the descending serotonergic system and the intrinsic tachykinin system were capable of plasticity in response to deafferentation, either by sprouting or increasing neurotransmitter or neuropeptide production, whereas the noradrenergic system did not show a plasticity response. The finding that two different intact systems (serotonergic and noradrenergic) reacted differently to the same injury led Wang *et al*[151] to suggest that the response of undamaged pathways to partial denervation is not random but is subject to some regulation. Further elucidation of the rules that govern plasticity in the adult CNS could prove important for the development of strategies aimed at inducing sprouting of undamaged pathways into denervated areas.

A recent study has highlighted the possibility of using molecules which neutralise inhibitory factors to uncover inherent plasticity in the adult nervous system.[81,82] In this study, the anatomical and functional effects of the IN-1 antibody were examined following a well defined lesion to the CST. The corticospinal projections of the non-lesioned CST and the corticorubral and corticopontine projections of the lesioned CST were examined and in both cases extensive branching was observed in lesioned rats treated with IN-1. In the brainstem, CST fibres from the lesioned side extended new branches into intact areas and in the spinal cord the unlesioned tract gave rise to new collaterals which appeared to reinnervate the denervated contralateral grey matter. In IN-1 treated rats they also observed apparently complete behavioural recovery on a number of tests of forelimb function. In contrast, there was a lack of structural plasticity and behavioural recovery in rats receiving control implants. Thus, neutralising inhibitory factors after a corticospinal tract lesion at the brainstem level promoted plastic changes at numerous sites in both the brain and spinal cord and was associated with recovery of forelimb function. The implication from this study is that the lesion induces some kind of sprouting tendency that is normally kept in check by whatever molecules are blocked by the antibody and that unmasking the potential for plasticity can be beneficial in promoting functional recovery. The authors claim, in light of these findings, that some of the observed functional recovery in previous experimental studies of SCI may have been due to compensatory plastic changes elsewhere rather than to the regeneration of damaged axons. However, it is unlikely that some of the robust regeneration that has been achieved with various experimental strategies (see Vertical Target 2) does not play a part in the observed functional recovery. To achieve regeneration of lesioned axons so that they form functional connections still has to be the major goal in SCI research. However, plastic changes and collateral sprouting no doubt play an important role in a CNS injury response and understanding the mechanisms involved may take us another step nearer the goal of functional recovery following SCI.

Some important questions have been raised by these recent studies on sprouting whose answers will be important for understanding mechanisms of CNS regeneration and plasticity. Firstly, what does a lesion do to axons that makes them sprout? Secondly, what are the mechanisms that cause unlesioned axons to put out new collaterals? They may undergo some intrinsic change that alters their growth properties, or they may simply be responding to external cues, perhaps diffusible factors released from nearby denervated tissue. Finally, what other factors or treatments can unmask the capacity of lesioned or intact axons to undergo compensatory collateral sprouting?

The type and the extent of plasticity that occurs following a CNS injury seems to depend upon a number of factors which include the stage of maturity of the neuronal pathway at the time of injury, the extent of injury incurred, the inherent nature of both the injured and intact systems and the environment at the injury site. In the clinic, rehabilitative efforts are often directed towards maximising compensatory activity in surviving systems. Experimentally, further elucidation of the rules and mechanisms that govern plasticity and sprouting after SCI in the adult is needed in order to develop treatment strategies aimed at inducing sprouting of undamaged pathways into denervated areas. However, as well as trying to harness any beneficial effects that could be achieved by promoting compensatory collateral sprouting it must also be noted that in some cases collateral sprouting may prove to be detrimental. For example, following a peripheral nerve injury aberrant collateral

sprouting of primary afferents occurs in the dorsal horn and has been associated with symptoms of pain.[152–154] Treatment with the trophic factors NGF and GDNF have been shown to reverse the aberrant dorsal horn sprouting observed after a peripheral axotomy.[126,127] Thus, in the case of SCI, treatment strategies may need to be designed which strike a balance between harnessing beneficial sprouting responses and controlling compensatory but unproductive sprouting of axon collaterals.

The ISRT objectives, therefore, are (i) to maximise the function of surviving spinal cord pathways in order to improve the quality of life of those with SCI, (ii) to maintain the function of effector and sensory systems after SCI in order to provide an optimal environment for subsequent regenerative interventions and (iii) to exploit surviving nerve tracts in guiding regenerating axons.

