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Electrophysiological evidence that olfactory cell transplants improve function after spinal cord injury

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Transplants of cells obtained from the olfactory system are a potential treatment for spinal cord injury and a number of clinical trials are in progress. However, the extent to which transplants improve recovery of function remains unclear and there are contradictory reports on the extent to which they support axonal regeneration. Here, we have used anatomical and electrophysiological techniques to investigate the repair promoted by olfactory cell transplants after a dorsal column lesion. Since the use of olfactory cells of varying type and origin may contribute to the differing outcomes of previous studies, regeneration of dorsal column axons was compared following transplants of pure olfactory ensheathing cells from neonatal animals and mixed olfactory cells from both neonatal and adult rats. Two to three months after lesioning, numerous regenerating fibres could be seen in each type of transplant. However, tracing of ascending dorsal column fibres showed that few regenerated beyond the lesion, even when transplanted with mixed olfactory cells from the adult olfactory bulb which have previously been reported to support regeneration which bridges a lesion. Despite the absence of axonal regeneration across the injury site, olfactory cell transplants led to improved spinal cord function in sensory pathways investigated electrophysiologically. When cord dorsum potentials (CDPs), evoked by electrical stimulation of the L4/L5 dorsal roots, were recorded from the spinal cord above and below a lesion at the lumbar 3/4 level, CDPs recorded from transplanted animals were significantly larger than those recorded from lesioned controls. In addition, sensory evoked potentials recorded over the sensorimotor cortex were larger and detectable over a more extensive area in transplanted animals. These results provide direct evidence that transplants of olfactory cells preserve the function of circuitry in the region of the lesion site and of ascending pathways originating near the injury. These actions, rather than axonal regeneration, may help ameliorate the effects of spinal cord injury.

Keywords: spinal cord injury; axonal regeneration; olfactory ensheathing cells; electrophysiology; cell transplantation

Abbreviations: CDPs = cord dorsum potentials; SEPs = sensory evoked potentials

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Introduction

Transplantation of cells from the olfactory system has emerged as a potential treatment for spinal cord injury (Raisman, 2001; Reier, 2004; Ruitenberg *et al*., 2006) and a number of centres have begun clinical translation of this approach to spinal cord repair (Feron *et al*., 2005; Guest *et al*., 2006; Dobkin *et al*., 2006; Ibrahim *et al*., 2006). However, experimental evidence for the efficacy of this procedure is mixed. Behavioural tests of functional recovery following olfactory cell transplants have produced inconsistent results. Improved recovery has been reported following lesions of the corticospinal tract (Li *et al*., 1997) and complete transection (Ramon-Cueto *et al*., 2000; Lu *et al*., 2001; Lopez-Vales *et al*., 2006). On the other hand, Steward *et al*. (2006) failed to see improved recovery following complete transection and recovery following contusion injuries is reported to be absent (Takami *et al*., 2002) or modest (Plant *et al*., 2003). One of the mechanisms most commonly suggested as an explanation for improved functional recovery after OEC transplants is axonal regeneration. However, reports on the extent of axonal regeneration promoted by OECs are also mixed. Some report long-distance regeneration across the injury

(Li *et al.*, 1998; Ramon-Cueto *et al.*, 2000; Lu *et al.*, 2001) while others report that regeneration is restricted to the transplant site (Andrews and Stelzner, 2004; Ruitenberg *et al.*, 2003, 2005; Ramer *et al.*, 2004*a*, *b*; Lu *et al.*, 2006).

The explanation for these contradictory results is not clear but the composition, source and methods used to prepare cells for transplantation is one notable variation between different studies (Ruitenberg *et al.*, 2006). For example, transplants of pure OECs have included cells obtained from adult and neonatal animals, from the olfactory bulb and the olfactory mucosa and purification has been performed by several different methods (Ramon-Cueto *et al.*, 2000; Riddell *et al.*, 2004; Ramer *et al.*, 2004*a*, *b*). In addition to purified OECs, mixed olfactory bulb cultures (Li *et al.*, 2003) and pieces of lamina propria (Lu *et al.*, 2001; Steward *et al.*, 2006) have also been investigated. The relative merits of these different approaches are not yet clear. The olfactory mucosa provides an accessible source of OECs which can be obtained without permanent detriment to the donor's sense of smell (Feron *et al.*, 1998). This tissue is therefore considered a convenient source of cells for autologous transplantation (Feron *et al.*, 2005). However, transplantation of purified OECs from the lamina propria has consistently failed to promote axonal regeneration when tested using lesions of different axonal pathways (Ramer *et al.*, 2004*a*, *b*; Lu *et al.*, 2006). On the other hand, mixed populations of cells obtained from the olfactory bulb have been reported to promote bridging axonal regeneration and functional recovery of reaching movements when transplanted acutely or after a delay (Li *et al.*, 1997, 1998; Keyvan-Fouladi *et al.*, 2005). Furthermore, it has been suggested that successful regeneration across a lesion depends critically on a mixture of OECs together with olfactory fibroblast-like cells (Raisman, 2001).

Apart from promoting axonal regeneration, olfactory cell transplants may contribute to spinal cord repair in several other ways. They are reported to reduce cavitation (Takami *et al.*, 2002; Ramer *et al.*, 2004*a*, *b*), improve vascularization (Lopez-Vales *et al.*, 2004; Ramer *et al.*, 2004*a*, *b*), myelinate experimentally demyelinated and regenerating axons (Franklin *et al.*, 1996; Sasaki *et al.*, 2004), protect neurons forming long descending axonal pathways (Sasaki *et al.*, 2006) and promote sprouting of intact axons near the transplant (Chuah *et al.*, 2004). All of these mechanisms could potentially improve recovery of function.

In this study we have investigated regeneration of the ascending branches of primary afferent fibres following transplantation of OECs into dorsal column lesions. Since contradictory reports on the extent of axonal regeneration might be due to the composition of the cell transplants used, we compared regeneration promoted by mixed olfactory bulb cells obtained from adult animals (Raisman, 2001) with purified OECs obtained from neonatal animals. Although axonal regeneration occurred within each type of transplant, none promoted significant regeneration beyond the lesion. We next used a unique electrophysiological approach to investigate whether olfactory cell transplants can improve the function of the injured spinal cord by mechanisms other than axonal regeneration. Our results demonstrate that transplants of olfactory cells can preserve the function of spinal cord circuitry in the region of the injury and of ascending sensory pathways originating close to the lesion site.

Material and methods

Cell culture

Purified neonatal

OECs isolated from the olfactory bulbs of 7-day old Fischer 344 rat pups were purified using the O4 antibody and flow sorting as previously described (Barnett *et al.*, 1993). Purified cells were placed in a mitogen mixture of fibroblast growth factor 2 (FGF2, 500 ng/ml, Peprotech, London, UK), heregulin β1 (50 ng/ml, R&D Systems, Abingdon, UK), forskolin (5×10^{-7} M, Sigma) and astrocyte conditioned medium (ACM, Noble and Murray, 1984) diluted 1 : 5 in modified serum-free medium (Bottenstein *et al.*, 1979, DMEM-BS) which was then diluted 1 : 1 with DMEM containing 10% FCS (Alexander *et al.*, 2002). Cells were grown on poly-L-lysine coated flasks until confluent and then passaged and maintained in the mitogen mix for up to 1 month. On the day of transplantation, cells were detached from flasks using 0.25% trypsin (Gibco), neutralized with soybean trypsin inhibitor (0.52 mg/ml) and DNAse (0.04 mg/ml) in L15 media. Cells were spun, re-suspended at around 50 000 cells/μl in a small PCR tube, placed on ice and transplanted normally within 1 h. A small sample of cells was removed and used to assess cell purity (see later).

Mixed olfactory bulb cultures

Olfactory bulbs from P7 and adult (10–12-week old) Fischer 344 rats were dissected, dissociated, plated directly onto PLL coated 12.5 cm^2 flasks and incubated with DMEM containing 10% foetal bovine serum as described by Jani and Raisman (2004). Cells were washed after 2 days and fed three times a week. After 2–3 weeks in culture cells were removed from the flask for transplantation, either as described for pure OECs or using a polythene cell scraper (Corning, The Netherlands) as described by Li *et al.* (2003). The latter procedure allowed cells and their extracellular matrix to be removed together for transplantation (see later).

Composition of cell cultures

To assess the purity of the P7 OEC cultures or the proportion of OECs in mixed cultures, cells were placed on PLL-coated cover slips and immunolabelled with an antibody to p75 nerve growth factor receptor ($p75^{NTR}$, hybridoma supernatant, 1 : 1; IgG1, Yan and Johnson, 1988). In a few cases, mixed cell cultures were also labelled with antibodies to smooth muscle actin (SMA, Sigma, 1 : 400, anti-mouse), laminin (Abcam, anti-rabbit, 1 : 25) and fibronectin (Dako, 1 : 100, anti-rabbit). Cell surface markers were incubated on living cells for 30–40 min followed by the class-specific antibody diluted 1 : 100 (Southern Biotechniques) for 30 min. Intracellular markers were incubated onto cells for 30–40 min after permeabilization with ice-cold methanol for 15 min, followed by their class-specific antibody (1 : 100).

