Nature Reviews Neuroscience | AOP, published online 7 March 2007; doi:10.1038/nrn2099

PERSPECTIVES

OPINION

Repair of neural pathways by olfactory ensheathing cells

Geoffrey Raisman and Ying Li

Abstract | Damage to nerve fibre pathways results in a devastating loss of function, due to the disconnection of nerve fibres from their targets. However, some recovery does occur and this has been correlated with the formation of new (albeit abnormal) connections. The view that an untapped growth potential resides in the adult CNS has led to various attempts to stimulate the repair of disconnectional injuries. A key factor in the failure of axonal regeneration in the CNS after injury is the loss of the aligned glial pathways that nerve fibres require for their elongation. Transplantation of cultured adult olfactory ensheathing cells into lesions is being investigated as a procedure to re-establish glial pathways permissive for the regeneration of severed axons.

The CNS endows the individual with its ability to adapt and respond to the features of the environment in which it finds itself. The brain is the ultimate organ of evolution, and as such the human brain enjoys the lion's share of genetic fine-tuning[1,2]. In order to adapt successfully to external conditions, the nervous system depends on the accuracy with which its constituent nerve cells establish and maintain their highly complex network of connections. It has long been a puzzle that function, which is essentially 'plastic' or adaptable, is carried out by connectional circuitry which seems to be fixed. It is a further paradox that severed nerve fibres in the brain and spinal cord seem incapable of effective regeneration of their lost connections.

It is crucial to pinpoint the source of the failure of regeneration. We propose that there are two levels of organization in the nervous system, with different rules governing them. The first is the white matter level, which is the subject of the black arrow school of the diagrams found in neuroanatomical textbooks, and which has a regular, predictable and fixed arrangement of nerve fibres and pathway glial cells within — and even largely between — species. The second is the fine 'neuropil' or grey matter level, which is where the functional connections between nerve cells are made, and whose structure may be in an as yet hardly detected state of continual change[3], indicating that nerve cells retain a degree of plasticity throughout adult life.

After disconnectional injury, regeneration fails at the white matter level. If interventions could be found to enable the severed nerve fibres to regenerate across the damaged site, the plasticity of the grey matter neuropil might allow for new functional connections to be restored. Many methods for repair have been tried. None has yet reached the level of routine application in clinical practice. This article explores the idea that the response of the glial cells to injury is a crucial contributor to the failure of axon regeneration in CNS white matter, and that repair of the glial pathway by transplantation might enable the inherent growth potential latent in adult nerve fibres to be expressed as regeneration across the injury site.

This approach, which we call the pathway hypothesis, is only a hypothesis. It does not exclude, and might well be complementary to, other approaches, such as attempts to introduce nerve growth factors, prevent secondary damage, encourage sprouting or neutralize inhibitory molecules, which have been dealt with elsewhere in extensive reviews[4–7].

Responses to injury

In contrast to the failure of regeneration in white matter tracts, the response to injury at the neuropil level is more positive. Loss of fibre inputs to the neuropil has resulted in replacement of the lost synapses by the formation of new connections in the denervated areas, commonly referred to as 'sprouting'[8,9]. These new connections are not formed by regeneration of the originally cut fibres, but consist of adventitious synaptic contacts made by intact fibres surviving in the denervated area. If the original pathway contained unique and irreplaceable information (such as the visual input from the retina), the new connections would not be able to restore such functions. However, in less dedicated pathways, the new neuropil circuits could result in beneficial effects. It is also possible that they could produce adverse effects[10], such as making sites unavailable for regeneration of the original connections[11] and causing abnormal sensations or abnormal movements. However, if therapeutic interventions at the pathway level could overcome the block to regeneration of the originally cut axons in the white matter tracts, the innate capacity for synaptogenesis at the neuropil level might provide a situation that would enable the regenerating fibres to re-establish functional connections with their original targets.

Although nerve fibres severed in their course through the white matter do not regenerate, they show an immediate local sprouting response[12–15]. In the CNS, this response is much weaker than it is in peripheral nerves, and depends on the distance of the axotomy from the cell body[16]. Moreover, after axons are lesioned in the white matter, the sprouts arising from the cut ends of the nerve fibres are frustrated in their attempts to advance. Instead, branching of the cut fibres in the area of the lesion produces localized neuromatous configurations[12,17] (FIG. 1). Although a proportion of these axotomized nerve cells atrophy or die[18], those neurons that survive offer an opportunity to devise procedures to reconnect them to their original targets. The strategy to be adopted will depend largely on what is perceived to be the cause of the failure of regeneration. Why do axons which are able to grow during development fail to do so when lesioned in the adult?

Figure 1 | **Sprouting at the cut ends of axons in the CNS.** Camera lucida drawings showing the variety and profusion of branching at the cut ends of three sample axons labelled by anterograde transport of biotin dextran between 9 and 13 weeks after lesion of the rat corticospinal tract. Modified, with permission, from REF. 17 © (1995) Elsevier Science.

Clues from development

In the embryo, the developing pattern of neuronal connections results from the cumulative action of a precise temporal and spatial hierarchy of a large number of specific molecular signals that populate the environment through which the nerve fibres grow[19–21]. The growing axons navigate by means of cues in the vicinity of the explorative filopodia extruded from the growth cones[22]. These signals are detected and responded to by appropriate ligand–receptor interactions with matching molecules on the nerve fibre surface[23]. The outcome of these interactions determines the decisions made by a growing nerve fibre to advance or retract, pause, turn, branch, and form or detach contacts[24,25], and determines the final pattern of connections that the nerve cell establishes.

The operation of developmental molecular signals is contingent in a stepwise fashion on preceding events. For example, once developing commissural spinal axons reach the floor plate they change their direction, abandoning their circumferential movement and turning rostrocaudally[26]. As development progresses, the pattern of evolving connections becomes increasingly responsive to inputs from within the body and from the external environment, as well as to the evolving patterns of neural circuit activity[27,28]. This enables the developing nervous system to adapt its pattern of connections to optimize its response to the specific circumstances of the environment that an individual encounters during development.

Ongoing experimental work is showing that, in addition to molecular changes affecting synaptic efficacy[29], a key mechanism in both developmental and adult plasticity[30,31] is the ability to change the anatomical patterns of connectivity[8,32]. To preserve the existing hierarchy of connections (and maintain previously acquired functions), ongoing changes must accommodated in a way that respects the same mechanisms and obeys the same rules as the original development of connectional patterns. Indeed, there is mounting evidence that many of the molecular signals acting during development either remain present or are elicitable in the adult[21,33].

Pinpointing the defect in the adult

One factor underlying the decrease in the ability of cut axons to elongate in the adult might be intrinsic developmental changes taking place during the maturation of adult neurons[34–37]. However, during development, as the neural tube develops into the adult brain, dramatic changes in the arrangement and size of its component parts occur. Even if adult neurons mount a growth response at the same level as their embryonic counterparts, the sprouts formed would need to navigate very different environments and elongate over much greater distances than did their embryonic counterparts[38].

The absence of growth may be a result of unfavourable extrinsic inputs, such as a lack of positive stimuli or inhibitory influences in the adult tissue environment[4,5,36,39,40]. The importance of tissue environmental factors in preventing axon regeneration is indicated by the observation that cut central axons that do not elongate after injury can do so when confronted with transplanted pieces of peripheral nerve, although even in this situation axons differ considerably in their growth potential, with some havng a notoriously low capacity for growth[41].

Glial pathways

Regeneration fails in all white matter tracts that have been studied, regardless of their different compositions or arrangements[12], indicating that this failure involves the operation of some common signals operating at the white matter level, shared by many different types of nerve fibres. This focuses attention on the interaction of the fibres with the cellular substrate of the tract, which in the embryo consists of the radial glia[42–44] and, in the adult, of the longitudinal array of astrocytic processes and oligodendrocytes in the CNS[45–47], and the Schwann cells and their precursors[48] in the PNS.

Why should adult white matter tract glial cells have the non-adaptive, even fatal function of denying the damaged CNS the benefits of axon regeneration? One reason could be that, in addition to their role in forming the permissive aligned substrate of the white matter tracts, astrocytes have another, equally vital, function that conflicts with their ability to provide for nerve fibre regeneration after damage. Astrocytes are asymmetrical cells. One surface provides the permissive membranes that form the substrate of the pathways along which nerve fibres grow. This surface is furnished with the communal molecular machinery needed for the advance of the growth cones of different types of nerve fibres[49,50]. The other surface has a closely apposed basal lamina, which forms where the astrocytes come into contact with fibroblasts. The basal lamina-covered surfaces provide the outer coverings of the brain and spinal cord (FIGS 2,3a). By sealing off the nervous system from the rest of the body, this arrangement maintains the unique ionic environment that the brain and spinal cord need in order to function[51].