Horizontal Capability 1: Developing representative animal models of SCI (Table 5)

Experimental models of SCI are crucial for the development of therapeutic strategies to treat the clinical problem. In order to reliably assess whether treatments can produce axonal regeneration and functional recovery it is essential to have a lesion model which has reproducible and consistent anatomical and functional outcome measures. Patrick D Wall, in a personal communication to the ISRT, has stated four characteristics that are required for an optimal model of SCI:

(1) *The nature and extent of the lesion should be precisely defined*. If there is doubt about the extent of a lesion or whether axons have been spared, then interpretations of regeneration can be misleading. Contusion injury models have met with this criticism but have been much improved since efforts have been made to standardise this model across multiple centres.
(2) *A histological method should be available to detect the growth of axons through the lesion*. For example, advantage should be taken of the various anterograde and retrograde tracers and intrinsic markers of different fibre types in order to visualise specific pathways and allow the comparison of different tracts following various treatments.
(3) *An electrophysiological method should be available capable of detecting functional synaptic transmission beyond the lesion*. If axons can be stimulated to regrow it will be essential to determine whether regenerating axons have formed functional synaptic connections.
(4) *A behavioural measure should be available capable of detecting restoration of known circuits*. If axon regeneration is achieved then it is essential to assess whether any recovery of function has occurred.

Table 5 Horizontal Capability 1: Developing representative animal models of SCI

Action plans		*Priority*
H1.1	Fund research to establish a mouse model of SCI, in order to allow the use of mouse-specific reagents, transgenics and other tools.	High
H1.2	Fund research to establish a rodent model of the 'massive compression/laceration' and 'solid core injury' types of human SCI.	High
H1.3	Fund work, both experimental and through literature reviews, that integrates the results from different methodologies and models, including the resolution of discrepancies and gaps.	High
H1.4	Fund research that integrates, in a rodent model of SCI, the introduction of bridging grafts, and the application of an optimal combination of trophic agents and antagonists of CNS inhibitory molecules, in preparation for a similarly integrated approach in the clinic.	High
H1.5	Fund research that integrates, in a primate model closely resembling human SCI, the introduction of bridging grafts, and the application of an optimal combination of trophic agents and antagonists of CNS inhibitory molecules, in preparation for a similarly integrated approach in the clinic.	High
H1.6	Encourage the use of genetically-modified animals (knock-outs, transgenics, etc.) in established SCI models, in order to gain additional insights into the key molecular and cellular components of SCI and its repair.	Medium
H1.7	Fund research aimed at cataloguing the repertoire of mediators, cellular events and other base parameters in animal models and, where possible, in man, to act as a basic definition of SCI.	Medium
H1.8	Fund the establishment of animal models of demyelination and remyelination in the spinal cord.	Medium
H1.9	Fund research to establish a cat model of SCI in order to harness the sophisticated electrophysiological capabilities available for this species.	Low
H1.10	Fund the supply of equipment necessary for the standardised use of selected models in laboratories funded by ISRT.	Low

Exclusions, future issues, etc.	*Proposed approach*
As noted under 'Objectives', work in non-spinal cord models should be explicitly justified.	Proposals should include plans for testing the validity of the results and conclusions in the spinal cord.

Two common approaches are, firstly, to use an experimental model that aims to mimic, as closely as possible, the type of SCI that is seen clinically. The most common model in this instance is the contusion (or weight-drop) injury. The second approach is to use a model in which specific axon pathways or projection systems are lesioned in order to study that particular system's response to injury and its regenerative capacity. There is merit in both types of strategy and also potential pitfalls with both.

Contusion-type lesion models

Most human spinal cord injuries are 'closed' injuries, without an open wound of the cord or dura matter, and result in acute contusion due to displacement of bone or disc into the spinal cord during fracture dislocation or burst fracture of the spine. Contusion injuries involving a weight-drop method, first described by Allen,[155] have been used in adult rats to provide an experimental model that closely mimics the outcome of many human injuries. The histological appearance of an experimentally contused spinal cord is usually characterised by an evolving core of central necrosis which results in the formation of large cysts. The central cavitation is surrounded by a rim of more intact-appearing white matter with the rim of spared tissue becoming smaller as the height of the weight drop is increased. A common criticism of the contusion model in adult rats has been that this type of injury is not sufficiently reproducible. Variable degrees of tissue damage and spared white matter makes it difficult to distinguish between regenerated and surviving axons, leading to difficulties in interpreting regeneration studies. The New York University weight-drop device (the NYU impactor model) has been designed to standardise contusion injury models.[5,156] A 10 g rod is released from incremental pre-set heights above the cord to produce increased levels of lesion severity. It supposedly delivers graded and precisely measured trauma to rat spinal cord, producing consistent lesion volumes and reproducible rims of spared white matter. Correlations have been made between cord compression rate, lesion volumes, spared white matter and locomotor recovery.[157–159]