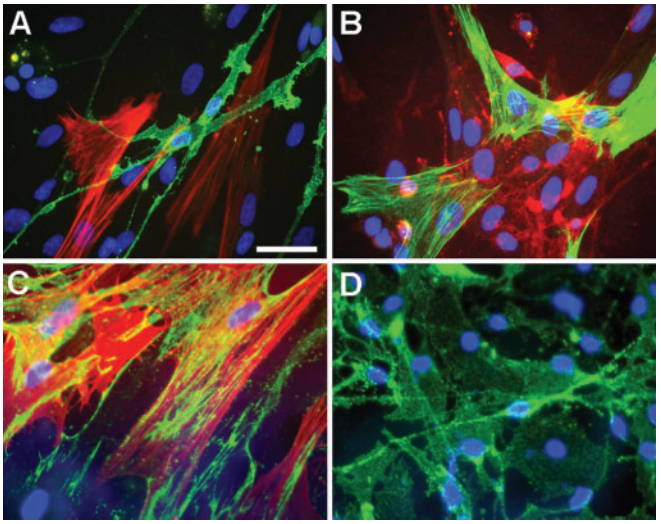

Fig. 1 Markers expressed by the different cell preparations in culture. **A**–**C** show the range of markers expressed by adult olfactory bulb cells in culture. (**A**) p75 (green) and smooth muscle actin (red). (**B**) Smooth muscle actin (green) and laminin (red). (**C**) Smooth muscle actin (red) and fibronectin (green). (**D**) Shows purified olfactory ensheathing cells expressing p75 (green). In all panels, nuclei are stained with DAPI (blue). Scale bar 50 μm applies to all panels.

The cover slips were mounted in anti-fade mountant (Vectashield, Vector Laboratories) containing 4,6-diamidino-2-phenylindole (DAPI, Sigma) to identify nuclei (Vector Laboratories, Peterborough, UK). Purified OECs were over 98% positive for $p75^{NTR}$ with 2% of the cells expressing thy1.1 which is expressed by a variety of cell types including neuronal cells, stem cells and connective tissues (Fig. 1D). The mixed olfactory cell cultures consisted of 10–50% (mean 30%) $p75^{NTR}$ expressing cells, together with other cells expressing varying amounts of fibronectin, SMA and laminin (Fig. 1A–C), indicating a mixture of olfactory bulb fibroblasts, muscle and endothelial cells from blood vessels and meningeal cells (Li *et al.*, 2003; Jani and Raisman, 2004; Andrews and Stelzner, 2004).

Retroviral labelling of OECs for identification in vivo

In order to investigate the distribution of OECs at the graft site, cells used in some experiments were engineered to express enhanced green fluorescent protein (EGFP, Clontech, Living Colors). Subconfluent cultures of each OEC preparation were infected overnight with transient supernatant taken from EGFP transfected phoenix cells shortly after purification, or in the case of mixed cell populations, 2–5 days after tissue dissociation. Between 20 and 30% of cells examined on cover slip samples taken at the time of transplantation were found to express EGFP.

Lesioning and cell transplantation

Seventy-three adult male Fischer 344 rats (Harlan, Loughborough, UK, 200–250 g) were used for *in vivo* assessment of OEC transplants; 8 to investigate the distribution and integration of transplanted cells, 23 to study regeneration of ascending dorsal column fibres and 42 for electrophysiological assessment. All experimental procedures were approved by the Ethical Review Panel of the University of Glasgow, and performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

Lesioning

Sixty-four of the 73 animals used in the study were subjected to a bilateral dorsal column lesion, while nine were used to obtain normal electrophysiological data. Animals were anaesthetized with halothane and the lumbar spinal cord exposed by laminectomy at the L1/T13 vertebral junction. The dorsal columns were lesioned close to the L3/4 segmental border using a wire knife (David Kopf Instruments, Tujunga CA, USA). The sheathed knife was inserted through a slit in the dura over the left dorsal root entrance zone, lowered to a depth of 950 μm and then protruded to form an arc (~1.5 mm diameter) encompassing the dorsal columns. This was then raised against a glass rod placed on the surface of the cord to cut through the dorsal columns. Because the glass rod does not contact the full circumference of the arc of the knife, lesioning of the most superficial fibres was ensured by pressing the compacted head of a pointed cotton bud into the arc of the knife for ~20 s. This procedure produces a clean transection of the dorsal columns without damage to the central vein at the surface. The left L3 dorsal root was cut in order to avoid the possibility of tracer being transported along anastomosing afferent fibres in these roots.

Cell transplantation

Forty-four of the 64 animals receiving a dorsal column lesion were transplanted with olfactory cells immediately after lesioning. Cells were transplanted either as a suspension ($n = 28$) or within their own matrix ($n = 16$). Cell suspensions were injected using a glass pipette with a fine bevelled tip (inside diameter 60–70 μm). Since cells were better retained within the lesion when injected as a thick suspension, they were concentrated to between 50 000 and 100 000 cells/μl prior to filling the pipette by removal of medium from above the cell pellet. The pipette was inserted into the lesion through small slits in the dura either side of the central vein to a depth of ~1000 μm. Cells were injected by applying brief (40 ms) pressure pulses (Picoinjector, WPI, Sarasota FL, USA) over the course of several minutes, during which the pipette was gradually raised to the surface. Cells were injected until they overflowed from the lesion, which required injection of up to 4.0 μl of cell suspension into each lesion.

To transplant cells in their matrix, cell monolayers were scraped from the base of the culture dish. Watchmaker's forceps were then used to tease the matrix into sub-mm^2 pieces and gently push them into the lesion through small slits in the dura.

Wounds were closed in layers and animals routinely received an analgesic (Vetergesic, Alstoe Ltd, UK; 0.06 mg, s.c.).

Anatomical assessment of regeneration

Twenty-three animals with dorsal column lesions and transplants of olfactory cells were investigated using tract tracing. Eight animals received mixed adult cells as a suspension, seven received mixed adult cells in matrix and eight received purified P7 cells as a suspension. Regeneration was assessed 2–3 months later using tract-tracing with either biotinylated dextran amine (BDA, 10 000 MW, product no. D-1956, Invitrogen, 11 animals, see Table 1) or the B subunit of cholera toxin (CTB, List Biological Laboratories, 12 animals). Only transplanted animals in which the histology showed a lesion that was well-filled with cells were included in the study.

Table 1 Assessment of the ability of different transplant types to support dorsal column axon regeneration

Animal number	Survival (months)	Proportion of sections with ingrowth	Total sections with ingrowth
Adult mixed cells, suspension			
1	3	5/7	17/29, 59%
2	2.5	2/8	
3	2	4/7	
4	2	6/7	
Adult mixed cells, matrix			
5	3	2/8	7/34, 21%
6	3	0/15	
7	2.5	5/11	
Neonatal pure cells, suspension			
8	3	4/5	5/19, 26%
9	3	1/6	
10	2	0/3	
11	2	0/5	

The proportion of sections with ascending dorsal column axon ingrowth determined using BDA tract tracing is shown for each of the transplant types compared. The data suggest that mixed adult cells more effectively promote regeneration than pure neonatal cells when both are transplanted as a suspension and that mixed adult cells more effectively promote regeneration when transplanted as a suspension than as a matrix.

Injection of tract tracer

For BDA injection, the left L4 DRG was exposed by laminectomy under surgical anaesthesia and injected with 3–4 µl of a solution of 20% BDA as described previously (Riddell *et al.*, 2004). For CTB injection, the left L4 and L5 spinal nerves were dissected under surgical anaesthesia and each injected with 3–4 µl of a 1% solution of CTB. Both tracer solutions contained fast green dye to detect any spillage during injections.

Histological processing

Three days after CTB injection, or 2 weeks after BDA injection, animals were deeply anaesthetized with intraperitoneal sodium pentobarbital (200 mg/ml Euthatal, Vericore Ltd, UK) and perfused through the left ventricle with mammalian Ringer containing 0.1% lidocaine followed by 4% paraformaldehyde in 0.1 M PB, pH 7.4. The lumbar spinal cord was removed and post-fixed overnight in the same fixative with 30% sucrose. Blocks containing the lesion were cut into 70 µm sagittal sections on a freezing microtome then incubated for 30 min in 50% ethanol. After washing for 10 min in 0.3 M phosphate buffered saline (PBS), sections were incubated for 72 h in streptavidin conjugated to dichlorotriazinylamino fluorescein (DTAF; 1 : 1000, Jackson, USA) to detect BDA or with goat anti-CTB (List, UK; 1 : 5000). Some sections were also incubated in rabbit antibody to glial fibrillary acidic protein (GFAP; 1 : 1000, Dako) to delineate the lesion. Sections were subsequently incubated for 2–4 h in fluorophore-conjugated species specific donkey IgG secondary antibodies. All antibodies were diluted in PBS with 0.3% Triton X-100. Sections were mounted in Vectashield (Vector Laboratories, UK) and stored at −20°C.

