

Medical sciences / Sciences médicales

# Repair of spinal cord injury by transplantation of olfactory ensheathing cells

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Received 22 December 2006; accepted after revision 23 March 2007

Available online 9 May 2007

Presented by Jean-François Bach and Nicole Le Douarin

## Abstract

Repair of spinal cord injury requires that severed axons are able to regenerate. Regrowth of axons is impeded by the loss of astrocytic pathways caused at the time of injury. Ensheathing glial cells cultured from the adult olfactory system can be transplanted into lesions and mediate both regeneration of axons and recovery of function. **To cite this article: G. Raisman, C. R. Biologies 330 (2007).**

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**Keywords:** Adult; CNS; Axotomy; Regeneration; Paraplegia; Stem cells

## 1. Introduction

There are two major ways in which the brain and spinal cord (the ‘central nervous system’, the CNS) can be damaged. Both are currently incurable. One is where nerve cells are lost, due to degeneration or injury. This accounts for conditions such as Alzheimer’s disease, motoneuron disease, Parkinson’s disease, Huntington’s disease and many kinds of head injury and stroke. The other is an injury peculiar to the nervous system: it occurs because the nerve cells give rise to long fibres that convey information to the next nerve cells in the circuit. If, during their course to their destination, these nerve fibres are interrupted, the information transfer is disabled, and function is lost, even though the cell bodies survive. The challenge is to make those cell bodies regenerate their fibres in such a way as they can return to desti-

nations that can enable function to resume. This paper will deal with the repair of nerve fibre injury (sometimes called ‘axotomy’) by transplantation of adult olfactory stem cells in rats, and the steps we are taking for its application to humans.

## 2. Spinal cord injury

Nerve fibre injury is seen at its most dramatic in spinal cord injury, and is perhaps most visible because it most often affects young active people with their lives ahead of them. Although spinal injured patients have a life expectancy equal to that of the uninjured population, they suffer a loss of control of movement, and sensation, as well as losing control of other bodily functions such as bladder, bowel, sexual function, and control of body temperature and blood pressure. They are confined to wheel chairs, and become dependent on carers for their most intimate bodily needs. According to the level of

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the damage, either the legs alone may be affected, or with progressively higher levels of injury (i.e. closer to the neck), function of the arms and hands may be lost, and the patient may not be able to breathe without assistance.

Nerve fibre injury is also seen in spinal root lesions, where the nerve fibres are torn out of the spinal cord, and in some of the most severe forms of stroke (affecting the bottleneck of fibres coming down from the cerebral cortex), as well as in blindness due to damage to nerve fibres (including glaucoma), and deafness due to damage to the auditory nerves, often during surgical removal of tumours. Discovery of how to repair nerve fibre injury in one of these situations will elucidate principles that are likely to lead to methods for repair of injury in the other situations.

Spinal cord injuries have received the most experimental attention. The loss of function is almost entirely the result of damage to long fibre pathways travelling in both directions between the spinal cord and the brain. The injury is one of disconnection of one group of nerve cells from another. Direct destruction of nerve cells in the spinal cord plays a lesser role in the overall pattern of disabilities. There are basically two (non-exclusive) approaches to repair of nerve fibre injuries in the spinal cord. The first concentrates on the ability of severed nerve fibres to grow, and provides them with stimulatory substances or removes inhibitory molecules from their path. It predicts basically a drug-related therapy. The second looks at the glial pathway along which nerve fibres need to grow, and attempts to reconstruct this pathway by a transplantation approach. This article will deal with the second of these.

### 3. Transplantation

The idea of transplantation arose from the observations firstly, that nerve fibres grow during embryonic life, and secondly that nerve fibres severed in the adult peripheral nervous system (the nerves of the limbs and body outside the spinal cord and brain) are able to regenerate for long distances. Furthermore, cut nerve fibres in the adult brain and spinal cord also sprout, often profusely in the region of the injury. The failure to repair is due to the inability of these sprouts to elongate and retrace their original pathways to reach their former destinations [1]. Such observations focus attention on the question of why the pathway is not permissive for regeneration of nerve fibres. They also led to the idea that cells transplanted from either embryonic nervous tissue, or from peripheral nerves (in both situations of which nerve fibres can grow) into areas of damage in

the adult brain and spinal cord might transfer with them the power to provide pathways for the growth of severed adult central nerve fibres.

In general, the transplantation of embryonic tissue has not given very promising results for encouraging adult nerve fibre growth. However, the transplantation of pieces of peripheral nerve or Schwann cells cultured from peripheral nerve (Schwann cells make up the glial pathway of peripheral nerves) shows a striking induction of growth of severed brain and spinal nerve fibres [2,3]. Such experiments indicate that the failure of regeneration is in some way related to their environment, and show that transplantation of pathway cells can induce growth. The problem with Schwann cells is that although they induce growth of cut nerve fibres out of the brain or spinal cord into the transplant, those fibres stop at the end of the transplant, they do not leave the transplant and re-enter the CNS, and therefore they cannot restore function.