A recent study analyzed the degree to which adult rat contusion injuries could serve as a model for human SCI.[159] Morphological, electrophysiological and functional outcome measures were used to compare rats lesioned with the NYU impactor model and human patients with chronic SCI. In both rats and humans close correlations were observed between lesion length and spinal cord atrophy (assessed by magnetic resonance imaging), prolonged latencies and reduced amplitudes in motor and somatosensory evoked potentials and impaired locomotor capacity (assessed by the 21-point BBB scale). This study demonstrated that techniques for evaluating the extent and severity of SCI in humans and animals can be comparable (see also Horizontal Capability 2) and that the rat contusion model provides us with a valuable tool for assessing the effects of new treatment strategies. Indeed, experimental studies which have assessed the effects of methylprednisolone[5,7,9,160] and other pharmacological interventions[161] after SCI have generally used contusion models.

Contusion lesions, therefore, are a valuable model when trying to mimic the human condition of SCI and for testing out therapeutic strategies. However, to further our understanding of mechanisms of repair and regeneration much can be learned from the responses of individual fibre tracts to injury that may not be derived from contusion models.

Transection lesion models

Equally important to the ISRT research strategy are models that teach us about specific spinal cord pathways and their varying capacities for regeneration. For such studies microsurgical transection lesions are often used. Complete transection lesions, although very rare clinically, provide a valuable tool for SCI research. In a study by Cheng *et al*[77] the spinal cord was transected and a length of cord removed. They used peripheral nerve grafts as bridges to re-route specific ascending and descending tracts from white matter to grey matter and vertebral shortening, fibrin glue and acidic fibroblast growth factor to provide stabilisation and trophic support. This approach is important because any fibre growth observed after a complete transection lesion must be due to regenerating or sprouting axons, as there can be no axon sparing in this model. Furthermore, this was a key study which emphasised the importance of a combination of strategies to promote regeneration, one of the prime objectives of the ISRT. A similar approach has been employed in the experiments of Bunge and colleagues who have removed segments of thoracic cord and used Schwann cell-filled guidance channels as artificial bridges to connect the proximal and distal transected stumps. They have used this model to assess the effects of various growth promoting strategies on inducing axon regeneration into and beyond the bridges.[8,162–165]

Other studies have used incomplete transections in order to lesion one half or more of the spinal cord. A wealth of studies have employed the hemisection model to investigate the effects of various treatments in promoting axon regeneration[98,166–173] and cell survival.[27–30,174,175]

Other lesion models target specific tracts or pathways. For example, lesions which target the dorsal columns have been used to sever the ascending dorsal column projection[99,176] and the descending dorsal corticospinal tract.[80,177] An electrolytic lesion technique was employed by Li *et al*[87] to produce a more selective dorsal corticospinal tract lesion. In this study they lesioned the corticospinal tract on one side of the cord to investigate whether olfactory ensheathing cell transplants could promote regeneration of this tract.

Similarly, Schwab and colleagues have employed a more precise surgical lesion than in previous studies to specifically lesion the corticospinal tract unilaterally by exposing the tract at the level of the pyramids in the brainstem so that it could easily be identified and transected with minimal damage to neighbouring pathways.[81,82] Dorsal rhizotomy lesions have also been employed to specifically study primary afferent regeneration into the cord.[100,101,178]

A major advantage of discreet lesions is that the functional relevance of specific pathways can be determined. For example, accurate performance in tasks requiring skilled forelimb function has been shown to be dependent on the integrity of the CST.[81,82,87]

The biggest potential problem with partial transection lesions or targeted lesions of specific tracts is that there is always the possibility that axons may have been spared in the pathway that is being investigated, which could lead to unlesioned axons being attributed as regenerating. In some cases these problems can be overcome. For example, with the electrolytic lesion employed by Li *et al*[87] it was possible to determine even after treatment whether the CST lesion had been complete and rats with incomplete lesions were discarded from the study. However, in other lesion models it may be impossible to verify whether the lesion was complete after the rat has been treated with a regeneration promoting strategy. The only way to overcome such problems is to adopt stringent controls. For example, if, after a particular lesion and no treatment, regenerating axons are never observed in the pathway of interest then any axons observed in the same model but after a particular treatment can be attributed as regenerating with some confidence.