Epifluorescence and confocal microscopy

Sections containing CTB or BDA-labelled fibres were examined carefully under the epifluorescence microscope (Nikon Eclipse E600) at low power. Sections in which BDA-labelled fibres appeared to enter the transplant or CTB labelling occurred in close proximity to the lesion were investigated further by serially scanning the tissue at 2 µm (×20) or 0.5 µm (×60, oil immersion) intervals using a Bio-Rad MRC1024 confocal system. Projections of confocal image stacks were formed using Confocal Assistant software (Todd Clark Brelje, University of Minnesota) in order to determine whether BDA or CTB-labelled profiles within the sections entered the cell transplant in the lesion site or passed rostrally of the dorsal column lesion.

Cell distribution and integration

Eight animals with dorsal column lesions received transplants of cells which had been labelled with EGFP (see earlier); six animals transplanted with mixed adult olfactory cells (three as a suspension and three as a matrix) and two with a transplant of pure OECs from P7 animals. These animals were perfused 2 weeks later and sections containing the lesion site were cut and processed as described earlier using the following primary antibodies in various combinations: rabbit anti-GFP antibody (1 : 4000, ab6556, Abcam), rabbit anti-GFAP, mouse anti-GFAP (1 : 1000, Sigma), mouse anti-neurofilament 200 kDa (NF200; 1 : 1000, Sigma), rabbit anti-laminin (1 : 100, Sigma) and mouse anti-smooth muscle actin (SMA; 1 : 400, Sigma). After incubation in appropriate combinations of species-specific secondary antibodies conjugated either to FITC or RRX, sections were examined using epifluorescence and confocal microscopy.

Electrophysiology

Surgical preparation

Animals were deeply anaesthetized, initially with halothane and subsequently with doses of sodium pentobarbital (10 mg/kg i.v.), given as required. Depth of anaesthesia was assessed by monitoring pedal withdrawal reflexes and blood pressure. During recording, when the animals were paralysed with

pancuronium (Faulding Pharmaceuticals PLC, Leamington Spa, UK; 0.1 mg i.v. at 40-min intervals) and artificially ventilated, anaesthetic was given at a frequency commensurate with that required before paralysis and the adequacy of anaesthesia monitored by continuously recording blood pressure and its response to noxious stimuli and observing pupil diameter. Core temperature was maintained close to 38°C, mean blood pressure >80 mmHg and pCO^2 within 4.0–4.5%. A laminectomy was performed from the Th13 to L4 vertebrae. The animal was transferred to a rigid spine and head holder and a craniotomy performed to expose the sensorimotor cortex on the right side. The dura over the brain and spinal cord was opened and exposed tissues covered with liquid paraffin maintained close to 37°C by radiant heat. The L4 DRG was located to identify the L4 dorsal root and this and the L5 dorsal root cut distally and mounted on bipolar silver-wire electrodes for electrical stimulation. The lesion was identified from a 10/0 marking suture left in the dura at the end of the lesion/transplant operation and by its darker appearance compared to the surrounding cord (in non-transplanted animals especially). Its location in relation to the L3/4 border was confirmed by following the dissected L4 dorsal root to its level of entry.

Recordings of cord dorsum potentials

To assess spinal cord function in the region of the lesion, cord dorsum potentials (CDPs) evoked by electrical stimulation of dorsal roots were recorded from a total of 40 animals: 9 normal animals, 18 animals with dorsal column lesions but no cell transplants (8 at 3–6 days post lesion, and 10 at 62–88 mean 77 days post lesion); and 13 animals with dorsal columns lesions transplanted with olfactory cells (at 60–91, mean 78 days post lesion; 7 transplanted with cell suspensions and 6 with cell matrix). Recordings were made using a silver-ball electrode placed on the dorsal surface of the cord while alternately stimulating the L4 dorsal root, L5 dorsal root and both roots simultaneously (supramaximal shocks, up to 60 μA, 0.2 ms). At each location, 10–20 recordings were averaged using a CED 1401+ interface and Signal software (Cambridge Electronic Design, Cambridge, UK) and measurements of CDP amplitude made using the same software.

Recordings of sensory evoked potentials

To assess the function of ascending pathways projecting to the sensorimotor cortex, sensory evoked potentials (SEPs) produced by electrical stimulation of dorsal roots were recorded from a total of 22 animals: 5 normal animals, 10 animals with dorsal column lesions but no cell transplant (4 at 3–6 days post lesion and 6 at 65–90 mean 78 days post lesion); and 7 animals with dorsal column lesions and olfactory cell transplants (at 60–88, mean 73 days post lesion; 4 transplanted with cell suspensions and 3 with cell matrix). SEPs were recorded using a monopolar silver-ball electrode placed on the surface of the sensorimotor cortex while supramaximal shocks were applied alternately to the L4 dorsal root, L5 dorsal root or both roots simultaneously. Up to 40–60 individual records were collected and averaged at each recording location using a CED 1401+ interface and Signal software (Cambridge Electronic Design, Cambridge, UK). Latencies and amplitudes of SEPs were measured and isopotential plots of SEP data were created using the 3D field contour plotting software (version 2.1.6, Vladimir Galouchko).

Histological processing

Experiments were terminated by transcardial perfusion with Ringer followed by paraformadehyde fixative. The lumbar spinal cord was removed and cryoprotected overnight in 30% sucrose in 4% paraformaldehyde. A block spanning the dorsal column lesion was prepared, 70 μm transverse sections cut on a freezing microtome and sections incubated with anti-NF200 and anti-GFAP. Mounted sections were inspected using an epifluorescence microscope in order to assess the lesion site (Fig. 8).

Results

Survival and distribution of transplanted cells

Lesions produced by the wire knife consisted of a cavity surrounded by an area of spinal cord tissue containing reactive astrocytes (a glial scar) labelling densely for GFAP (Figs 2A and 8). To confirm survival of each of the transplanted cell preparations and their ability to fill and bridge the lesion cavity, five animals were transplanted with cells modified to produce EGFP. Two weeks after transplantation, numerous labelled cells could be observed at the lesion site (Fig. 2B–E) and smooth muscle actin labelling reminiscent of blood vessels was seen in and around the transplant (Fig. 2F). Cells transplanted as a suspension (both mixed cells from adult animals and pure OECs from neonatal animals) were evenly distributed throughout the lesion site, effectively filling the lesion cavity (Fig. 2B and D). Cells transplanted in pieces of matrix were also widely distributed within the lesion. However, in animals receiving transplants by this method, small areas devoid of labelled cells occurred at the lesion perimeter, indicating the presence of some unfilled gaps at the transplant/lesion interface (Fig. 2C). A similar tendency was observed at longer survival times (2–3 months) in animals used for tract tracing and electrophysiology (see later). These animals were transplanted with non-labelled cells but small cavities (recognizable under dark field illumination or as areas devoid of background immunolabelling) occurred more commonly in the matrix transfer animals than animals where cells were delivered by injection.

Axonal regeneration

As a first indication of the ability of the different cell preparations to provide a growth supportive substrate for regenerating axons, we investigated the extracellular matrix component laminin and neurofilament-labelled axons associated with the transplants. Two weeks after lesioning, non-transplanted lesions were largely devoid of laminin apart from that associated with blood vessels and tissue capping the lesion cavity (Fig. 3A). In contrast, 2 weeks after cell transplantation, laminin immunolabelling was seen throughout the transplant site; both in association with newly formed blood vessels, and also more diffusely (e.g. Fig. 3B–D). Interestingly, the pattern and density of laminin staining produced by mixed cells

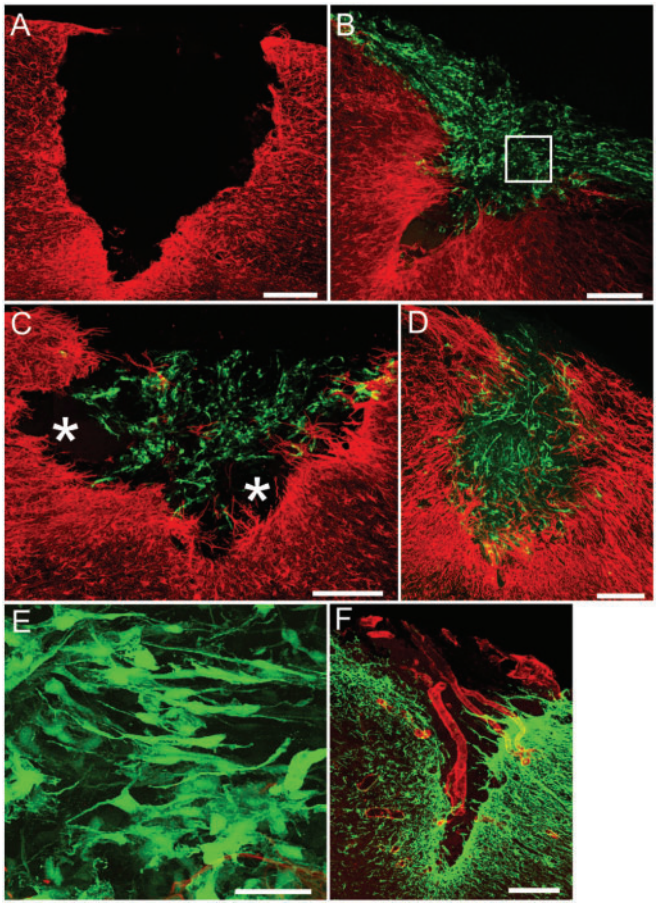

Fig. 2 Survival and integration of transplanted cells is accompanied by blood vessel formation at the lesion site. Confocal microscope images of sagittal sections through dorsal column lesions and cell transplants. (**A**) Animals not transplanted with cells developed a strongly GFAP-positive (red) glial scar around the lesion cavity. (**B**–**E**) Transplanted EGFP-expressing cells (green) at the lesion site. (**B**) Mixed adult cells transplanted as a suspension. (**C**) Mixed adult cells transplanted within a matrix. (**D**) Pure neonatal OECs. (**E**) Higher power (X60) view of the boxed area shown in **B**. Cells transplanted by suspension effectively filled the lesion site while transplant by matrix transfer sometimes led to small areas devoid of cells at the lesion perimeter (marked by asterisks in **C**). (**F**) Smooth muscle actin-positive blood vessels (red) within a transplanted lesion delineated by GFAP labelling (green). Scale bars: **A**–**D** and **F**, 200 μm, **E**, 50 μm. Images **A**–**D** and **F** are composites of several confocal microscope fields of view.