When injury occurs, the superficial astrocytic covering of the nervous system is broken open. This could occur as a result of penetration from outside, or be caused by events within the CNS, such as vascular accidents, tumours, infections or immune attack. This leads to the breakdown of nervous tissue, opening of the blood–brain barrier and exposure of nervous tissue to

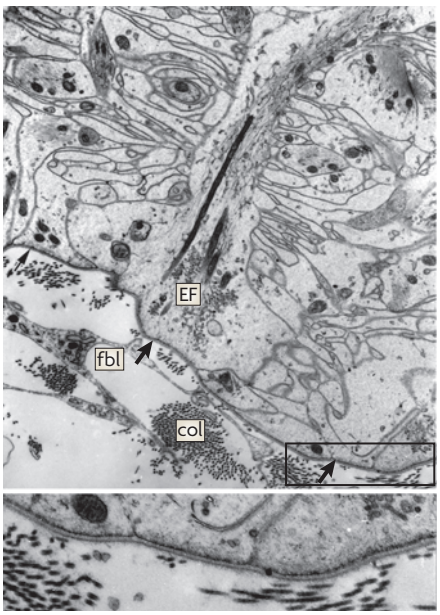

Figure 2 | **Asymmetrical coating of astrocytic surfaces.** An electron micrograph showing the basal lamina-covered surface (arrows; enlarged in lower panel) of an astrocytic end foot (EF) facing the fibroblast (fbl)- and collagen (col)-containing meningeal space of the ventral pial surface of the rat forebrain.

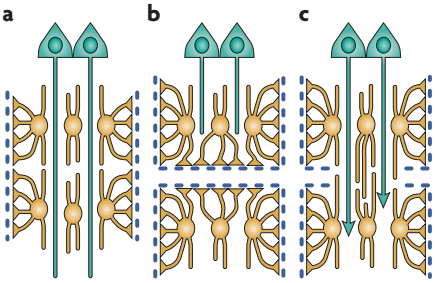

Figure 3 | **The pathway hypothesis of repair.** Schematic diagram showing the key features of the pathway hypothesis. **a** | Normal arrangement of aligned astrocytic processes (yellow) forming the longitudinal channels associated with nerve fibres (green) and astrocytic end feet covered by the basal lamina (blue dashed line). **b** | An astrocytic scar formed after a lesion and resulting in sealing of the outer surface by basal lamina-lined end feet and abrogation of any through channel for nerve fibres. **c** | Opening of the glial scar is required to reconstitute open longitudinal channels for regeneration of the severed nerve fibres. Modified, with permission, from REF. 84 © (2005) Kluwer Academic.

non-neural connective tissue elements. In these circumstances astrocytic processes become highly motile, and their immediate response is to seal off the breach. A basal lamina is formed on those outward-facing astrocytic surfaces that abut onto non-neural cells such as fibroblasts and endothelial cells, and which come to form a newly erected external wall of the nervous system[52].

The astrocytic reaction to injury preserves the ionic environment of the nervous system and prevents further invasion by damaging organisms, cells or other extraneous material. As such, it is a life-saving response[36,53]. But in doing so, the astrocytes and their processes congeal into a scar-like configuration that abrogates the pathways needed for the sprouts formed at the cut ends of severed nerve fibres to regenerate to their original destinations (FIG. 3b). Therefore, the penalty for this essential emergency measure is loss of the ability to repair nervous connections. According to this view, a requirement for the regeneration of the severed nervous connections would be that the astrocytic scar must somehow be re-opened, so as to provide the aligned astrocytic pathways[54,55] needed for the intrinsic growth capacity of the nerve cells to be expressed (FIG. 3c).

Repair by transplantation

Schwann cells. Cajal[12] proposed that, as severed nerve fibres are able to regenerate in adult peripheral nerves, there was something in the PNS environment that is permissive to their growth, and that by transplanting pieces of peripheral nerve into CNS lesions it might be possible to transfer this growth-permissive capacity to severed central fibres that otherwise would not regenerate. This seminal proposal led to the discovery of nerve growth factors[56], and also to the transplantation of peripheral nerve grafts[57] or cultured Schwann cells into disconnectional injuries of the CNS[58]. So far, however, neither approach has led to a completely effective repair strategy. Both strategies induce elongative growth of severed central axons within the grafted tissue, but few axons are able to leave the graft and re-enter the astrocytic territory of the host[59]. They are therefore unable to restore a functionally significant level of connections.

This valve-like effect of allowing axon growth into the graft but not out of it is comparable to the barrier faced by cut dorsal root axons as they regenerate through the Schwann cell territory of the central branches of the dorsal roots but, on reaching the surface of the spinal cord, are unable to enter the glial territory of the CNS[60]. The reasons for the difficulty axons face in growing from a Schwann cell to an astrocytic territory are unknown. In the case of the transplants, one possibility is that the grafted tissue expresses a higher concentration of axon-attracting factors than the surrounding CNS tissue, forming a sink that traps the axons. Another possibility is timing: grafts owe their growth-promoting effects to living cells[61], and their incorporation into the host tissue is a dynamic process, changing with time. During the period required for axons to grow through the grafts, the evolution of events in the participating cells could somehow make the graft–host interface impenetrable for exit. Until these phenomena are better understood, Schwann cells remain only a potential future component of a repair strategy, either alone or combined with other treatment options[62].

Olfactory ensheathing cells (OECs). Cajal's idea of transferring something from a part of the nervous system that can sustain axon growth into brain and spinal cord lesions where cut axons fail to grow had led him logically to peripheral nerves as a source of reparative tissue. A century later, even more impressive axon growth has been found in the olfactory system. The primary olfactory projection is the only part of the nervous system known to retain the property of axon growth throughout adult life, an embryonic feature that is also associated with the continued expression of markers present in developing neural tissue[63].

Whereas most of the nervous system develops by physically continuous outgrowth from the neural tube and crest, the olfactory system develops from a separate placode on the surface of the body[64]. The developing olfactory nerves therefore tunnel their way through intervening mesenchyme to reach the future olfactory bulbs on the rostral surface of the telencephalic hemispheres. Their pathway is pioneered by a specialized type of glial cell, the OECs[65–70].

Throughout adult life, the olfactory neuroepithelium retains the embryonic capacity for continual renewal of olfactory receptor neurons from adult stem cells located in the depths of the epithelium[71,72]. As olfactory receptor neurons die, they are continually replaced[73–75], and the newly formed neurons grow axons that traverse the olfactory nerve bundles and enter the olfactory bulbs of the adult brain. If the adult olfactory nerves are severed, the axotomized neurons undergo rapid retrograde cell death, the process of cell generation in the olfactory epithelium is greatly accelerated[76] and newly formed neurons grow axons that cross the lesion and re-establish contact with the brain[77–79]. After damage to the olfactory nerves in the adult, the OECs persist and continue to provide open channels along which the regenerating olfactory nerves grow back to the olfactory bulbs[80,81].

Electron microscopy studies show that each OEC consists of a thin cytoplasmic sheet that is curved over to enclose a tunnel-like space through which run approximately 1000 olfactory fibres bundled into a labyrinth of interconnected locular channels, formed by the ingrowth of sheet-like processes from the enclosing perimeter[82] (FIG. 4). The olfactory nerves consist of an end-to-end series of these OEC tunnels conveying the nerve fibres from the mucosa through the cribriform plate to the olfactory bulbs. At the entry point into the bulbs, the OECs interact with the astrocytic processes covering the pial surface of the brain so that the closure of the surface of the CNS provided elsewhere by the sub-pial astrocytic processes is opened here to provide a growth-permissive pathway, enabling the olfactory axons to enter the olfactory bulbs[66,83].

Based on reasoning analogous to that of Cajal, taking OECs from the olfactory nerves and transferring them into lesions of CNS tracts could transfer the property allowing regenerating axons to re-enter the astrocytic environment of the CNS[84]. As in the olfactory nerve, this would involve constituting a continuous growth-permissive channel so that the inner, astrocytic surfaces,

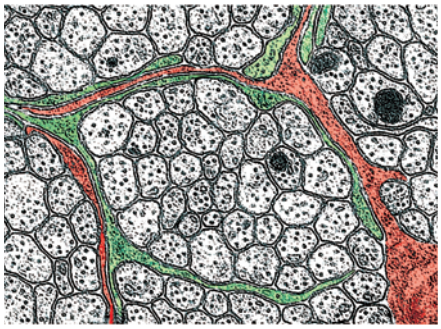

Figure 4 | **Olfactory ensheathing cell processes enclosing olfactory axons.** Electron micrograph of a cross section through a bundle of over 30 olfactory axons (small circular profiles) ensheathed in a channel formed by the interweaving of the sheet-like olfactory ensheathing cell processes (red and green) arising from adjacent olfactory ensheathing cell bodies (out of the picture). Modified, with permission, from REF. 82 © (2003) Kluwer Academic.

which are permissive to the growth of nerve fibres, become re-aligned to form a bridging pathway that allows the severed nerve fibres to cross the lesion (FIG. 5).