Two common problems with all current injury models are that in patients, ventral compression from burst fractures or circumferential cord compression from fracture dislocation is very common. Whereas most animal models use a dorsal approach through a laminectomy to create the injury. Also, the majority of human injuries occur in a closed vertebral system, whereas most animal models use an open laminectomy for lesioning. However, there seems no easy way to get around these problems.

Experimental animal models of SCI have proved vital in furthering our understanding of the pathological events that occur following SCI and the challenges that need to be overcome to achieve repair and regeneration. Models that aim to mimic human SCI, such as contusion injuries will be particularly valuable for examining functional and morphological changes after SCI and for testing pharmacological and other therapeutic interventions. However, we do not recommend that every centre should use this model. Equally important to the ISRT research strategy are models that teach us about specific spinal cord tracts and their varying capacities for regeneration. Transection and targeted lesions are likely to be vital for the discovery of molecular mechanisms involved in CNS repair and regeneration. So, in the first instance, researchers must continue using a variety of different models in order to learn more about the regenerative capacity and plasticity of different systems. In the second instance, contusion models will be important for trying out new treatment strategies before higher testing is carried out in primates and finally in the clinic.

The ISRT also want to stress the importance of accumulating and integrating available information from multiple laboratories. An effort needs to be made to integrate the results from different laboratories so that models can be standardised and results can be comparable. We need to consider the reality of standardising models across centres, although efforts have already been made with the NYU impactor model which has now been standardised across multiple centres.[179,180]

Finally, whatever model is used, whether it be a complete or partial transection or a contusion injury, it is essential that the injury is consistent and has reproducible anatomical and behavioural outcomes. The following ISRT objectives are aimed at establishing well characterised models of acute and chronic spinal cord damage and repair: (i) To delineate the basic operational and descriptive parameters (precise nature of the lesion, including location and proportion of the CNS axonal fibres that are dislocated, etc) that should be established for all animal models used in SCI research, in order to facilitate the transfer of models between laboratories, to enhance the reproducibility of results and to promote the integration of results from different studies. (ii) To promote the adoption, by ISRT-funded researchers, of the most informative models applicable to the study being undertaken. These may include models of systems outside the SC if they offer unique insights and/or accessibility for the study, but every attempt should then be made to relate the results back to their relevance in the SC. (iii) To delineate animal models representing the main formats of SCI seen in man, and to promote their 'tertiary' use to evaluate the likely relevance of results obtained in other experimental systems.

Horizontal Capability 2: Developing methods for measuring CNS axonal regrowth and restoration of function (Table 6)

The ability to accurately determine the extent of regeneration and functional restitution is an obvious and crucial requirement if the ISRT is to be successful in determining a feasible repair strategy. The ability to detect (at a minimum) substantial recovery would only be useful if this were likely to occur, and while there is much optimism that in the near future robust regeneration will become a reality, for the time being tests must be devised to detect modest increments of improvement (or indeed, deterioration).

Table 6 Horizontal Capability 2: Developing methods for measuring CNS axonal regeneration and the restoration of function