(Fig. 3B and C) was qualitatively similar to that produced by transplants consisting of pure OECs (Fig. 3D) and there was little difference between the mixed cells transplanted as a suspension (Fig. 3B) and within a matrix (Fig. 3C). Consistent with this, there was also no obvious difference in the results obtained with neurofilament labelling of regenerating axons. Numerous axons were seen extending throughout each of the transplant types (Fig. 3E–H), indicating that each are capable of supporting robust axonal regeneration.

Dorsal column axons have ascending and descending branches and may enter the transplant from either rostral or caudal directions. Since individual neurofilament-labelled axons cannot easily be followed for any great length, this method does not allow identification of axons traversing the lesion site. In order to determine whether any of the cell transplants allowed regenerating dorsal column axons to extend all the way across the lesion site, tract tracing of lumbar afferent fibres entering below the lesion was performed by injection of tracer. Injections of BDA (see Table 1) or CTB were made into animals transplanted with each of the different cell types. Figure 4 shows examples of BDA labelling in sagittal sections spanning the lesion site, identified by dense immunolabelling for GFAP (Fig. 4A, B and D) or neurofilament (Fig. 4C). Below the level of the lesion (right-hand side of images shown in Fig. 4), there was extensive labelling of the intact portions of primary afferent fibres entering through the L4 dorsal root. BDA labels axons throughout their course and in animals injected with this tracer, labelled axons are seen running rostrocaudally in the dorsal columns, giving rise to collateral branches which extend ventrally into the grey matter (Fig. 4A–C). In many of the sections from animals injected with BDA, labelled fibres could be seen extending from below the lesion into the lesion site (Fig. 4A–C). To obtain a semiquantitative indication of whether the different transplant types differed in their ability to support regeneration of dorsal column axons, for each animal, the number of sections with BDA-labelled fibres extending into the transplant site was counted and expressed as a proportion of the total number of sections containing the lesion (Table 1). The results of this analysis suggest that mixed adult cells may more effectively enable regeneration of dorsal column axons than pure neonatal OECs when both are transplanted as a suspension. The analysis also suggests that when mixed adult cells are transplanted in a matrix, they support regeneration less effectively than when injected as a suspension. This latter finding may be related to the poorer integration of matrix transplants described earlier. Although numerous examples of labelled axons growing into the transplant filled lesion site were observed, there was very little evidence that axons were able to reach the rostral side. Only two sections (from different animals) containing BDA-labelled axons extending beyond the rostral lesion margin were observed (Fig. 4B).

Since BDA traces only a small fraction of ascending dorsal column axons, we also performed tract tracing with CTB in order to ensure that we were not missing bridging regeneration because of the tracer employed. CTB binds to the Gm1 ganglioside and therefore labels a higher proportion of DRG neurons than BDA, but it labels predominantly the terminals of afferent fibres rather than axons. Injection of this tracer therefore results in a dense region of labelled boutons corresponding to the grey matter of the dorsal horn and also labels retraction bulbs formed by axotomized fibres in the dorsal columns on the caudal margin of the lesion (Fig. 4D). Despite observing dense labelling below the lesion site, CTB-labelled boutons could

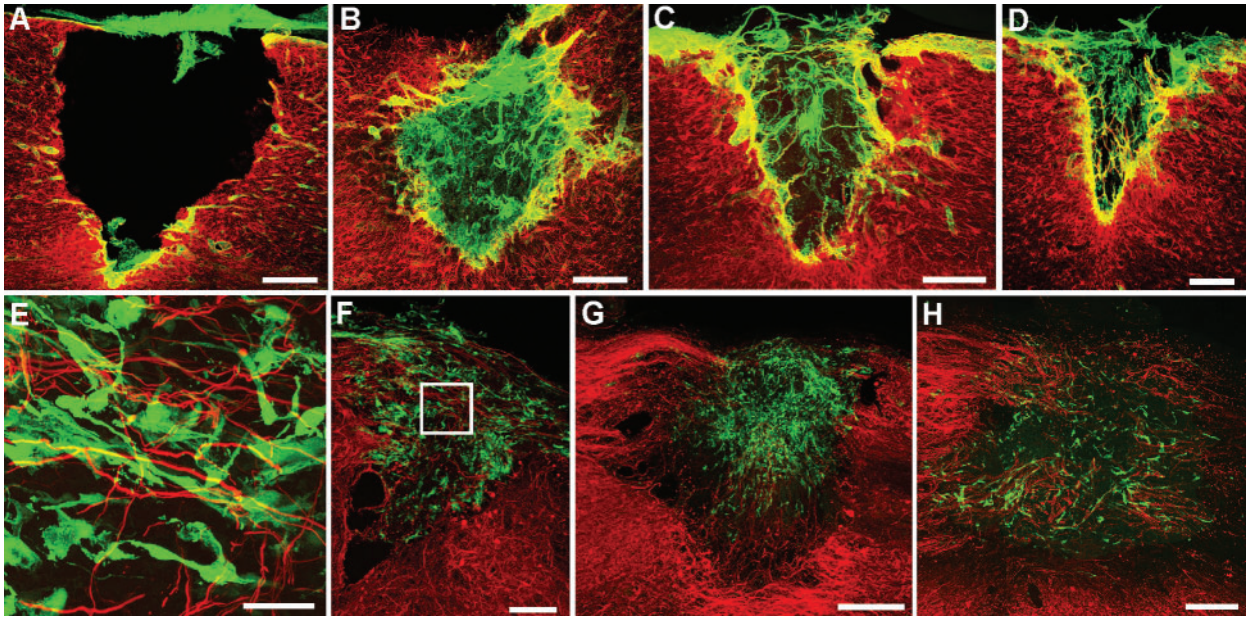

Fig. 3 Olfactory cell transplants deposit a laminin-rich matrix and support axonal growth. Confocal microscope images of sagittal sections through lesions and cell transplant sites. (**A**–**D**) Sections immunolabelled for GFAP (red) to delineate the lesion site and laminin (green). (**A**) Lesion site without transplanted cells showing laminin labelling restricted mainly to blood vessels at the edge of the lesion and tissue capping the lesion cavity. **B**, **C** and **D** show dense deposition of laminin within transplants of mixed adult cells transplanted as a suspension (**B**) or matrix (**C**) and pure p7 OECs (**D**). Each of the transplants exhibit diffuse laminin staining, distinct from that of the basal lamina of blood vessels. In addition, GFAP-positive astrocytic processes, which are strongly laminin-positive, project into each type of transplant (co-labelling visible in yellow). (**E**–**H**) Sections labelled for GFP-expressing transplanted cells (green) and neurofilament-200 containing axons (red). Mixed adult cells transplanted as a suspension (**F**) or matrix (**G**) and pure p7 OECs (**H**). (**E**) Magnified image (X60 scan) of the boxed area in **F**. Note the robust ingrowth of neurofilament-labelled fibres into each of the transplant types. Scale bars: **A**–**D** and **F**–**H**, 200 μm, **E**, 50 μm. Images **A**–**D** and **F**, **G** are composites of several confocal microscope fields of view.

not be detected rostral to the lesion (Fig. 4D). The CTB evidence is therefore consistent with the results obtained using BDA. Together they suggest that none of the transplant types promote significant axonal regeneration across the type of lesion used in this investigation.

Spinal cord function assessed by electrophysiology

Since few ascending dorsal column fibres grow across the transplanted lesion site in this model (see earlier), little or no functional improvement is likely to occur by this mechanism. To determine whether olfactory cell transplants can improve spinal cord function by mechanisms other than axonal regeneration, we next used an electrophysiological approach to investigate the synaptic actions of afferents entering the spinal cord through the L4 and L5 dorsal roots, caudal to the dorsal column lesion. The anatomy of these afferent fibres is illustrated schematically in Fig. 5A. Potentials evoked by these afferents were recorded from normal animals, from dorsal column lesioned animals without cell transplants (studied after a short and long survival time to assess possible spontaneous recovery) and from dorsal column lesioned animals receiving olfactory cell transplants. Mixed olfactory cells from adult animals transplanted both as a suspension and as a matrix were used in this group of animals.