Transplantation of OECs

OECs are thought to be generated from a self-renewing population of stem cells in the mucosa[85,86]. They can be cultured from the adult olfactory bulb or mucosa[68,69,87–90], and a number of groups have independently provided evidence that transplantation of cultured adult OECs into lesions induces regeneration of axons in long fibre tracts[88,91–102] and regeneration of dorsal root axons into the spinal cord[60,103].

In contrast to transplanted Schwann cells, OECs do not seem to trap regenerating axons in the grafted tissue, possibly because they cause less expression of inhibitory molecules by the host astrocytes[104–107]. The ability of transplanted OECs to allow axons to leave the graft and re-enter the host tissue of the spinal cord[91,92,94,108–110] would place the regenerating axons in a position to establish functional connections with the host tissue. Re-entry of regenerating axons from OEC transplants parallels the ability of OECs *in situ* to mediate the entry of the olfactory axons through the glia–pial surface of the brain and into the astrocytic territory of the olfactory bulb glomeruli[66,83,111].

A number of authors[91,93,97,108,112,113] have reported that transplantation of OECs results in the return of lost functions. However, the correlation of recovery of function with the presence of nerve fibres regenerating through the graft and re-entering the host spinal cord[94] remains a subject for further investigation. In a quantitative study, we found that restoration of paw retrieval movements was associated with regeneration of as few as 0.5% of corticospinal tract fibres in the rat[108]. In this study we saw regenerating fibres crossing the transplant and entering the caudal part of the corticospinal tract to arborize in appropriate terminal areas in the spinal grey matter. However, we do not know how far the regenerating fibres can travel, nor to what extent they establish normal patterns of connections at appropriate levels of the spinal cord. A working hypothesis is that the CNS might have sufficient adaptability to take functional advantage of a small number of abnormally distributed connections, provided that they can re-establish a flow of unique information to roughly appropriate zones of the neuropil.

Apart from mediating the reconnection of severed fibres, the transplanted cells have other beneficial effects, such as the promotion of remyelination[114–116], secretion of growth factors[80,117], vascularization, sparing from secondary damage[118] and enhancement of sprouting[119], all of which may have an important bearing on functional recovery. However, since the time of the classical nineteenth century neurological studies (such as the description of the Brown-Sequard syndrome[120]), our understanding of spinal, and indeed CNS function, makes the assumption that disconnectional injuries causing severance of specific pathways are the cause of the specific loss of the functions mediated by those pathways. Therefore, we propose that, until proved otherwise, the primary hypothesis to explain the benefits observed after transplantation of OECs is the re-establishment of functionally useful neural circuitry as a result of the formation of connections by regeneration of lesioned axons.

Conflicting results

In addition to the spinal cord and spinal roots, the encouraging indications for the potential reparative properties of OECs in the optic nerve[121] and the PNS[122–124] have led to considerable interest, as a result of which a number of uncertainties have been identified. Several groups have failed to demonstrate repair either in spinal tracts or in dorsal roots[125–127], and in a number of cases the same authors have reported both positive[92,95,103,118,128–130] and negative outcomes[126,131–135]. At this stage, these difficulties remain to be resolved.

One possible source of these discrepancies is the tissue used to obtain the OECs. There is a lively discussion about whether there are significant differences between OECs derived from the olfactory bulb and those from the olfactory mucosa[135,136]. Another is the purity of the cells. Most groups use purified OECs[69]. In our experience[94,108,137], effective transplants require the OECs to be co-transplanted with the olfactory nerve fibroblasts (ONFs), with which they are intimately associated in their parent tissues[81,128,139,140]. Culture of the

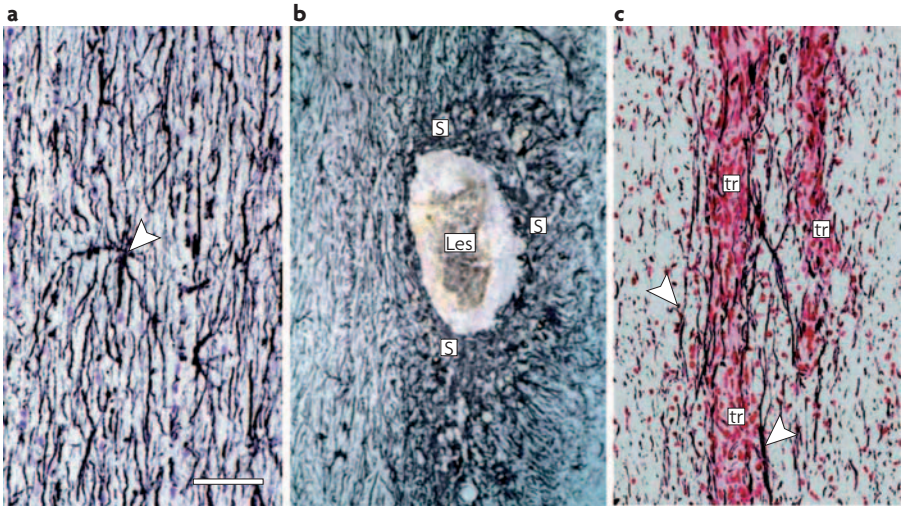

Figure 5 | **Closure of pathway by astrocytic scar and re-opening by OECs.** Repair of the astrocytic pathway by transplantation of olfactory ensheathing cells (OECs) into an electrolytic lesion of the adult rat corticospinal tract. **a** | The normal longitudinally aligned parallel array of astrocytic processes (fine black lines running vertically). Arrowhead shows an astrocytic cell body. **b** | Dense scar tissue (S) formed by astrocytic processes congealing to form a dense mass around a lesion (Les). **c** | Reorganization of the astrocytic processes to form a newly aligned pathway (fine black lines; for examples, see arrowheads) across a lesion into which OECs have been transplanted (tr). Scale bar, 50 μm. Modified, with permission, from *Spinal Cord* REF. 180 © (2006) Macmillan Publishers Ltd.

OECs and ONFs together results in the cells becoming embedded in an endogenously produced semi-solid gel-like matrix, which also provides an efficient method of removing the donor cells from the tissue culture dish, placing them accurately and retaining them without loss in the host transplantation site[137]. After transplantation, the ONFs associate with the OECs in the form of a perineurial-like outer sheath[141].

Whether or not Schwann cells contaminate the cell preparations[142,143] (which seems unlikely, at least in the case of OECs derived from the olfactory bulb), and whether they or endogenous Schwann cells also contribute to remyelination will only be settled definitively once there is a clear method for distinguishing OECs from Schwann cells[144–146]. There is considerable discussion about whether OECs can myelinate host axons[143,147–149]. Two groups have published convincing double labelling experiments[114,115,150] showing peripheral type myelin formed by labelled OECs transplanted into the spinal cord. A review of the literature on this topic is beyond the scope of the present article. Similarly, there is still no consensus on the extent to which transplanted OECs migrate within the host tissue[101,124,133,135,151], nor on whether migration would be needed for their reparative effects.

There is also considerable variation in the outcome measures being reported. The desired final outcome is recovery of function. Promising intermediate outcomes are restoration of connections, remyelination, revascularization and sparing of tissue from secondary damage. Less convincing is the use of computer-assisted graphics programmes to assess the size of the lesion. As the spinal cord can accommodate damaged or cystic areas of considerable size without corresponding tissue loss, the amount of spared tissue would be a much more convincing measure than the size of the lesion.

Most investigations have studied the effects of transplantation of OECs or other therapeutic interventions carried out at or shortly after the time of the injury. This necessitates the use of a control group to distinguish the benefits of the interventions from the spontaneous recovery that occurs after any injury. In the immediate post-lesion period, recovery could also be obtained as a result of interventions having non-specific beneficial effects, such as protection of spinal tissue against secondary damage.

The delayed transplantation paradigm makes it possible to establish that the effects of the lesion are permanent, and that the glial scar has already formed before the intervention is carried out. This has the advantage that the pre-transplantation data from the individual animals serves as its own control, and the benefits are more likely to be due to reconstruction of specific pathways, rather than protection from secondary damage.