Action plans		*Priority*
H2.1	Fund research to develop the utility of non-invasive methodologies, including imaging and spectroscopy, for assessing SCI and repair/regeneration in animals and in man.	High
H2.2	Fund studies to compare and delineate robust experimental methodologies able to distinguish true CNS axonal regeneration from collateral sprouting, surviving fibres, central rewiring and other mechanisms that could explain the demonstration of additional function or morphological evidence of new outgrowth.	High
H2.3	Fund studies that develop new approaches to selectively labelling defined axonal tracts in order to facilitate subsequent analyses of regeneration after SCI (e.g. by tract-specific transfection of neuronal populations with readily traced markers).	High
H2.4	Fund studies that closely couple, in animal models, the measurement of physical CNS axonal outgrowth, the formation of morphological re-innervation and synaptic connectivity patterns, and the assessment of functional restoration through behavioural studies.	High
H2.5	Fund work to develop functional assessment systems and parameters that offer objective, quantitative and high resolution information about re-innervation, to replace methodologies that measure broad functions (e.g. simple stepping systems) that are susceptible to multiple possible re-innervation/collateral sprouting/synaptic plasticity mechanisms. Work should eventually be confirmed in species with well-developed higher cortical modulation of behaviour.	High
H2.6	Fund work – experimental, through literature reviews and through the organisation of cross-disciplinary discussion fora – that correlates measurement systems used in animal models and in man; foster the associated interface between scientists and clinicians.	High
H2.7	Fund research that aims to establish comprehensive, sensitive and detailed characterisations of the state of spinal cord damage, synaptic connectivity and residual function in SCI patients – a new 'integrated package' of baseline data.	High
H2.8	Fund work to identify the human SCI sub-types, originally categorised by morphological studies post-mortem, according to non-invasive parameters (including the use of imaging).	High
H2.9	Fund studies that closely couple, in a primate model closely resembling human SCI, the measurement of physical CNS axonal outgrowth, the formation of morphological re-innervation patterns and synaptic connectivity patterns, and the assessment of functional restoration through behavioural studies.	Medium (because the work needs to be developed first in lower species)
H2.10	Fund studies of existing standardised methods for assessing residual function in the SCI patient, in order to estimate their progression with time post-SCI, their intrinsic variability during longitudinal studies, and their sensitivity to different degrees of SCI (and thence their ability to track partial repair).	Medium
H2.11	Fund longitudinal studies of the new integrated package (H2.7) of methods for assessing function and connectivity in the SCI patient, in order to estimate their progression with time post-SCI, their intrinsic variability, and their sensitivity to different degrees of SCI (and thence their ability to track partial repair).	Medium (awaits progress in other H2 Action Plans; direct comparisons with existing methods – H2.10 – would also be desirable in due course)

Exclusions, future issues, etc.	*Proposed approach*
Funding imaging and other major equipment in SCI centres.	Refer to alternative funding mechanisms, including NHS.
Refining image analysis software to be more suitable for evaluating SCI and CNS axonal repair.	Seek support from imaging equipment manufacturers and encourage application for Research Council funding.
Establishing new centres for basic electrophysiological research, or building electrophysiological capabilities *de novo* into laboratories providing other skills pertinent to ISRT's goals. The same exclusions apply to other skills/facilities better accessed from existing centres.	Seek support from imaging equipment manufacturers and encourage application for Research Council funding.

Anatomical studies

In the laboratory, anatomical measures of regeneration must be robust and quantitative, and they must ideally distinguish real regeneration from compensatory reactions (like sprouting) of intact systems.

Intrinsic proteins expressed within different subsets of axons may be useful as tags for demonstrating structural changes reflecting regeneration or remodelling. For example, separate populations of axons within the spinal cord express separate markers: descending corticospinal tract axons contain an isoform of protein-kinase C (PKCγ[181]), axons from neurons located in locus coeruleus express the enzyme tyrosine hydroxylase,[149] and a separate population of

bulbospinal axons express serotonin.[182] This means that separate axonal phenotypes can be identified, and their responses to injury and treatment can be studied. Minimally overlapping populations of primary sensory neurons express calcitonin gene-related peptide and the purinoceptor $P2X_3$, and have served useful in the study of primary afferent regeneration into the spinal cord[88,100] and of primary afferent plasticity following cord deafferentation[183] or spinal cord injury.[184]

Intrinsic markers, however, have disadvantages as in some cases there are other populations of spinal cord neurons which also express them (interneurons in the dorsal horn, for example, also express PKCγ[181]), or their expression may be modulated as a result of injury, or by treatment with pharmacological agents such as neurotrophic factors (e.g. [127,185]). This means that comparisons of axonal distributions between the intact state, the injury state, and injury-plus-treatment state can be easily muddied. A slightly different approach that has been used recently involves the xenotransplantation of mouse neurons from a green fluorescent protein-expressing transgenic line into the rat spinal cord.[69] Some of the above disadvantages are overcome since green fluorescent protein is constitutively expressed in the transplanted mouse neurons, and because it is expressed nowhere in the host tissue.