Cord dorsum potentials

Normal animals

Electrical stimulation of dorsal roots produces an afferent volley followed by a CDP at the surface of the spinal cord (Fig. 5B). These were recorded at 1 mm intervals from 6 mm rostral to 6 mm caudal to the L3/L4 border (Fig. 5C). In a normal animal, CDPs are largest at the segment of entry of the stimulated root, but potentials of gradually declining amplitude can be detected for several segments above and below (Fig. 5D). The mean amplitudes of CDPs recorded in the group of normal animals are plotted in Fig. 6A for stimulation of the L4 dorsal root and Fig. 6B for stimulation of the L5 dorsal root (black line in each plot).

Short survival, lesioned group

Examples of CDPs recorded from a representative animal of this group are shown in Fig. 5D and the distribution of averaged CDPs in all animals of the group is shown in Fig. 6A and B (green lines). Above the lesion, CDPs were virtually absent due to axotomy of the ascending branches

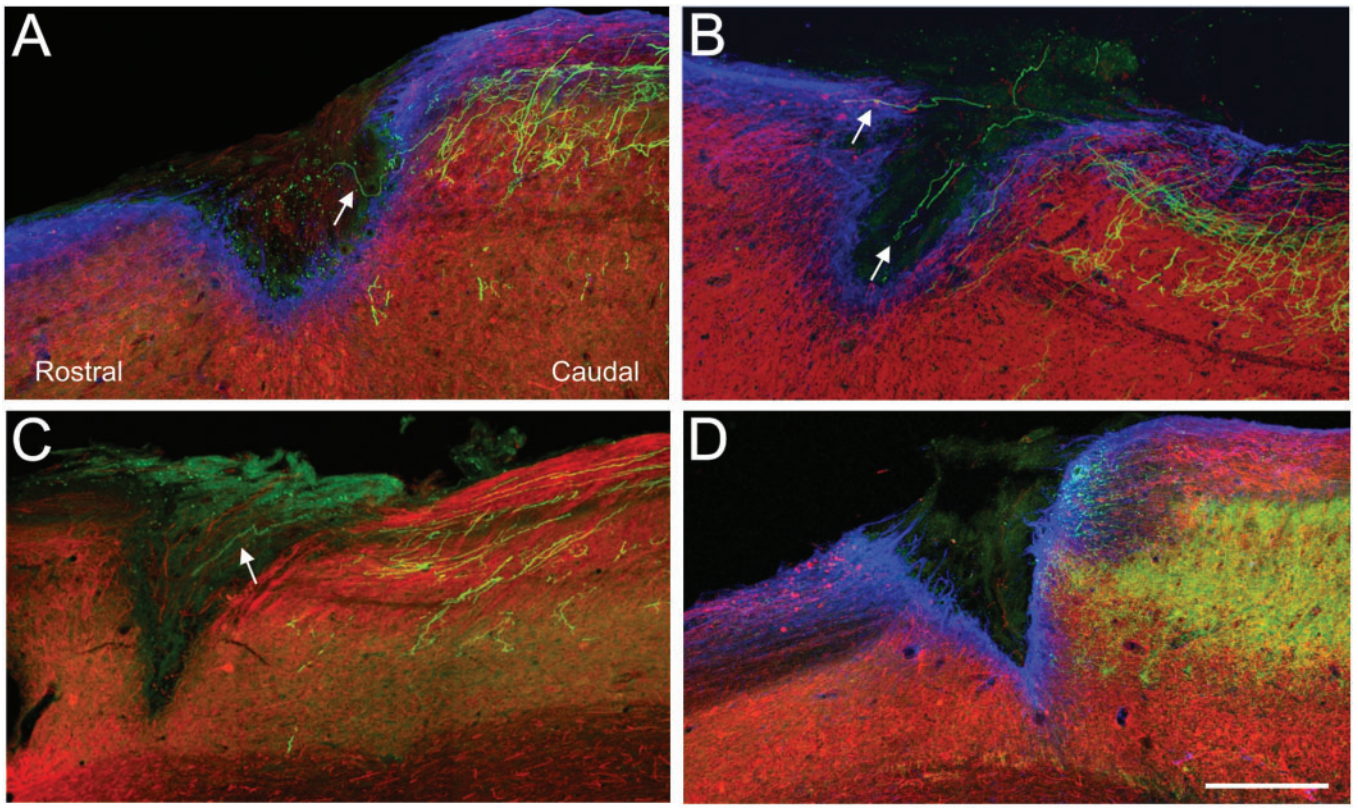

Fig. 4 Ascending primary afferent fibres grow into but not beyond the cell transplants. Confocal microscope images of sagittal sections through lesions transplanted with cells from animals injected with a tract-tracer to label dorsal column fibres. In **A**, **B** and **C** primary afferents were labelled using biotinylated dextran amine (BDA) while in **D** they were labelled using the cholera toxin, B subunit (CTB). In each section, labelled fibres are shown in green and neurofilament immunolabelling in red. In **A**, **B** and **D**, GFAP immunolabelling of the glial scar is shown in blue. In **A**, **B** and **C**, BDA-labelled fibres are seen in the dorsal columns caudal to the lesion and collateral branches from these project ventrally into the adjacent grey matter. Labelled fibres (arrows) can also be seen regenerating within the transplant filled lesion. Most fibres remained within the transplant, as in **B** and **C**, but **B** shows a rare example of a labelled fibre which has reached the rostral side of the lesion. In **D**, there is dense CTB labelling of afferent fibre terminals within the grey matter and labelled end bulbs can be seen in the dorsal columns at the caudal margin of the lesion. However, no terminal labelling is evident rostral to the lesion. Scale bar 500 μm. Images are composites of several fields of view.

of primary afferent fibres on which these potentials largely depend. However, CDPs below the lesion were also substantially depressed, even up to 6 mm or more from the lesion site. This is surprising since axon collaterals of L4 and L5 dorsal root afferents contacting spinal cord neurons below the level of the lesion were not directly damaged by the lesion (Fig. 5A).

Long survival, lesioned group

CDPs from an animal of this group are shown in Fig. 5D and the average amplitudes of CDPs in the group as a whole are shown in Fig. 6A and B (blue line). Above the lesion, CDPs are virtually absent, as in the short survival animals. Within a 3–4 mm region immediately below the level of the lesion, CDP amplitudes were also depressed to a similar extent as the short survival animals. However, more caudal to the lesion (6–7 mm), CDPs were significantly larger than those of the short survival animals and in fact were of virtually normal amplitude.

Long survival, lesioned and olfactory cell transplanted group

Example CDPs recorded from a transplanted animal are shown in Fig. 5D and the average amplitudes of CDPs in the group as a whole are shown in Fig. 6A and B (red lines). CDPs in the animals with transplants were significantly larger than those recorded from the long survival non-transplanted animals at virtually every recording location (significance levels as indicated by asterisks on plots in Fig. 6A and B). In the region extending 3 mm immediately below the lesion, averaged CDPs in the transplanted animals were appreciably (2–4 times) larger than those of non-transplanted animals. The bar graphs in Fig. 6C and D compare the average amplitudes of CDPs recorded in each group of animals at −3 mm, the position where the difference between the transplanted and non-transplanted animals was most marked. In this graph, the amplitudes of CDPs recorded from animals injected with a cell suspension and animals transplanted by transferring