### 4. Olfactory system

In this impasse, the breakthrough came from an unexpected quarter. In the 1970s, with the advent of labelling techniques for identifying dividing cells in tissue, it was found that there is one part of the adult nervous system in which nerve cells are in a state of continual turnover, and there are stem cells which can entirely replace the population if lost [4]. Current investigations suggest that this stem cell population consist of the so-called dark horizontal basal cells located in pockets in the deepest part of the olfactory mucosa immediately adjacent to the underlying basal lamina [5,6]. This is the olfactory mucosa. Since the nerve cells in the olfactory mucosa send their fibres through the floor of the skull and into the olfactory bulbs of the brain, this means that these nerve fibres are capable of regenerating along the whole of their course, reaching their correct destinations in the brain, and maintaining normal function.

The glial cells making up the pathway along which the olfactory nerve fibres travel are known as olfactory ensheathing cells (OECs). Once these cells had been identified and cultured, it became possible to test whether transplanting them into areas of damage in the spinal cord might also – like Schwann cell transplants – show the property of inducing growth of cut nerve fibres. However, perhaps, in addition, transplants of OECs might also allow the regenerating fibres to re-enter the host spinal cord in the same way that they permit entry of olfactory nerve fibres into the olfactory bulb. These predictions were indeed justified by obser-

vations, and the transplantation of cultured OECs has now been shown to restore both connections and function in a number of different experiments with spinal cord injury [7–10]. Transplantation of cultured adult OECs is currently one of the most promising routes for repair of human spinal cord injury, and in the remainder of this article, I will summarise some of our own experimental work on the transplantation of these cells in adult rat spinal cord.

## 5. Transplantation of olfactory ensheathing cells

We have transplanted cultured olfactory ensheathing cells into complete unilateral lesions of the upper cervical corticospinal tract in adult rats [11]. We found that the grafted cells encourage the growth of the cut nerve fibres, and suppress the excessive neuromatous branching found in untreated lesions. The grafted cells take up an elongated shape, and align to form a bridge between the ends of the cut fibre tract. The regenerating nerve fibres enter the graft and cross this new bridge. Within the bridge, the nerve fibres are intimately ensheathed by the Schwann-like cells, and enclosed in an outer, perineurial-like sheath of fibroblasts. Once they reach the end of the graft, they re-enter the host spinal cord, and continue along the distal part of the corticospinal tract to form terminal arborisations in their normal target areas. During their course through the transplant, the fibres are myelinated by peripheral myelin formed by the Schwann-like cells, and when they re-enter the spinal cord, they are myelinated by host oligodendrocytes. The effect is to put a patch over the lesion, restoring the integrity of the original pathway, and results in the functional recovery of a learned forepaw retrieval task.

This repair can be achieved by transplanting at two months after the time of injury in rats at which time the original wound is fully scarred over by astrocytic processes [12].

To study the repair of a larger lesion, we carried out a second series of investigations [13] with complete hemisection of one half of the spinal cord at the upper cervical level. These lesions result in a deficit in the placing of the ipsilateral forepaw during a climbing test, and the disappearance of the supraspinal respiratory rhythm in the ipsilateral phrenic nerve and hemidiaphragm. A specific matrix transfer method was used to achieve the efficient transfer of cells and their retention in these much larger, open cavities. Both the climbing and the respiratory functions were restored after transplantation of cultured OECs.

In experiments on the repair of avulsed dorsal and ventral spinal roots in collaboration with Thomas Carlstedt, Royal National Orthopaedic Hospital Trust, Stanmore, we have shown that the matrix transfer method of transplanting OECs allows major regeneration of severed dorsal root axons into the spinal cord, where they both form terminal fields in the dorsal horn and also long regenerating axons ascend in the dorsal columns [14]. In the ventral roots, the transplanted OECs give a 4–5 fold increase in the number of fibres regenerating from the spinal cord into the root [15].

In experiments with the severed optic nerve, we found transplanted OECs induce regeneration of cut adult retinal ganglion cell axons [16], although in this case the loss of the astroglial pathways in the distal stump of the nerve make further penetration of the regenerating axons impossible.

These observations, and those from several other laboratories, suggest that transplantation of adult OECs is a promising method for repair of nerve fibre injuries in the spinal cord, and may well be effective also for repairing nerve fibre damage in other sites in the brain and cranial nerves. The development of methods for culturing OECs from biopsy samples taken under local anaesthesia from the adult human nasal lining opens the way to use of these cells in an autograft procedure where the patient will act as his/her own donor for repair of spinal cord or spinal root injuries.

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