An alternative to the use of intrinsic markers is the application of exogenous molecules or viral vectors in tract-tracing experiments. Cortical injections of high molecular weight dextran-amines have been used to successfully label axons in the rat corticospinal tract.[163] Distinct populations of primary sensory axonal terminations within the spinal cord can be labelled with (for example) the β-fragment of cholera toxin (CTB[186]), and lectins from *triticum vulgaris* (WGA) and *bandaeria simpifolicia* (IB4),[187] and thus differential responsiveness of these populations to different treatments can, in principle, be determined. Viral vectors might also be used to deliver green fluorescent protein (for example) to specific populations of neurons either via stereotactic injections into defined nuclei, or by directed targeting of different neuronal surface epitopes, allowing long term, permanent, or even inducible marker expression.[188] However, these tract tracers possess some of the same disadvantages as intrinsic markers, as their uptake and/or transport properties may be modulated by injury and/or treatment: IB4 transport is impaired,[189] and CTB is transported by a broader, less specific population of axons following peripheral nerve injury.[186] Tracing experiments, as with the use of intrinsic markers, are plagued with the difficulty that some 'regrowth' may be indistinguishable from collateral sprouting of uninjured (yet labelled) axons.

Physiological studies

Of course, the mere demonstration of regrowth anatomically (and even the formation of synapse-like structures) does not necessarily imply that useful consequences ensue. At a minimum it should be demonstrated that the regrowing axons are capable of re-connecting with neurons in the spinal cord and in supraspinal structures and, ideally, it should be possible to distinguish connections made by regrowing axons from compensatory re-modelling within the cord. Post-synaptic recordings either from the surface of the spinal cord, or from individual units within the cord have shown that synaptic re-connection is indeed possible.[100,101] Further downstream, ventral root recordings have shown that in some cases, spinal reflex activity can be restored following dorsal root transection and transplantation of olfactory ensheathing cells.[88] Recent electrophysiological studies in spinal cord slice preparations have demonstrated occupation of vacated synapses by sprouting axons following peripheral nerve injury[190] and it should be possible to use similar techniques in SCI. It will, of course, be important to determine if effector responses regain some semblance of normality, and so in animal models (as well as in humans) baseline measurements of (for example) sensory, EMG and autonomic function will need to be rigorously and quantitatively categorised and, just as importantly, where multiple descending and ascending systems are disrupted, distinguished from one another.

Behavioural studies

In animal models of SCI, the study of functional restoration is particularly difficult for a number of reasons. A significant one in the rat is the remarkable capacity for spontaneous recovery of function after lesions to major tracts such as the dorsal columns. These contain central branches of large diameter primary afferent axons, and are thus responsible for transmission of much low threshold sensory information to the brainstem, as well as descending corticospinal axons that are thought to control fine movement of the distal parts of the limbs. Surprisingly, the behavioural deficits in reaching movements following dorsal column lesions affecting both the ascending and descending components are relatively minor, and detailed video analysis has been required for reasonable quantification.[191] Even then, it is uncertain to what extent the deficits are a result of the loss of sensory information ascending toward the brainstem, or of descending cortical impulses. The scarcity of information with respect to mechanisms of deficits and recovery is a problem in most open field-type behavioural tests, especially since the outcome measure involves movement or locomotion, in situations where both sensory and motor systems have been impaired.

In order to determine differential regeneration of separate axonal systems, there is the need to selectively and completely damage individual tracts within the spinal cord, which it is nearly impossible to do (see also Horizontal Capability 1). Another approach therefore has been to lesion axons outside the spinal

cord (primary afferents within dorsal roots), thus removing sensory input while leaving motor output intact. In this way not only can potential sensory reinnervation of the spinal cord be evaluated,[100] but the contribution of peripheral input to fine movements can also be determined.[192] However, it has been argued that dorsal root lesions do not have all of the same sequelae as direct CNS damage, and thus may not be representative of CNS lesions in humans. One of the aims of the ISRT in this respect is to help fund the development of high resolution functional assessment systems, in order to distinguish deficits in (and recovery of) sensory *versus* motor systems, and to discriminate recovery resulting from regeneration from that resulting from synaptic reorganisation following SCI.