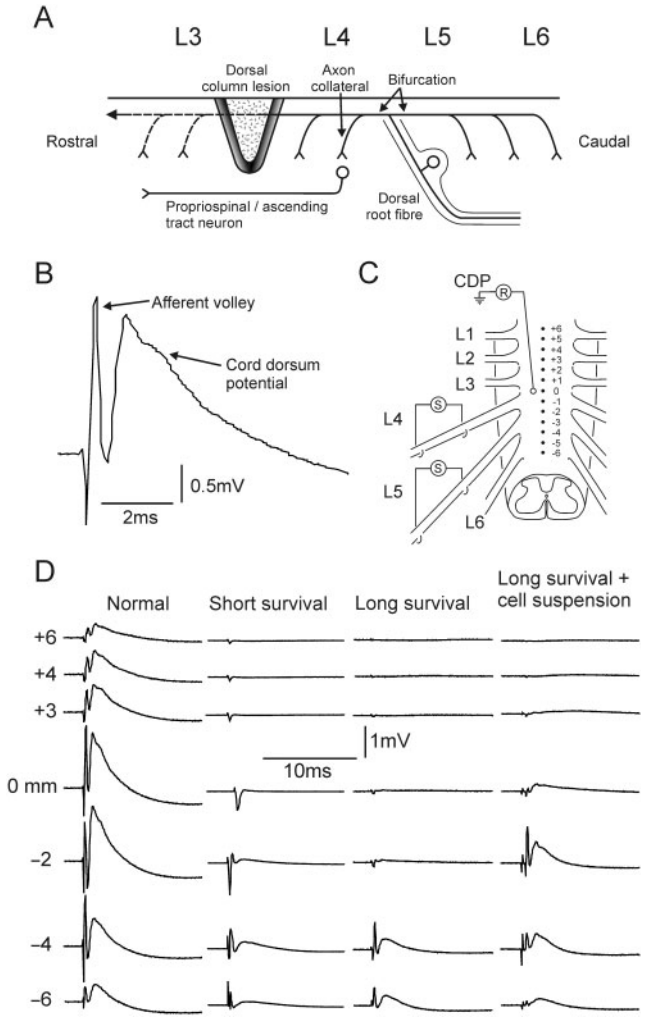

Fig. 5 Cord dorsum potentials recorded from the surface of the spinal cord. (**A**) Schematic diagram showing the anatomy of the lumbar dorsal root afferent fibres which were electrically stimulated during the electrophysiological experiments. Dorsal root fibres enter the cord and bifurcate into ascending and descending branches which run in the dorsal columns. These main axons give rise to regular axon collateral branches with terminations in the grey matter. Lesioning the dorsal columns at L3/4 severs the main ascending axons of fibres entering through the L4 and L5 dorsal roots but the connections formed by collaterals below the lesion (with propriospinal or ascending tract neurons, for example) remain intact. (**B**) Example of the electrical activity recorded from the surface of the lumbar spinal cord following dorsal root stimulation. The potentials consist of two components: an 'afferent volley' which represents electrical impulses travelling along the axons arriving at the spinal cord and a negative post-synaptic potential called a 'cord dorsum potential' which reflects current flow at synapses formed by collateral branches of the axons. (**C**) Schematic diagram showing the arrangement of stimulating (S) and recording (R) electrodes for investigating CDPs. The L4 and L5 dorsal roots were stimulated and recordings made in 1 mm steps along the surface of the lumbar spinal cord. (**D**) Shows representative examples of CDPs recorded above and below the L3/4 border (0 mm) in normal animals and in the different groups of animals with dorsal column lesions made close to the L3/4 border (~+1 mm). For further description, see text. In **B** and **D**, negativity is upwards. All traces are averages of between 10 and 20 sweeps.

cells within their matrix are shown separately. From this it is apparent that the CDPs were significantly larger in both suspension and matrix transplanted animals than non-transplanted controls. Above the lesion, small CDPs were just detectable but remained far smaller than normal.

Sensory evoked potentials

Normal animals

Electrical stimulation of dorsal roots produces sensory evoked potentials (SEPs) on the surface of the sensorimotor cortex. Typical examples of SEPs recorded from a normal animal are shown in Fig. 7B (black line). The earliest component of the SEP [sharp positive (downward) deflection, ~6 ms latency] is mediated by the ascending dorsal column system (Angel and Berridge, 1974). SEPs were investigated by recording at each position on a grid covering the sensorimotor cortex using bregma as a reference point (Fig. 7C). Three quantitative measures relating to the earliest positive component of the SEP were obtained from each animal: (1) the largest amplitude (maximal) potential within the recording grid, (2) the latency of the maximal potential and (3) the area of cortex containing potentials exceeding 50 μV. Since results obtained while stimulating the L4 and L5 dorsal roots separately and together were closely similar, only data obtained using stimulation of both roots together is presented. The bar charts in Fig. 7D show the mean maximal amplitude and latency of the early positive component of the SEP recorded from normal animals. A 50 μV isopotential plot constructed from the mean amplitudes of potentials recorded at all positions on the grid is shown in Fig. 7C (black line).

Short survival, lesioned group

In all animals of this group, the early positive component seen in normal animals was completely absent. However, in two of the four animals investigated, a small positive potential of longer latency (~13 ms rather than 6 ms) was observed (see Fig. 7B, green trace and Fig. 7D). This was largest within 1 mm of the usual position where maximal short latency SEP components were found in normal animals.

Long survival, lesioned group

The normal early (6 ms latency) positive component of the SEP remained absent in animals of this group but the novel positive potential of longer latency (~16 ms) seen in only two of the four short survival animals was clearly apparent in all six long survival animals (see Fig. 7B, blue trace). Furthermore, the mean maximal amplitude of this component was more than double that seen in the short survival animals (Fig. 7D). This difference, and other comparisons described later, were analysed using ANOVA with Tukey–Kramer *post hoc* testing ($P < 0.05$) and found to be significant. These potentials were maximal within 1 mm of the position where maximal short latency SEP components

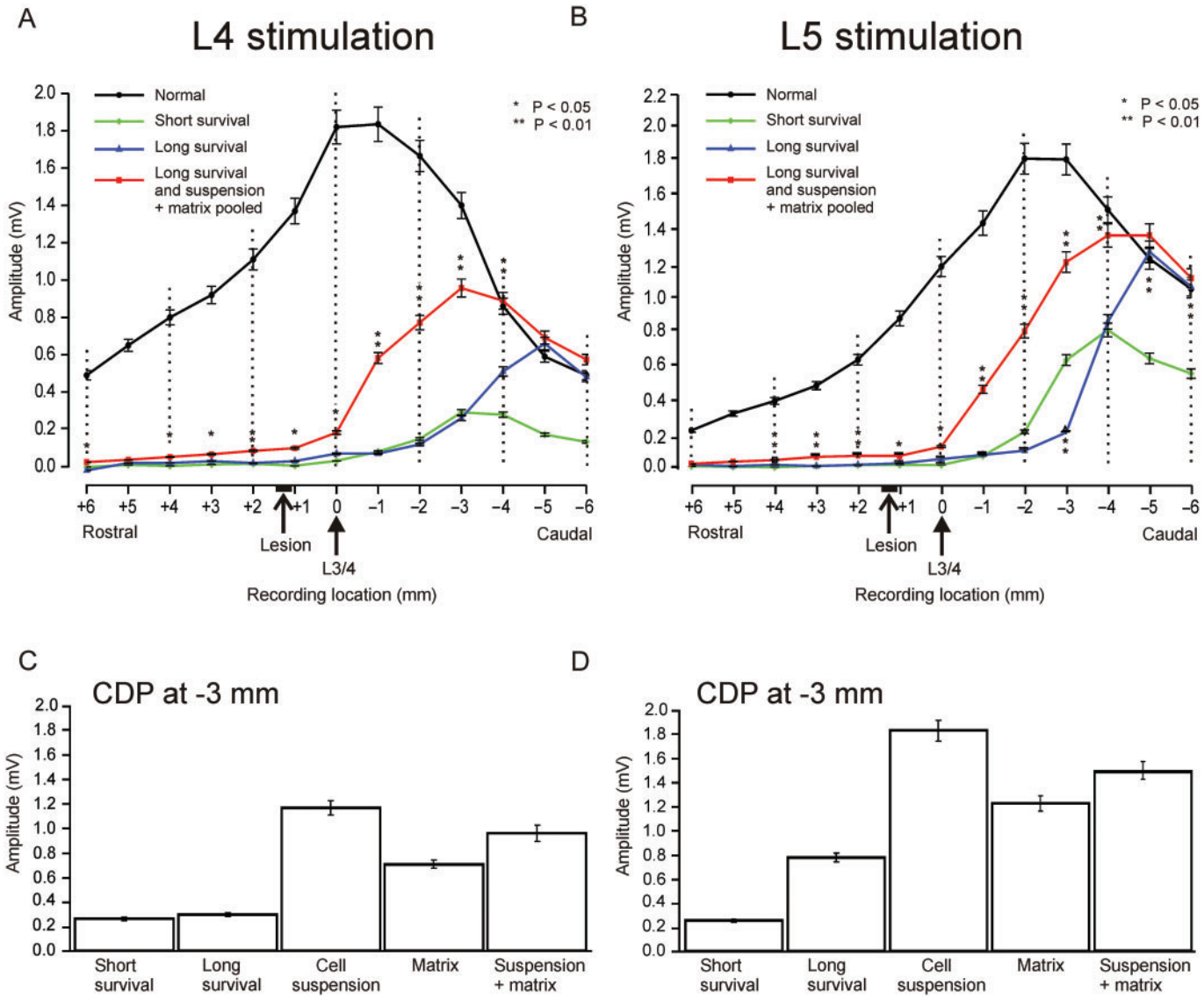

Fig. 6 Plots of cord dorsum potentials evoked by simulation of L4 or L5 dorsal roots in normal and dorsal column lesioned groups of animals. (**A** and **B**) Plots of the average amplitudes of CDPs recorded from each group of animals at 1 mm intervals extending 6 mm above and below the L3/4 border (0 mm). **A** shows plots for stimulation of the L4 dorsal root and **B** shows plots for stimulation of the L5 dorsal root. Plots representing each of the animal groups are colour coded: normal (black), short survival (green), long survival (blue) and long survival with cell transplant (red). The asterisks indicate positions at which the mean amplitude of CDPs recorded from animals with olfactory cell transplants (red) differs significantly from that of CDPs recorded from the long survival control animals (blue). (**C** and **D**) Histograms showing the mean maximal amplitudes of CDPs recorded at the −3 mm position following L4 (**C**) and L5 (**D**) dorsal root stimulation. Data for animals transplanted by cell injection and matrix transfer are shown separately to allow comparison. Numbers of animals in each condition: short survival = 8, long survival = 10, cell suspension = 7, matrix = 6, suspension + matrix = 13. CDP amplitudes were calculated from averages of between 10 and 20 individual sweeps per recording location for each animal in the experimental conditions. All error bars represent SEM, statistical significance at stated levels is based on ANOVA with Tukey–Kramer post-hoc analysis.

were found in normal animals but the area within which potentials exceeding 50 μV were evoked was much more restricted (see Fig. 7C, blue line).