A consensus opinion on the value or otherwise of transplantation of OECs will only be reached once comparisons are made using a similar source and mode of cell preparation, so that equivalent populations of cultured cells are transplanted using comparable surgical procedures, into exactly similar experimental lesions, with comparable anatomical or functional outcome measures. Finally, it must be cautioned that the present evidence for a positive effect of OEC transplants rests on only a few specific pathways. To what extent these results can be generalized to other pathways will depend on a case-by-case investigation.

Conclusions

While subject to confirmation, there are sufficient positive experimental observations to raise the hope that transplantation of OECs will have a role in developing methods for repair of disconnectional injuries to white matter tracts in the adult CNS. The pathway hypothesis proposes that reconstruction of aligned glial pathways would allow regenerating axons to cross lesions in adult white matter tracts, and take advantage of the ongoing plasticity in the denervated neuropil to establish functionally useful connections. This remains a hypothesis. The evidence for such a mechanism will require anatomical (including ultrastructural) demonstration that transplantation of OECs into a lesion site does indeed open up such a pathway through the glial scar, and that the pathway is provided with the appropriate secreted and membrane-bound growth-promoting molecules.

This proposal is not incompatible with the wider field of endeavours to bring about repair of CNS injuries by interventions to reduce secondary damage[118,152], growth factors to trigger, maintain or restore axon growth[118,153], or methods to open up pathways for the regeneration of cut axons by neutralization of inhibitory molecules associated with astrocytes[36,154], oligodendrocytes[4,5,7] or fibroblasts[40]. That such molecular interventions would also require reconstitution of a structural pathway is becoming accepted[62,155].

Likewise, the present proposal that pathway reconstruction on its own could be sufficient for at least a degree of repair does not imply that growth factors and removal of inhibitory influences do not have a role, only that the transplanted cells are able to provide the needed growth factors[156,157], and that the anatomical re-arrangements induced by the transplants provide a sequestered growth-permissive channel which results in the inhibitory surfaces of the astrocytes being turned aside, and the axons being shielded from non-astrocytic inhibitory influences such as myelin or fibroblasts present in the tissue.

Box 1 | **Clinical outlook**

For the purposes of clinical application, a number of groups have demonstrated the production of olfactory ensheathing cells (OECs) by tissue culture of adult olfactory neuroepithelium obtained from biopsy samples of the upper nasal lining[71,169]. This avoids the need for craniotomy and opens the way for use of the patient's own autografted material for transplantation repair. An initial published clinical trial has shown no adverse effects one year after transplanting autologous cultured adult human mucosal OECs into spinal injuries[170]. At the time of writing, two neurosurgical teams[171,172] have now gone a step further and adopted transplantation of olfactory tissue as a clinical procedure for treating patients with spinal cord injury (as well as for other conditions). With regards to the cell source, the Lisbon team[172] uses direct transplantation of minced olfactory mucosa without culture, and the Beijing team[171] uses allografts of cells cultured from human embryonic olfactory bulbs.

In setting up a clinical trial to validate these or any other procedures, it would be difficult to justify a full-control surgical procedure in which patients would be asked to submit to the considerable risks of transplantation of a neutral, non-reparative cell type. However, a randomized double-blind controlled trial with at least some surgical intervention[168,173–176] would be the route for transplantation of OECs to gain general acceptance as a basis for a future treatment. In the meantime, however, it would be valuable if the safety aspects[177] and independent longitudinal pre- and post-operative neurological assessments were available for as many of the patients currently being treated with OEC transplants as possible.

Clinical demonstration of the effectiveness of adult OEC autografts would be an important stimulus to research in this area, and would open the door to treating a wide variety of currently incurable injuries of the brain, spinal cord and cranial and spinal nerves[166]. Arising from the rat experiments[60,124], one possibility we are exploring is a trial of OECs in brachial plexus avulsion, a situation where the prognosis is clear, surgical procedures are already in practice[178,179] and sufficient numbers of cells are available for transplantation.

Although the interaction of OECs with astrocytes is central to the pathway hypothesis, our understanding of the expression profile of the OECs[146] and the signalling events by which OECs interact with neurons[158] and astrocytes[104,105] is in its infancy. The elucidation of the molecular basis of the interaction of OECs with astrocytes in CNS lesions, their interaction with Schwann cells in root and peripheral nerve lesions and their link to the cytoskeletal motors needed to carry out the re-alignment of astrocytic processes could be a key to a novel and productive approach to reparative molecular interventions. Further elucidation of the molecular signals that govern the regeneration of severed nerve fibres and the re-establishment of neural connections will provide information for future combinatorial approaches[6], which could enhance the effectiveness of cell transplants in the repair of disconnectional injuries.

Looking to the future, initial success will doubtless stimulate the development of more effective transplants by genetic modification of OECs[118,130,159], cells derived from other tissues[160–162], or enhancing the currently limited regenerative benefits of Schwann cells[138,163]. In addition to the use of an endogenous matrix, various biomaterials are being investigated[128,164,165], and these could enhance the orientation and retention of the small numbers of cells currently available for transplantation.

From the point of view of patients, clinicians and the lay public, the current plethora of peer-reviewed publications[6] claiming repair of spinal cord injury in laboratory animals, which often discounts the striking spontaneous recovery that occurs without intervention in both animals and man[166–168], serves as a warning that dead rats tell no tales. Only once the first benefits can be convincingly and reproducibly demonstrated in human clinical trials (BOX 1) will the research reach the level of credibility needed to accelerate our limping progress into this new phase of neuroscientific history. But whatever the remaining difficulties, we should not doubt the importance of the goal. Repair of brain and spinal cord injury would be one of the most significant contributions that the field of neuroscience could make to mankind.

Geoffrey Raisman and Ying Li are at the Spinal Repair Unit, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK.
Correspondence to G.R.
e-mail: G.Raisman@ion.ucl.ac.uk