Clinical studies

Initial clinical studies must focus on establishing standardised baseline measurements which will be sensitive enough to detect regeneration over one or two spinal segments. In humans, many of the difficulties associated with behavioural (neurological) testing in rats are avoided simply because patients are able to tell the examiner about what they do or don't feel. In this respect, sensory testing is easily done, and indeed all of the tools for thorough quantitative sensory evaluation of lesion extent are in place. In terms of skeletomotor function, however, previous work has focused on limb musculature (e.g. [193]), and there is now the need for more emphasis on making detailed analyses of the muscles of the trunk, as patients with mid-thoracic lesions are likely to be the first to receive potential restorative treatment. While it is not possible to do tract-tracing experiments in patients, high resolution imaging techniques such as magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and positron emission tomography (PET), which have previously been applied to the brain, can in theory be applied to the spinal cord. Indeed, important strides have recently been made in spinal cord imaging with both PET[194] and MRI,[159] and eventually, an 'integrated package' of baseline measurements of the state of spinal cord damage, synaptic connectivity and residual function should be assembled.

The usefulness of the techniques outlined above will be greatest where longitudinal and correlative studies are performed. The ISRT is particularly interested in funding studies which determine, in the same animals or patients, what the anatomical (via histological and imaging techniques) and functional (via physiological and behavioural assessment) deficits are following SCI and how they may change with experimental or therapeutic interventions. The principle objectives of the Trust in this respect are (i) to delineate practicable, sensitive, quantitative and preferably non-invasive methods for the evaluation of CNS axonal regeneration *in vivo*, in chronic animals and in humans (work should at a minimum rigorously define the specific CNS tracts being manipulated in animal studies); (ii) to delineate standardised methods for assessing the restoration of functional innervation *in vivo*, in chronic animals and in humans, with adequate sensitivity to support longitudinal studies; and (iii) to develop the use of imaging techniques, in chronic animals and in humans, for SCI and its treatment. See also Table 6.

Horizontal Capabilities 3 and 4: Establishing the capabilities to conduct clinical trials of new therapeutic interventions in SCI and promoting cross-disciplinary education and training and collaborative research and clinical practises focused on SCI and its treatment (Tables 7 and 8)

At the outset of this review, we emphasised that the cure for SCI was dependent not only upon successful demonstration of regeneration in the laboratory, but also success in bringing basic research findings to the point where they can be practised in patients. As encouraging as the recent advances in SCI research are, they mean very little to those suffering from SCI unless there is some hope of the results being applied to them. The question most often asked of basic researchers when a major advance is made is 'How soon will human sufferers of SCI benefit from this treatment?' In order that this question can be answered in any meaningful way, the chasm that has traditionally existed between basic and clinical research needs to be traversed – a bridge must be built between the laboratory, where potential treatments for SCI are discovered, and the clinic, where these discoveries may be put into practice as viable therapies. The ISRT is committed to supporting the transition of important findings in animal models into clinical applications, and thus its objectives are: (i) to establish the networks of skills and experience necessary to plan, conduct, and interpret meaningful clinical trials of new treatments (surgical, pharmacological, etc.) for SCI; (ii) to identify and then fill any gaps in the methodologies, reagents and training necessary to implement effective clinical interventions; (iii) to mutually inform clinicians and basic scientists of each others' advances and obstacles in developing effective repair strategies, and to promote the fruitful co-operative application of multiple clinical and scientific and technological expertise in a shared SCI problem-solving manner; (iv) to take a lead in promoting the investigation, in SCI, of emerging new treatments, including those that may be primarily intended for other indications (see also Table 7). It should be pointed out that as few other organisations have attempted to act in this 'translational' capacity, and thus some of the challenges remain unknown, this horizontal capability of the ISRT strategy will continue to be refined.

It is obvious that progress in the laboratory and the clinic would not be possible in the absence of

Table 7 Horizontal Capability 3: Establishing the capability to conduct clinical trials of new interventions for SCI

Action plans		*Priority*
H3.1	Initiate fora in which current clinical capabilities and gaps are identified, and co-operative approaches to SCI are explored.	High
H3.2	Design and promote meaningful experimental protocols for clinical trials by engaging the co-operation of relevant experts.	High
H3.3	Promote the exchange of insights between the clinical and research communities: encourage the consideration of basic scientific advances from the perspective of their potential clinical impact and utility, and the exploration of key clinical problems through appropriately designed laboratory studies, including through the organisation of joint scientist-clinician discussion meetings.	High
H3.4	Develop links with potential sources (including commercial organisations) of new treatments for SCI.	Medium
H3.5	Consider the promotion of SCI, if an adequately robust clinical trial protocol can be developed and tested, as a surrogate marker for the evaluation of treatments aimed at other, allied neurological applications, as a means of obtaining additional support and accessing candidate treatments.	Medium (awaits progress in other H3 Action Plans)
H3.6	Keep under review, and explore options for sharing with other medical charities – especially in the neurosciences, the appointment of additional ISRT staff to manage clinical trial activities (including the promotion of relevant 'enabling' research) on a day-to-day basis.	Medium
H3.7	Establish links with other national and international organisations (including, where relevant, those targeting non-SCI neuro-muscular syndromes) in order to share learnings and to establish common standards of clinical and ethical practice.	Medium
H3.8	Develop, if appropriate in concert with other medical charities, methods for the effective communication of scientific and clinical advances to spinally injured people and the wider community, in order to promote a realistic understanding of the prospects and anticipated timing of new treatments.	Medium