Long survival, lesioned and olfactory cell transplanted group

The early (6 ms latency) positive dorsal column mediated component of the SEP remained absent in these animals but the novel short latency component (at 15 ms), which occurred consistently in long survival non-transplanted animals, was significantly larger in transplanted animals (Fig. 7B, red trace; Fig. 7D). The area of cortex over which this component exceeded 50 μV in amplitude was also significantly expanded compared to animals without transplants (Fig. 7C). A break down of the animals into suspension and matrix transplanted groups showed that this effect was

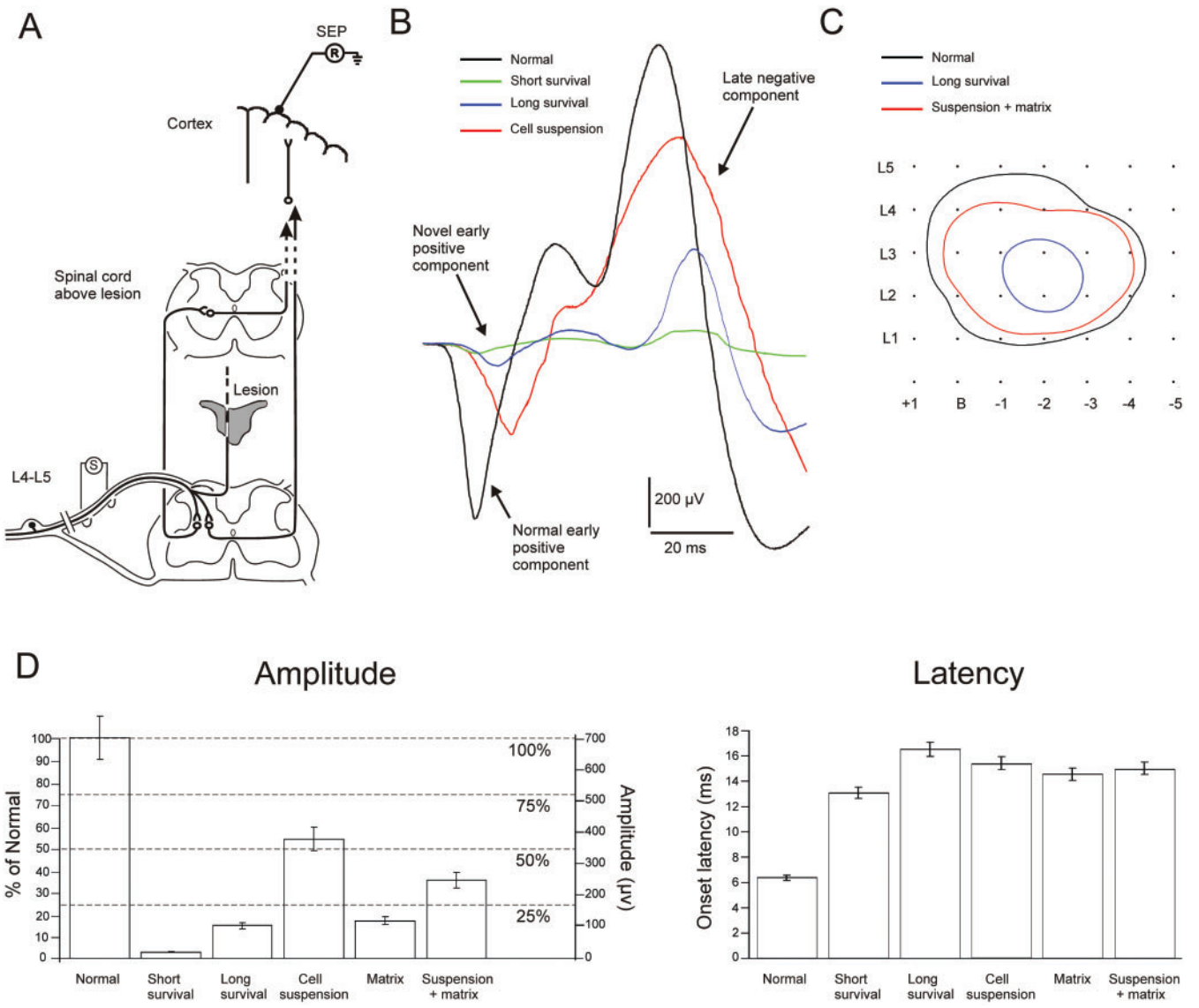

Fig. 7 Analysis of sensory evoked potentials. (**A**) Schematic diagram showing the experimental arrangement for recording SEPs evoked by stimulation of the L4 and L5 dorsal roots. This diagram also shows putative alternative (non-dorsal column) pathways by which sensory activity entering the spinal cord below the dorsal column lesion may reach the sensorimotor cortex. (**B**) Shows representative examples of SEPs recorded from a normal animal and animals from the different groups with dorsal column lesions (see key for colour coding). Each trace represents the average of between 40 and 60 individual sweeps. Further explanation of the different components of the SEP is provided in the text. (**C**) Isopotential plots constructed from the amplitudes of early positive components of SEPs recorded in a grid pattern (as shown) from the surface of the cortex. 'B' represents bregma. The isopotential lines represent the area within which early positive SEP components exceeded 50 µV in amplitude. For further explanation, see text. (**D**) Bar graphs showing the average amplitude (left) and latencies (right) of the early positive SEP components in each of the animal groups. Data for animals transplanted by cell injection and matrix transfer are shown separately to allow comparison. Number of animals in each condition: normal = 5, short survival = 4, long survival = 6, cell suspension = 4, matrix = 3, suspension + matrix = 7. Error bars represent SEM.

explained by larger potentials in the suspension transplanted group.

Lesion location and properties in different animal groups

All dorsal column lesions were verified at the end of electrophysiological experiments and found to be within the caudal half of the L3 segment. Furthermore, the distribution of the lesion sites did not differ systematically between animal groups (Table 2) and did not therefore contribute to differences in the CDPs and SEPs seen in the different groups. Lesion and transplant sites were also subsequently inspected histologically. An indication of the extent of the lesion cavity in non-transplanted animals and any gaps indicative of incomplete filling of the cavity in transplanted animals was obtained by counting the number of 70 µm sections in which these features occurred (Table 2).

Table 2 Incidence and location of lesion cavities

	Lesion and short survival	Lesion and long survival	Lesion and long survival + cell transplant
Position of lesion relative to L3/4 border (mm rostral)	+1.3	+1.1	+1.4
Proportion of animals with cavity	8/8, 100%	10/10, 100%	6/13, 46%
Cavity length (μm)	740–1050, mean 910 μm	840–>2000, mean 1335 μm	140–490, mean 292 μm

The table shows the mean location, relative to the L3/4 border, of the centre of the dorsal column lesions in the different animal groups investigated electrophysiologically. There is no systematic difference between groups. Also shown is the proportion of animals in each group for which a lesion cavity was detected histologically and an estimate of the length of the cavity from the number of transverse sections in which it was observed. Cavities could be seen in all of the lesioned animals which did not receive transplants but were longer in the long survival animals compared to the short, suggesting some expansion of cavity size with time. In contrast, less than half of the transplanted animals showed any visible form of cavity and, when present, they were much smaller than in the non-transplanted animals.

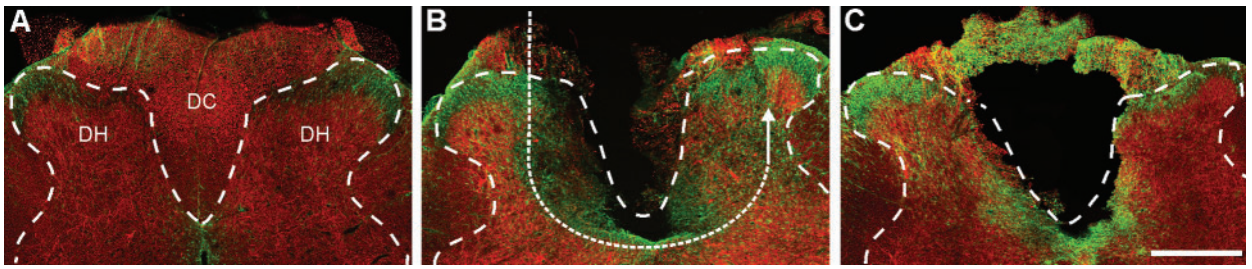

Fig. 8 Examples of wire knife dorsal column lesions. Confocal microscope images from transverse sections of spinal cord from the caudal L3 segment. The images show immunolabelling for neurofilament (red) and GFAP (green) in the dorsal half of the spinal cord. The dashed line outlines the border between the white and grey matter. (**A**) Shows a section from a normal animal while **B** and **C** show sections taken from close to the centre of the lesion in two dorsal column lesioned animals (survival times 84 and 88 days, respectively). The dotted line in **B** shows the dimensions and the positioning of the wire knife used to make the lesions. DH: dorsal horn; DC: dorsal columns. Scale bar 200 μm.

Cavities were seen in all of the lesioned animals which did not receive transplants (Fig. 8). However, the mean cavity length was greater in the long survival animals than in the short, suggesting some expansion of cavity size with time. In contrast, less than half of the transplanted animals showed any visible form of cavity or discontinuity within the transplant. Where small holes or gaps within the transplant could be seen, they were much smaller than the lesion cavities defined by the GFAP border in non-transplanted animals. Most of the transplanted animals in which small cavities were detected were transplanted with matrix.