doi:10.1038/nrn2099
Pubished online 7 March 2007

1. Amadio, J. P. & Walsh, C. A. Brain evolution and uniqueness in the human genome. *Cell* **126**, 1033–1035 (2006).
2. Pollard, K. S. *et al.* An RNA gene expressed during cortical development evolved rapidly in humans. *Nature* **443**, 167–172 (2006).
3. Briggman, K. L. & Denk, W. Towards neural circuit reconstruction with volume electron microscopy techniques. *Curr. Opin. Neurobiol.* **16**, 562–570 (2006).
4. Filbin, M. T. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nature Rev. Neurosci.* **4**, 703–713 (2003).
5. Yiu, G. & He, Z. Glial inhibition of CNS axon regeneration. *Nature Rev. Neurosci.* **7**, 617–627 (2006).
6. Thuret, S., Moon, L. D. & Gage, F. H. Therapeutic interventions after spinal cord injury. *Nature Rev. Neurosci.* **7**, 628–643 (2006).
7. Schwab, M. E. Nogo and axon regeneration. *Curr. Opin. Neurobiol.* **14**, 118–124 (2004).
8. Raisman, G. Neuronal plasticity in the septal nuclei of the adult rat. *Brain Res.* **14**, 25–48 (1969).
9. Bareyre, F. M. *et al.* The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nature Neurosci.* **7**, 269–277 (2004).
10. Turner, J. P., Sauvé, Y., Varela-Rodriguez, C., Lund, R. D. & Salt, T. E. Recruitment of local excitatory circuits in the superior colliculus following deafferentation and the regeneration of retinocollicular inputs. *Eur. J Neurosci.* **22**, 1643–1654 (2005).
11. Tan, M. M. & Harvey, A. R. A comparison of postlesion growth of retinotectal and corticotectal axons after superior colliculus transections in neonatal rats. *J. Comp. Neurol.* **386**, 681–699 (1997).
12. Ramón y Cajal, S. *Degeneration and Regeneration of the Nervous System* (Hafner, New York, 1928).
13. Dinocourt, C., Gallagher, S. E. & Thompson, S. M. Injury-induced axonal sprouting in the hippocampus is initiated by activation of trkB receptors. *Eur. J. Neurosci.* **24**, 1857–1866 (2006).
14. Kerschensteiner, M., Schwab, M. E., Lichtman, J. W. & Misgeld, T. *In vivo* imaging of axonal degeneration and regeneration in the injured spinal cord. *Nature Med.* **11**, 572–577 (2005).
15. Bareyre, F. M., Kerschensteiner, M., Misgeld, T. & Sanes, J. R. Transgenic labeling of the corticospinal tract for monitoring axonal responses to spinal cord injury. *Nature Med.* **11**, 1355–1360 (2005).
16. Fernandes, K. J. L., Fan, D. P., Tsui, B. J., Cassar, S. L. & Tetzlaff, W. Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: differential regulation of GAP-43, tubulins, and neurofilament-M. *J. Comp. Neurol.* **414**, 495–510 (1999).
17. Li, Y. & Raisman, G. Sprouts from cut corticospinal axons persist in the presence of astrocytic scarring in long-term lesions of the adult rat spinal cord. *Exp. Neurol.* **134**, 102–111 (1995).
18. Kwon, B. K. *et al.* Survival and regeneration of rubrospinal neurons 1 year after spinal cord injury. *Proc. Natl Acad. Sci. USA* **99**, 3246–3251 (2002).
19. O'Leary, D. D. M. & Wilkinson, D. G. Eph receptors and ephrins in neural development. *Curr. Opin. Neurobiol.* **9**, 65–73 (1999).
20. Charron, F. & Tessier-Lavigne, M. Novel brain wiring functions for classical morphogens: a role as graded positional cues in axon guidance. *Development* **132**, 2251–2262 (2005).
21. Harel, N. Y. & Strittmatter, S. M. Can regenerating axons recapitulate developmental guidance during recovery from spinal cord injury? *Nature Rev. Neurosci.* **7**, 603–616 (2006).
22. Huber, A. B., Kolodkin, A. L., Ginty, D. D. & Cloutier, J. F. Signaling at the growth cone: ligand–receptor complexes and the control of axon growth and guidance. *Annu. Rev. Neurosci.* **26**, 509–563 (2003).
23. Rodger, J. *et al.* Expression of ephrin-A2 in the superior colliculus and EphA5 in the retina following optic nerve section in adult rat. *Eur. J. Neurosci.* **14**, 1929–1936 (2001).
24. O'Leary, D. D. M., Ruff, N. L. & Dyck, R. H. Development, critical period plasticity, and adult reorganizations of mammalian somatosensory systems. *Curr. Opin. Neurobiol.* **4**, 535–544 (1994).
25. Stanfield, B. B., Nahin, B. R. & O'Leary, D. D. M. A transient postmamillary component of the rat fornix during development: implications for interspecific differences in mature axonal projections. *J. Neurosci.* **7**, 3350–3361 (1987).
26. Placzek, M., Tessier-Lavigne, M., Jessell, T. & Dodd, J. Orientation of commissural axons *in vivo* in response to a floor plate-derived chemoattractant. *Development* **110**, 19–30 (1990).
27. Del, R. T. & Feller, M. B. Early retinal activity and visual circuit development. *Neuron* **52**, 221–222 (2006).
28. Hooks, B. M. & Chen, C. Distinct roles for spontaneous and visual activity in remodeling of the retinogeniculate synapse. *Neuron* **52**, 281–291 (2006).
29. Collingridge, G. L. & Bliss, T. V. Memories of NMDA receptors and LTP. *Trends Neurosci.* **18**, 54–56 (1995).
30. Wu, C. W. & Kaas, J. H. Reorganization in primary motor cortex of primates with long-standing therapeutic amputations. *J. Neurosci.* **19**, 7679–7697 (1999).
31. Polley, D. B., Steinberg, E. E. & Merzenich, M. M. Perceptual learning directs auditory cortical map reorganization through top-down influences. *J. Neurosci.* **26**, 4970–4982 (2006).
32. Callaway, E. M. Should I stay or should I go? Presynaptic boutons in the adult cortex still haven't made up their minds. *Neuron* **49**, 780–783 (2006).
33. Pasterkamp, R. J. & Verhaagen, J. Semaphorins in axon regeneration: developmental guidance molecules gone wrong? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **361**, 1499–1511 (2006).
34. Li, D., Field, P. M. & Raisman, G. Failure of axon regeneration in postnatal rat entorhino-hippocampal slice co-culture is due to maturation of the axon, not that of the pathway or target. *Eur. J. Neurosci.* **7**, 1164–1171 (1995).
35. Blackmore, M. & Letourneau, P. C. Changes within maturing neurons limit axonal regeneration in the developing spinal cord. *J. Neurobiol.* **66**, 348–360 (2006).
36. Silver, J. & Miller, J. H. Regeneration beyond the glial scar. *Nature Rev. Neurosci.* **5**, 146–156 (2004).
37. Fernandes, K. J., Fan, D. P., Tsui, B. J., Cassar, S. L. & Tetzlaff, W. Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: differential regulation of GAP-43, tubulins, and neurofilament-M. *J. Comp. Neurol.* **414**, 495–510 (1999).
38. Shatz, C. J. Form from function in visual system development. *Harvey Lect.* **93**, 17–34 (1997).
39. Schwab, M. E. & Bartholdi, D. Degeneration and regeneration of axons in the lesioned spinal cord. *Physiol. Rev.* **76**, 319–370 (1996).
40. Niclou, S. P., Franssen, E. H., Ehlert, E. M., Taniguchi, M. & Verhaagen, J. Meningeal cell-derived semaphorin 3A inhibits neurite outgrowth. *Mol. Cell. Neurosci.* **24**, 902–912 (2003).
41. Rossi, F., Jankovski, A. & Sotelo, C. Differential regenerative response of Purkinje cell and inferior olivary axons confronted with embryonic grafts: environmental cues versus intrinsic neuronal determinants. *J. Comp. Neurol.* **359**, 663–677 (1995).
42. Easter, S. S. Jr, Ross, L. S. & Frankfurter, A. Initial tract formation in the mouse brain. *J. Neurosci.* **13**, 285–299 (1993).
43. Sobkowicz, H. M., Waclawik, A. J. & August, B. K. The astroglial cell that guides nerve fibers from growth cone to synapse in organotypic cultures of the fetal mouse spinal cord. *Synapse* **59**, 183–200 (2006).
44. Hatten, M. E. New directions in neuronal migration. *Science* **297**, 1660–1663 (2002).
45. Suzuki, M. & Raisman, G. Multifocal pattern of postnatal development of the macroglial framework of the rat fimbria. *Glia* **12**, 294–308 (1994).
46. Suzuki, M. & Raisman, G. The glial framework of central white matter tracts: segmented rows of contiguous interfascicular oligodendrocytes and solitary astrocytes give rise to a continuous meshwork of transverse and longitudinal processes in the adult rat fimbria. *Glia* **6**, 222–235 (1992).
47. Barry, D. & McDermott, K. Differentiation of radial glia from radial precursor cells and transformation into astrocytes in the developing rat spinal cord. *Glia* **50**, 187–197 (2005).
48. Wanner, I. B. *et al.* Invariant mantling of growth cones by Schwann cell precursors characterize growing peripheral nerve fronts. *Glia* **54**, 424–438 (2006).
49. Walsh, F. S. & Doherty, P. Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. *Annu. Rev. Cell Dev. Biol.* **13**, 425–456 (1997).