Exclusions, future issues, etc.	*Proposed approach*
Funding infrastructure such as buildings, major equipment, permanent clinical posts.	Refer to the relevant responsible body, such as the NHS.

This component of the Research Strategy is currently under additional active debate within the Trust's Clinical Initiative, and this provisional analysis will therefore be continue to be significantly refined.

Table 8 Horizontal Capability 4: Promoting cross-disciplinary training and collaborative research and clinical networks

Action plans		*Priority*
H4.1	Fund research fellowships/studentships (Nathalie Rose Barr Awards) for both basic scientist and clinical researcher tracks, with an explicit requirement that they support collaborative work between centres providing substantively different skills or methodologies, including between basic science and clinical centres.	High
H4.2	Fund, usually within a broader award, the sharing or transfer of skills, reagents, dedicated equipment and methodologies between centres working on SCI research or its treatment, e.g. through the specific support of travel or temporary secondments. This may include small grants to allow reagents or systems to be further developed in order to make them more applicable to key SCI models or SCI-relevant species, including man.	High
H4.3	Fund, and where appropriate initiate or organise, cross-disciplinary discussion fora such as symposia (including the Bermuda conference series) and seminars, in order to educate participants and disseminate insights into SCI and its treatment.	High
H4.4	Produce regular reports (including the Annual Research Review) of ISRT-funded work, and ensure their distribution to key opinion-leaders and potential recruits to the SCI research/treatment community.	High
H4.5	Ensure all ISRT-initiated communication initiatives are accurately informed by reliable scientific and clinical assessments of the current status and realistic future prospects for treating SCI.	High
H4.6	Build effective working relationships with other institutions (including other medical charities) and fora relevant to SCI and its treatment.	High

Exclusions, future issues, etc.	*Proposed approach*
The majority of 'public' communications from ISRT are aimed at non-technical audiences and fall outside the direct responsibility of the Research Division.	Ensure effective liaison as per Action Plans H3.8 and H4.5, above.

highly skilled personnel. The ISRT of course realises this and is committed to increasing the number, quality, and skill diversity of scientists and clinicians working on SCI. On the other hand, merely increasing the size of the body working on SCI does not necessarily imply that a cure will be achieved any more expeditiously. Thus the ISRT is also keenly interested in promoting fruitful interaction between disciplines and is dedicated to promoting the sharing of methodologies, insights and information pertinent to SCI and its treatment between the relevant scientific, clinical and other healthcare communities. The ISRT's specific objectives with respect to this horizontal capability are listed in Table 8.

Conclusion

This refined research strategy for the ISRT has attempted to provide firstly, a review of some of the current issues and major advances that have been made in the field of repair and regeneration following traumatic injury to the spinal cord and secondly, a structured framework of targets that the ISRT believe to be important for the development and application of treatment strategies that will benefit spinal injured patients. The vertical components of the strategy, which address the key biological problems, ultimately all involve understanding the balance and integration between potentially opposing influences: (1) The acute sequelae of SCI: issues of whether inflammation and immediate tissue 'clearance' events are deleterious or potentially growth promoting; (2) Trophic *versus* inhibitory factors: the balance between maximising the growth potential of damaged neurons and minimising inhibitory factors present in the lesion environment; (3) Guiding axonal growth: the possibility of conflicts of intent between directing axonal outgrowth to achieve appropriate re-innervation of effector tissues and impeding synaptic plasticity which can restore function even if physical connectivity is scrambled; (4) Maximising residual function: the balance between utilising remaining fibre systems and stimulating deleterious spasticity.

Thus, the ultimate goal requires a major shift in thinking, from 'influences in isolation' to a truly integrated, and therefore necessarily complex, understanding of multiple influences.

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