Discussion

We show that pure neonatal OECs and mixed adult cultures containing OECs support axonal regeneration within a transplant, but not significant regeneration of dorsal column fibres beyond the transplanted lesion. We also show that, despite the absence of functional regeneration, transplants of olfactory cells enhance the function of the neuropil in the region of a spinal cord lesion and of pathways projecting to the sensorimotor cortex.

Axonal regeneration promoted by olfactory cell transplants: significance of different cell types and transplant method

Although it is clear that regeneration of various axon types occurs within OEC transplants, there are contradictory reports on the extent of regeneration beyond a transplanted lesion, including lesions of the dorsal columns. Imaizumi *et al.* (2000*a*, *b*) describe electrophysiological evidence for regeneration across a dorsal column lesion transplanted with acutely prepared neonatal OECs from rats and transgenic pigs. However, Andrews and Stelzner (2004) showed using a tract tracing approach that there is minimal growth of dorsal column axons beyond lesions transplanted with adult mixed and OEC-enriched cultures and Lu *et al.* (2006) have recently failed to see regeneration across transplants of pure OECs prepared from the lamina propria. Our results are in agreement with these latter anatomical studies since we saw no significant regeneration across dorsal column lesions either using BDA (which is estimated to label one-third of DRG neurons when injected into the ganglion; Novikov, 2001), or with CTB (which labels most myelinated fibres). This was despite the lesions being at a lumbar level, close to the normal termination sites of the lesioned fibres, which should

maximize the contribution of any target derived signalling to regeneration. The electrophysiological results obtained by Imaizumi *et al.* (2000*a*, *b*) may be explained by stimulus spreading from below the lesion to excite fibres above the lesion, since stimuli were applied very close (1 mm) to the lesion site.

The methods used to obtain, prepare and transplant olfactory cells vary widely between laboratories and this could potentially contribute to contradictory results on the extent of axonal regeneration (see section 'Introduction'). One laboratory consistently reporting long-distance regeneration uses transplants of OECs mixed with fibroblast-like cells prepared from the adult olfactory bulb and considers this mixture of cells necessary for successful regeneration (Raisman, 2001). In the present study, although it appeared that mixed adult cultures might support more regeneration within the transplant than pure neonatal OECs, neither resulted in significant regeneration beyond the transplanted lesion. These and other results in the literature (see Discussion of Riddell *et al.*, 2004) suggest that the source and composition of transplanted olfactory cells are not crucial in determining whether successful long-distance axonal regeneration is obtained.

We used two methods to transplant mixed adult cells: injection of a cell suspension and matrix transfer (Li *et al.*, 2003). Cells transplanted by both methods survived and integrated into the lesion site, but cells transferred in a matrix filled the lesion cavity less effectively at the transplant–lesion interface. This may be because suspended cells can flow freely within the lesion cavity and form a more closely apposed graft–host interface than the more solid pieces of matrix.

Effects of dorsal column lesions on the electrophysiology of primary afferent pathways

Dorsal root-evoked CDPs below the level of the dorsal column lesion were profoundly depressed within days of lesioning. This was an unexpected finding because the branches of L4 and L5 dorsal root fibres terminating in the L4/L5 segments are not directly damaged by lesioning at the L3/4 level (Fig. 5A) and CDPs over the L4/5 segments are relatively normal in amplitude immediately after lesioning (A. Toft, D. T. Scott, J. S. Riddell, unpublished observations). The potentials were depressed at up to 6 mm from the lesion (Fig. 6A and B) and it seems improbable that disruption of blood supply or inflammatory products would explain effects at this distance. These observations therefore raise the possibility that axotomy of the main ascending branch of primary afferent fibres in the dorsal columns triggers changes in the fibre that affect the efficacy of transmission at synapses formed by their intact collaterals in more caudal segments (Fig. 5A). Other long pathways in the spinal cord have a similar branched morphology, including the corticospinal tract, and it will therefore be interesting to determine whether they are affected in a similar way. The depressed CDPs showed some spontaneous recovery with time since 2–3 months after lesioning, root-evoked potentials below the lesion approached normal amplitudes at some locations. However, recovery was not seen close to the lesion where progressive secondary damage may have occurred.

Dorsal column lesions abolished the short latency positive component of SEPs mediated by the ascending dorsal column pathway. These did not recover in long survival animals and were not restored by transplants of OECs. However, a longer latency positive component was unmasked after lesioning. The pathway responsible for this is not certain. Preliminary evidence suggests that it may travel in the lateral white matter since in two experiments, unilateral lesions of the dorsolateral funiculus reduced its amplitude by half and bilateral lesions virtually abolished it (A. Toft, D. T. Scott, J. S. Riddell, unpublished observations). However, we cannot entirely rule out the possibility that a few spared fibres travelling around the edge of the lesion contribute to these potentials. Both the longer latency positive component and a later negative component of the SEP were larger in long compared to short survival animals. The increase in amplitude of these potentials might involve plasticity in the brain but is more likely explained by more effective activation of the postsynaptic ascending tract neurons in the spinal cord that are the origin of the potentials (Fig. 7A), as reflected by the larger CDPs. Similarly, the slightly larger CDPs recorded above the dorsal column lesion in transplanted animals are probably explained by more effective activation of propriospinal neurons with cell bodies below the lesion and axons projecting rostrally through intact white matter of the lateral or ventral funiculi (Fig. 5A).

Enhanced function promoted by olfactory cell transplants and the possible mechanisms involved

The lesion made using the wire knife is designed to ensure complete destruction of the dorsal columns (necessary for the anatomical assessment of regeneration) and therefore inevitably encroaches on the adjacent grey matter—primarily of the medial dorsal horn (Fig. 8). The wire knife creates a gap in the spinal cord which we show by histology performed at short (<1 week) and long (up to 3 months) survival times, expands with time. This process likely leads to death of some dorsal horn neurons and die back of primary afferent fibres in the dorsal columns, such that they form fewer collateral branches in the dorsal horn and make fewer connections with dorsal horn neurons. The electrophysiological assessment we have performed involves stimulation of dorsal roots which produces impulses in afferent fibres and leads to activity in the dorsal horn neurons onto which they connect. Using this procedure we show that less activity is evoked in dorsal horn neurons in

the region of the injury in dorsal column lesioned compared to normal animals but that there is significantly greater activity if the lesion is transplanted with OECs. The anatomical findings of this study show that regeneration of axons across the lesion is unlikely to explain the enhanced potentials detected above the injury at spinal and cortical levels, and such regeneration is irrelevant to the enhanced CDPs produced by the intact branches of L4/L5 dorsal root fibres below the lesion (Fig. 5A). Two alternative mechanisms might explain the enhanced function. The transplants could induce some form of plasticity; local sprouting of primary afferent collaterals or increased synaptic efficacy at their connections with spinal cord neurons, or they could exert a neuroprotective effect, limiting progression of the secondary damage that occurs over time in the neuropil surrounding the injury.

There is currently little evidence that OEC transplants promote sprouting of intact fibres. Chuah *et al.* (2004) reported that when the main component of the corticospinal tract is interrupted by a dorsal column lesion, the minor component projecting through ventral white matter has more collateral branches near an OEC transplanted compared to non-transplanted lesion. This was interpreted as evidence for sprouting of the ventral corticospinal component. However, since the number of collateral branches in transplanted animals was not significantly greater than normal, an equally valid interpretation would be that the transplants prevent a loss of branches that otherwise occurs in control lesioned animals. Several sets of observations suggest that transplants of OECs might be neuroprotective. OECs from both bulb and mucosa are reported to reduce cavity formation at contusion and transection injuries (Takami *et al.*, 2002; Ruitenberg *et al.*, 2003; Ramer *et al.*, 2004*a*, *b*). OECs in culture are reported to produce a number of substances that might contribute to a neuroprotective effect, including both neurotrophic (Woodhall *et al.*, 2001; Lipson *et al.*, 2003) and angiogenic factors (Au and Roskams, 2003). Consistent with the latter, OEC transplants have been shown to promote angiogenesis in and around transection and photochemical lesions of the spinal cord (Lopez-Vales *et al.*, 2004; Ramer *et al.*, 2004*a*, *b*). It has also recently been reported that OEC transplants protect corticospinal tract neurons with fibres projecting to a transplanted lesion (Sasaki *et al.*, 2006). A neuroprotective action is therefore the most likely explanation for the improved electrophysiological function that we see in our transplanted animals.

Our results raise the possibility that the same mechanisms that underlie the enhanced electrophysiological function in transplanted animals might explain the improvements in sensorimotor function reported by some following behavioural testing of transplanted animals (Li *et al.*, 1997; Ramon-Cueto *et al.*, 2000; Plant *et al.*, 2003). In a clinical context, the results suggest that olfactory cell transplants could be useful in preserving function within the zone of partial preservation surrounding a spinal cord injury and might therefore ameliorate the degree of disability that results. It will therefore be interesting to determine whether the enhanced function demonstrated in this study following acute transplantation is maintained in a delayed transplant paradigm.

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