50. Lemons, M. L. & Condic, M. L. Combined integrin activation and intracellular cAMP cause Rho GTPase dependent growth cone collapse on laminin-1. *Exp. Neurol.* **202**, 324–335 (2006).
51. Janzer, R. C. & Raff, M. C. Astrocytes induce blood–brain barrier properties in endothelial cells. *Nature* **325**, 253–257 (1987).
52. Reier, P. J., Stensaas, L. J. & Guth, L. in *Spinal Cord Reconstruction* (eds Kao, C. C., Bunge, R. P. & Reier, P. J.) 163–195 (Raven, New York, 1983).
53. Faulkner, J. R. *et al.* Reactive astrocytes protect tissue and preserve function after spinal cord injury. *J. Neurosci.* **24**, 2143–2155 (2004).
54. Li, Y. & Raisman, G. Long axon growth from embryonic neurons transplanted into myelinated tracts of the adult rat spinal cord. *Brain Res.* **629**, 115–127 (1993).
55. Davies, S. J. A., Field, P. M. & Raisman, G. Long interfascicular axon growth from embryonic neurons transplanted into adult myelinated tracts. *J. Neurosci.* **14**, 1596–1612 (1994).
56. Cowan, W. M. Viktor Hamburger and Rita Levi-Montalcini: the path to the discovery of nerve growth factor. *Annu. Rev. Neurosci.* **24**, 551–600 (2001).
57. Vidal-Sanz, M., Bray, G. M., Villegas-Pérez, M. P., Thanos, S. & Aguayo, A. J. Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. *J. Neurosci.* **7**, 2894–2909 (1987).
58. Paíno, C. L. & Bunge, M. B. Induction of axon growth into Schwann cell implants into lesioned adult rat spinal cord. *Exp. Neurol.* **114**, 254–257 (1991).
59. Carter, D. A., Bray, G. M. & Aguayo, A. J. Regenerated retinal ganglion cell axons can form well-differentiated synapses in the superior colliculus of adult hamsters. *J. Neurosci.* **9**, 4042–4050 (1989).
60. Li, Y., Carlstedt, T., Berthold, C.-H. & Raisman, G. Interaction of transplanted olfactory-ensheathing cells and host astrocytic processes provides a bridge for axons to regenerate across the dorsal root entry zone. *Exp. Neurol.* **188**, 300–308 (2004).
61. Berry, M., Rees, L., Hall, S., Yiu, P. & Sievers, J. Optic axons regenerate into sciatic nerve isografts only in the presence of Schwann cells. *Brain Res. Bull.* **20**, 223–231 (1988).
62. Fouad, K. *et al.* Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. *J. Neurosci.* **25**, 1169–1178 (2005).
63. Pasterkamp, R. J., Ruitenberg, M. J. & Verhaagen, J. Semaphorins and their receptors in olfactory axon guidance. *Cell. Mol. Biol. (Noisy-le-grand)* **45**, 763–779 (1999).
64. Baker, C. V. H. & Bronner-Fraser, M. Vertebrate cranial placodes I. Embryonic induction. *Dev. Biol.* **232**, 1–61 (2001).
65. Doucette, J. R. The glial cells in the nerve fiber layer of the rat olfactory bulb. *Anat. Rec.* **210**, 385–391 (1984).
66. Raisman, G. Specialized neuroglial arrangement may explain the capacity of vomeronasal axons to reinnervate central neurons. *Neuroscience* **14**, 237–254 (1985).
67. Valverde, F. & Lopez-Mascaraque, L. Neuroglial arrangements in the olfactory glomeruli of the hedgehog. *J. Comp. Neurol.* **307**, 658–674 (1991).
68. Barnett, S. C., Hutchins, A.-M. & Noble, M. Purification of olfactory nerve ensheathing cells from the olfactory bulb. *Dev. Biol.* **155**, 337–350 (1993).
69. Ramón-Cueto, A. & Nieto-Sampedro, M. Glial cells from adult rat olfactory bulb: immunocytochemical properties of pure cultures of ensheathing cells. *Neuroscience* **47**, 213–220 (1992).
70. Ramón-Cueto, A. & Valverde, F. Olfactory bulb ensheathing glia: a unique cell type with axonal growth-promoting properties. *Glia* **14**, 163–173 (1995).
71. Murrell, W. *et al.* Multipotent stem cells from adult olfactory mucosa. *Dev. Dyn.* **233**, 496–515 (2005).
72. Huard, J. M. T., Youngentob, S. L., Goldstein, B. J., Luskin, M. B. & Schwob, J. E. Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells. *J. Comp. Neurol.* **400**, 469–486 (1998).
73. Moulton, D. G. Dynamics of cell populations in the olfactory epithelium. *Ann. NY Acad. Sci.* **237**, 52–61 (1974).
74. Graziadei, P. P. C. & Montigraziadei, G. A. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *J. Neurocytol.* **8**, 1–18 (1979).
75. Mackay-Sim, A. & Kittel, P. W. On the life span of olfactory receptor neurons. *Eur. J. Neurosci.* **3**, 209–215 (1991).
76. Carr, V. M. & Farbman, A. I. Ablation of the olfactory bulb up-regulates the rate of neurogenesis and induces precocious cell death in olfactory epithelium. *Exp. Neurol.* **115**, 55–59 (1992).
77. Schwob, J. E., Youngentob, S. L., Ring, G., Iwema, C. L. & Mezza, R. C. Reinnervation of the rat olfactory bulb after methyl bromide-induced lesion: timing and extent of reinnervation. *J. Comp Neurol.* **412**, 439–457 (1999).
78. Graziadei, P. P. C. & Montigraziadei, G. A. Neurogenesis and neuron regeneration in the olfactory system of mammals. 3. Deafferentation and reinnervation of the olfactory bulb following section of the fila-olfactoria in rat. *J. Neurocytol.* **9**, 145–162 (1980).
79. Barber, P. C. & Raisman, G. Replacement of receptor neurones after section of the vomeronasal nerves in the adult mouse. *Brain Res.* **147**, 297–313 (1978).
80. Williams, S. K., Franklin, R. J. & Barnett, S. C. Response of olfactory ensheathing cells to the degeneration and regeneration of the peripheral olfactory system and the involvement of the neuregulins. *J. Comp. Neurol.* **470**, 50–62 (2004).
81. Li, Y., Field, P. M. & Raisman, G. Olfactory ensheathing cells and olfactory nerve fibroblasts maintain continuous open channels for regrowth of olfactory nerve fibres. *Glia* **52**, 245–251 (2005).
82. Field, P. M., Li, Y. & Raisman, G. Ensheathment of the olfactory nerves in the adult rat. *J. Neurocytol.* **32**, 317–324 (2003).
83. Valverde, F., Santacana, M. & Heredia, M. Formation of an olfactory glomerulus: morphological aspects of development and organization. *Neuroscience* **49**, 255–276 (1992).
84. Li, Y., Li, D. & Raisman, G. Interaction of olfactory ensheathing cells with astrocytes may be the key to repair of tract injuries in the spinal ord: the 'pathway hypothesis'. *J. Neurocytol.* **34**, 343–351 (2005).
85. Beites, C. L., Kawauchi, S., Crocker, C. E. & Calof, A. L. Identification and molecular regulation of neural stem cells in the olfactory epithelium. *Exp. Cell Res.* **306**, 309–316 (2005).
86. Carter, L. A., MacDonald, J. L. & Roskams, A. J. Olfactory horizontal basal cells demonstrate a conserved multipotent progenitor phenotype. *J. Neurosci.* **24**, 5670–5683 (2004).
87. Devon, R. & Doucette, R. Olfactory ensheathing cells do not require L-ascorbic acid *in vitro* to assemble a basal lamina or to myelinate dorsal root ganglion neurites. *Brain Res.* **688**, 223–229 (1995).
88. Boyd, J. G., Skihar, V., Kawaja, M. & Doucette, R. Olfactory ensheathing cells: historical perspective and therapeutic potential. *Anat. Rec. B New Anat.* **271**, 49–60 (2003).
89. Jani, H. R. & Raisman, G. Ensheathing cell cultures from the olfactory bulb and mucosa. *Glia* **47**, 130–137 (2004).
90. Au, E. & Roskams, A. J. Olfactory ensheathing cells of the lamina propria *in vivo* and *in vitro*. *Glia* **41**, 224–236 (2003).
91. Lu, J., Féron, F., Mackay-Sim, A. & Waite, P. M. E. Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. *Brain* **125**, 14–21 (2002).
92. Ramón-Cueto, A., Plant, G. W., Avila, J. & Bunge, M. B. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. *J. Neurosci.* **18**, 3803–3815 (1998).
93. Ramón-Cueto, A., Cordero, M. I., Santos-Benito, F. F. & Avila, J. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. *Neuron* **25**, 425–435 (2000).
94. Li, Y., Field, P. M. & Raisman, G. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science* **277**, 2000–2002 (1997).
95. Ramer, L. M. *et al.* Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. *J. Comp. Neurol.* **473**, 1–15 (2004).
96. Plant, G. W., Christensen, C. L., Oudega, M. & Bunge, M. B. Delayed transplantation of olfactory ensheathing glia promotes sparing/regeneration of supraspinal axons in the contused adult rat spinal cord. *J. Neurotrauma* **20**, 1–16 (2003).
97. Lopez-Vales, R., Fores, J., Navarro, X. & Verdu, E. Chronic transplantation of olfactory ensheathing cells promotes partial recovery after complete spinal cord transection in the rat. *Glia* **55**, 303–311 (2007).
98. Andrews, M. R. & Stelzner, D. J. Modification of the regenerative response of dorsal column axons by olfactory ensheathing cells or peripheral axotomy in adult rat. *Exp. Neurol.* **190**, 311–327 (2004).
99. Lu, J. & Ashwell, K. Olfactory ensheathing cells: their potential use for repairing the injured spinal cord. *Spine* **27**, 887–892 (2002).
100. Smale, K. A., Doucette, R. & Kawaja, M. D. Implantation of olfactory ensheathing cells in the adult rat brain following fimbria-fornix transection. *Exp. Neurol.* **137**, 225–233 (1996).
101. Bartolomei, J. C. & Greer, C. A. Olfactory ensheathing cells: bridging the gap in spinal cord injury. *Neurosurgery* **47**, 1057–1069 (2000).
102. Perry, C., Mackay-Sim, A., Féron, F. & McGrath, J. Olfactory neural cells: an untapped diagnostic and therapeutic resource. The 2000 Ogura Lecture. *Laryngoscope* **112**, 603–607 (2002).
103. Ramón-Cueto, A. & Nieto-Sampedro, M. Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. *Exp. Neurol.* **127**, 232–244 (1994).
104. Lakatos, A., Franklin, R. J. M. & Barnett, S. C. Olfactory ensheathing cells and Schwann cells differ in their *in vitro* interactions with astrocytes. *Glia* **32**, 214–225 (2000).
105. Lakatos, A., Barnett, S. C. & Franklin, R. J. Olfactory ensheathing cells induce less host astrocyte response and chondroitin sulphate proteoglycan expression than Schwann cells following transplantation into adult CNS white matter. *Exp. Neurol.* **184**, 237–246 (2003).
106. Plant, G. W., Bates, M. L. & Bunge, M. B. Inhibitory proteoglycan immunoreactivity is higher at the caudal than the rostral Schwann cell graft-transected spinal cord interface. *Mol. Cell. Neurosci.* **17**, 471–487 (2001).
107. Verdu, E. *et al.* Effects of ensheathing cells transplanted into photochemically damaged spinal cord. *NeuroReport* **12**, 2303–2309 (2001).
108. Keyvan-Fouladi, N., Raisman, G. & Li, Y. Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats. *J. Neurosci.* **23**, 9428–9434 (2003).
109. Pearse, D. D. *et al.* Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: does pretreatment with methylprednisolone and interleukin-10 enhance recovery? *J. Neurotrauma* **21**, 1223–1239 (2004).
110. Imaizumi, T., Lankford, K. L., Burton, W. V., Fodor, W. L. & Kocsis, J. D. Xenotransplantation of transgenic pig olfactory ensheathing cells promotes axonal regeneration in rat spinal cord. *Nature Biotechnol.* **18**, 949–953 (2000).
111. Doucette, J. R. PNS–CNS transition zone of the first cranial nerve. *J. Comp. Neurol.* **312**, 451–466 (1991).
112. Nash, H. H., Borke, R. C. & Anders, J. J. Ensheathing cells and methylprednisolone promote axonal regeneration and functional recovery in the lesioned adult rat spinal cord. *J. Neurosci.* **22**, 7111–7120 (2002).
113. Polentes, J., Stamegna, J. C., Nieto-Sampedro, M. & Gauthier, P. Phrenic rehabilitation and diaphragm recovery after cervical injury and transplantation of olfactory ensheathing cells. *Neurobiol. Dis.* **16**, 638–653 (2004).
114. Sasaki, M. *et al.* Molecular reconstruction of nodes of Ranvier after remyelination by transplanted olfactory ensheathing cells in the demyelinated spinal cord. *J. Neurosci.* **26**, 1803–1812 (2006).
115. Radtke, C. *et al.* Remyelination of the nonhuman primate spinal cord by transplantation of H-transferase transgenic adult pig olfactory ensheathing cells. *FASEB J.* **18**, 335–337 (2004).
116. Smith, P. M., Lakatos, A., Barnett, S. C., Jeffery, N. D. & Franklin, R. J. M. Cryopreserved cells isolated from the adult canine olfactory bulb are capable of extensive remyelination following transplantation into the adult rat CNS. *Exp. Neurol.* **176**, 402–406 (2002).
117. Lipson, A. C., Widenfalk, J., Lindqvist, E., Ebendal, T. & Olson, L. Neurotrophic properties of olfactory ensheathing glia. *Exp. Neurol.* **180**, 167–171 (2003).
118. Ruitenberg, M. J. *et al.* NT-3 expression from engineered olfactory ensheathing glia promotes spinal sparing and regeneration. *Brain* **128**, 839–853 (2005).

119. Chuah, M. I. *et al.* Olfactory ensheathing cells promote collateral axonal branching in the injured adult rat spinal cord. *Exp. Neurol.* **185**, 15–25 (2004).
120. Laporte, Y. Charles-Edouard Brown-Sequard: an eventful life and a significant contribution to the study of the nervous system. *C. R. Biol.* **329**, 363–368 (2006).
121. Li, Y., Sauvé, Y., Li, D., Lund, R. D. & Raisman, G. Transplanted olfactory ensheathing cells promote regeneration of cut adult rat optic nerve axons. *J. Neurosci.* **23**, 7922–7930 (2003).
122. Guntinas-Lichius, O. *et al.* Transplantation of olfactory mucosa minimizes axonal branching and promotes the recovery of vibrissae motor performance after facial nerve repair in rats. *J. Neurosci.* **22**, 7121–7131 (2002).
123. Choi, D. & Raisman, G. Disorganization of the facial nucleus after nerve lesioning and regeneration in the rat: effects of transplanting candidate reparative cells to the site of injury. *Neurosurgery* **56**, 1093–1100 (2005).
124. Li, Y., Yamamoto, M., Raisman, G., Choi, D. & Carlstedt, T. An experimental model of ventral root repair showing the beneficial effect of transplanting olfactory ensheathing cells. *Neurosurgery* (in the press).
125. Riddell, J. S., Enriquez-Denton, M., Toft, A., Fairless, R. & Barnett, S. C. Olfactory ensheathing cell grafts have minimal influence on regeneration at the dorsal root entry zone following rhizotomy. *Glia* **47**, 150–167 (2004).
126. Gomez, V. M. *et al.* Transplantation of olfactory ensheathing cells fails to promote significant axonal regeneration from dorsal roots into the rat cervical cord. *J. Neurocytol.* **32**, 53–70 (2003).
127. Ramer, L. M., Richter, M. W., Roskams, A. J., Tetzlaff, W. & Ramer, M. S. Peripherally-derived olfactory ensheathing cells do not promote primary afferent regeneration following dorsal root injury. *Glia* **47**, 189–206 (2004).
128. Deumens, R. *et al.* Olfactory ensheathing cells, olfactory nerve fibroblasts and biomatrices to promote long-distance axon regrowth and functional recovery in the dorsally hemisected adult rat spinal cord. *Exp. Neurol.* **200**, 89–103 (2006).
129. Verdú, E. *et al.* Effects of ensheathing cells transplanted into photochemically damaged spinal cord. *NeuroReport* **12**, 2303–2309 (2001).
130. Ruitenberg, M. J. *et al. Ex vivo* adenoviral vector-mediated neurotrophin gene transfer to olfactory ensheathing glia: effects on rubrospinal tract regeneration, lesion size, and functional recovery after implantation in the injured rat spinal cord. *J. Neurosci.* **23**, 7045–7058 (2003).
131. Takami, T. *et al.* Schwann cell but not olfactory ensheathing glia transplants improve hindlimb locomotor performance in the moderately contused adult rat thoracic spinal cord. *J. Neurosci.* **22**, 6670–6681 (2002).
132. Deumens, R. *et al.* Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing cell/olfactory nerve fibroblast–biomatrix bridges. *J. Neurosci. Res.* **83**, 811–820 (2006).
133. Lu, P. *et al.* Olfactory ensheathing cells do not exhibit unique migratory or axonal growth-promoting properties after spinal cord injury. *J. Neurosci.* **26**, 11120–11130 (2006).
134. Steward, O. *et al.* A re-assessment of the consequences of delayed transplantation of olfactory lamina propria following complete spinal cord transection in rats. *Exp. Neurol.* **198**, 483–499 (2006).
135. Richter, M. W., Fletcher, P. A., Liu, J., Tetzlaff, W. & Roskams, A. J. Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. *J. Neurosci.* **25**, 10700–10711 (2005).
136. Kumar, R., Hayat, S., Felts, P., Bunting, S. & Wigley, C. Functional differences and interactions between phenotypic subpopulations of olfactory ensheathing cells in promoting CNS axonal regeneration. *Glia* **50**, 12–20 (2005).
137. Li, Y., Decherchi, P. & Raisman, G. Transplantation of olfactory ensheathing cells into spinal cord lesions restores breathing and climbing. *J. Neurosci.* **23**, 727–731 (2003).
138. Keyvan-Fouladi, N., Raisman, G. & Li, Y. Delayed repair of corticospinal tract lesions as an assay for the effectiveness of transplantation of Schwann cells. *Glia* **51**, 306–311 (2005).
139. Lakatos, A., Smith, P. M., Barnett, S. C. & Franklin, R. J. Meningeal cells enhance limited CNS remyelination by transplanted olfactory ensheathing cells. *Brain* **126**, 598–609 (2003).
140. Barnett, S. C. & Chang, L. Olfactory ensheathing cells and CNS repair: going solo or in need of a friend? *Trends Neurosci.* **27**, 54–60 (2004).
141. Li, Y., Field, P. M. & Raisman, G. Regeneration of adult rat corticospinal axons induced by transplanted olfactory ensheathing cells. *J. Neurosci.* **18**, 10514–10524 (1998).
142. Boyd, J. G., Doucette, R. & Kawaja, M. D. Defining the role of olfactory ensheathing cells in facilitating axon remyelination following damage to the spinal cord. *FASEB J.* **19**, 694–703 (2005).
143. Plant, G. W. *et al.* Purified adult ensheathing glia fail to myelinate axons under culture conditions that enable Schwann cells to form myelin. *J. Neurosci.* **22**, 6083–6091 (2002).
144. Fairless, R., Frame, M. C. & Barnett, S. C. N-cadherin differentially determines Schwann cell and olfactory ensheathing cell adhesion and migration responses upon contact with astrocytes. *Mol. Cell. Neurosci.* **28**, 253–263 (2005).
145. Ibanez, C., Ito, D., Zawadzka, M., Jeffery, N. D. & Franklin, R. J. Calponin is expressed by fibroblasts and meningeal cells but not olfactory ensheathing cells in the adult peripheral olfactory system. *Glia* **55**, 144–151 (2007).
146. Vincent, A. J., Taylor, J. M., Choi-Lundberg, D. L., West, A. K. & Chuah, M. I. Genetic expression profile of olfactory ensheathing cells is distinct from that of Schwann cells and astrocytes. *Glia* **51**, 132–147 (2005).
147. Devon, R. & Doucette, R. Olfactory ensheathing cells myelinate dorsal root ganglion neurites. *Brain Res.* **589**, 175–179 (1992).
148. Boyd, J. G., Lee, J., Skihar, V., Doucette, R. & Kawaja, M. D. LacZ-expressing olfactory ensheathing cells do not associate with myelinated axons after implantation into the compressed spinal cord. *Proc. Natl Acad. Sci. USA* **101**, 2162–2166 (2004).
149. Li, Y., Li, D. & Raisman, G. Transplanted Schwann cells but not olfactory ensheathing cells myelinate optic nerve fibres. *Glia* **55**, 312–316 (2007).
150. Dunning, M. D. *et al.* Superparamagnetic iron oxide-labeled Schwann cells and olfactory ensheathing cells can be traced *in vivo* by magnetic resonance imaging and retain functional properties after transplantation into the CNS. *J. Neurosci.* **24**, 9799–9810 (2004).
151. Ruitenberg, M. J. *et al.* Viral vector-mediated gene expression in olfactory ensheathing glia implants in the lesioned rat spinal cord. *Gene Ther.* **9**, 135–146 (2002).
152. Dusart, I. & Schwab, M. E. Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur. J. Neurosci.* **6**, 712–724 (1994).
153. Tuszynski, M. H. *et al.* NT-3 gene delivery elicits growth of chronically injured corticospinal axons and modestly improves functional deficits after chronic scar resection. *Exp. Neurol.* **181**, 47–56 (2003).
154. Shearer, M. C. & Fawcett, J. W. The astrocyte/meningeal cell interface — a barrier to successful nerve regeneration? *Cell Tissue Res.* **305**, 267–273 (2001).
155. Geller, H. M. & Fawcett, J. W. Building a bridge: Engineering spinal cord repair. *Exp. Neurol.* **174**, 125–136 (2002).
156. Boruch, A. V. *et al.* Neurotrophic and migratory properties of an olfactory ensheathing cell line. *Glia* **33**, 225–229 (2001).
157. Woodhall, E., West, A. K. & Chuah, M. I. Cultured olfactory ensheathing cells express nerve growth factor, brain-derived neurotrophic factor, glia cell line-derived neurotrophic factor and their receptors. *Brain Res. Mol. Brain Res.* **88**, 203–213 (2001).
158. Chung, R. S. *et al.* Olfactory ensheathing cells promote neurite sprouting of injured axons *in vitro* by direct cellular contact and secretion of soluble factors. *Cell. Mol. Life Sci.* **61**, 1238–1245 (2004).
159. Cao, L. *et al.* Olfactory ensheathing cells genetically modified to secrete GDNF to promote spinal cord repair. *Brain* **127**, 535–549 (2004).
160. Davies, J. E. *et al.* Astrocytes derived from glial-restricted precursors promote spinal cord repair. *J. Biol.* **5**, 7 (2006).
161. Liu, Y. *et al.* Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function. *J. Neurosci.* **19**, 4370–4387 (1999).
162. Lu, P., Jones, L. L., Snyder, E. Y. & Tuszynski, M. H. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp. Neurol.* **181**, 115–129 (2003).
163. Xu, X. M., Guénard, V., Kleitman, N. & Bunge, M. B. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. *J. Comp. Neurol.* **351**, 145–160 (1995).
164. King, V. R., Henseler, M., Brown, R. A. & Priestley, J. V. Mats made from fibronectin support oriented growth of axons in the damaged spinal cord of the adult rat. *Exp. Neurol.* **182**, 383–398 (2003).
165. Sterne, G. D., Brown, R. A., Green, C. J. & Terenghi, G. Neurotrophin-3 delivered locally via fibronectin mats enhances peripheral nerve regeneration. *Eur. J. Neurosci.* **9**, 1388–1396 (1997).
166. Ibrahim, A., Li, Y., Raisman, G. & el Masry, W. S. Olfactory ensheathing cells: ripples of an incoming tide? *Lancet Neurol.* **5**, 453–457 (2006).
167. Frankel, H. L. *et al.* The value of postural reduction in the initial management of closed injuries of the spine with paraplegia and tetraplegia. I. *Paraplegia* **7**, 179–192 (1969).
168. Fawcett, J. W. *et al.* Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. *Spinal Cord* 19 Dec 2006 (doi:10.1038/sj.sc.3102007).
169. Féron, F., Perry, C., McGrath, J. J. & Mackay-Sim, A. New techniques for biopsy and culture of human olfactory epithelial neurons. *Arch. Otolaryngol. Head. Neck Surg.* **124**, 861–866 (1998).
170. Féron, F. *et al.* Autologous olfactory ensheathing cell transplantation in human spinal cord injury. *Brain* **128**, 2951–2960 (2005).
171. Huang, H. *et al.* Influence of patients' age on functional recovery after transplantation of olfactory ensheathing cells into injured spinal cord injury. *Chin. Med. J. (Engl.)* 1488–1491 (2003).
172. Lima, C. *et al.* Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study. *J. Spinal Cord Med.* **29**, 191–203 (2006).
173. Anderson, D. K. *et al.* Recommended guidelines for studies of human subjects with spinal cord injury. *Spinal Cord* **43**, 453–458 (2005).
174. Steeves, J. D. *et al.* Guidelines for the conduct of clinical trials for spinal cord injury (SCI) as developed by the ICCP panel: clinical trial outcome measures. *Spinal Cord* 19 Dec 2006 (doi:10.1038/sj.sc.3102008).
175. Tuszynski, M. H. *et al.* Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP Panel: clinical trial inclusion/exclusion criteria and ethics. *Spinal Cord* 19 Dec 2006 (doi:10.1038/sj.sc.3102009).
176. Lammertse, D. *et al.* Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: clinical trial design. *Spinal Cord* 19 Dec 2006 (doi:10.1038/sj.sc.3102010).
177. Dobkin, B. H., Curt, A. & Guest, J. Cellular transplants in China: observational study from the largest human experiment in chronic spinal cord injury. *Neurorehabil. Neural Repair* **20**, 5–13 (2006).
178. Htut, M., Misra, P., Anand, P., Birch, R. & Carlstedt, T. Pain phenomena and sensory recovery following brachial plexus avulsion injury and surgical repairs. *J. Hand Surg. [Br.]* **31**, 596–605 (2006).
179. Kato, N., Htut, M., Taggart, M., Carlstedt, T. & Birch, R. The effects of operative delay on the relief of neuropathic pain after injury to the brachial plexus: a review of 148 cases. *J. Bone Joint Surg. Br.* **88**, 756–759 (2006).
180. Raisman, G. Repair of spinal cord injury: ripples of an incoming tide, or how I spent my first forty years in research. *Spinal Cord* **44**, 406–413 (2006).

Acknowledgements
The British Neurological Research Trust, Spinal Research, and Henry Smith Charity.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Raisman's laboratory:
http://www.ion.ucl.ac.uk/research/hbir/spinal_repair_unit.htm
Access to this links box is available online.