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EDITORIAL

Stellar Performance in the Offing

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Repair of damaged brains and spinal cords, ranks as one of the most compelling problems that science can solve. The needs are immense and urgent, and the outlook is now realistic.

For the past two centuries medical neurologists have said that brains do not repair themselves. People have been trapped inside paralysed bodies, or enfeebled by stroke or degenerative disease with no hope of resuming normal functions. But, on closer examination, damaged central neurons do regrow and even proliferate after damage. The problem comes from crossing the scar tissue and voids left by the lesion, and finding and recognising

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Repairing Damaged Brains and Spinal Cords: Crucial Roles for Olfactory Ensheathing Cells

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Injuries of the nervous system often cause devastating long-term impacts on patients. In particular the central nervous system has a very limited capacity to regenerate after injury such that spinal cord injuries often result in permanent loss of function. The main factor hampering neuronal regeneration after injury is the inability of regenerating neurons to reach their target. It is therefore important that we determine the best approaches for developing neural regeneration therapies. One approach is to transplant glia, which are supporting cells crucial for the survival and growth of all neurons.

Olfactory ensheathing cells (OECs) are the glia of the olfactory system and populate the olfactory nerve and outer layers of the olfactory

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their former targets. The new cells set out, as it were, without steering or a road map to their destination.

Now things are starting to move. Olfactory ensheathing cells are being studied as a means of providing missing mechanisms for bridging scar tissue and either pulling growing nerves together in bundles or spreading them. This is an important step forward. The inspiration comes from the daily turnover and targeting of olfactory receptor cells from the nasal epithelium, whose regenerating nerve fibres (axons) travel from the nasal cavity, up through the skull's cribriform plate in bundles and onto the surface of the olfactory bulb, where they spread out and find their correct connections among thousands in the spherical glomeruli of the "smell retina".

It is a privilege to have edited this issue of ChemoSense, containing one of the most important papers we have ever carried: the review by Jenny Ekberg and James St John, proving several crucial roles for olfactory ensheathing cells in central neuronal regeneration.

The way is being opened for the release of so many accident and stroke victims from their hellish prison of paralysis and disability. Olfactory science is on centre stage for a stellar performance ■

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bulb. The OECs have properties that are thought to underlie the unique ability of the peripheral olfactory nervous system to continuously regenerate itself, and allow olfactory sensory axons to constantly breach the border between the peripheral and central nervous systems. It is for these reasons that transplantation of OECs to injury sites in other regions of the nervous system are being trialled for neural repair therapies. While there have been some very promising results, the neuroanatomical and functional outcomes are variable and improvements need to be achieved before effective therapies are developed for humans. Understanding the biology and function of OECs in more detail is likely to lead to better use of OECs for neural regeneration therapies.

Why are OECs suitable for neural repair therapies?

Following injury to the central nervous system, axons often sprout and regrow but cannot reach their targets due to inflammation, inhibitory cues or physical barriers. Neural repair therapies need to address these problems to create an environment that is conducive for axon growth. Glial cells are supporting cells crucial for the survival and growth of all neurons and one approach for neural repair is to use transplanted glial cells to help stabilise the injury site and to provide an amenable cellular bridge for the axon growth.

The majority of trials using glial cells have used Schwann cells (peripheral glia), OECs, or combinations of both. Schwann cells enwrap and myelinate individual axons after transplantation [1], but do not integrate well within the central nervous system [2-3]. OECs on the other hand wrap up groups of unmyelinated axons and can integrate well in both peripheral nervous system (PNS) and central nervous system (CNS), since they are naturally present in the primary olfactory nervous system at the PNS-CNS interface. It is possible that transplanted OECs are able to myelinate axons in the right conditions [4] but other studies suggest that it does not occur [1].

OECs populate the olfactory nerve and outer layers of the olfactory bulb (Fig. 1) and unlike other glia, they are able to migrate from the PNS into the brain. It is for these reasons that OECs are considered to be good candidates for use in neural repair therapies.

Transplanted OECs as a therapy for neuronal injuries

OECs have been trialled for neural regeneration with some promising results. In humans, a Phase I clinical trial of spinal cord injury repair demonstrated that transplantation of OECs obtained from the patient themselves (autologous transplantation) is feasible and safe [5-6]. In preclinical animal trials it has been shown that transplanted OECs can survive and migrate within the injured spinal cord of rats [7-8], reduce scar and cavity formation [9-10], lead to improved functional locomotor and hindlimb recovery [11-12] and can restore breathing and climbing ability [13] even when complete transections of the spinal cord have occurred [12, 14]. Similarly, Schwann cells have been shown to promote axon growth in spinal injury models [15] although they are not always as effective as OECs [16]. In other CNS regions transplanted OECs can migrate along the optic nerve and ensheathe retinal ganglion cell axons [17] and restore sensory input in brachial plexus injuries [18].

The use of growth factors and other cell types in combination with glia has also

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continued

been trialled. In an optic nerve injury model, OECs together with soluble glial-derived neurotrophic factor (GDNF) have been shown to enhance axon regeneration although the mechanism by which GDNF acted was not investigated [19]. Alternatively, OECs can be genetically engineered to express GDNF to promote nerve repair [20]. The integration of transplanted glia and their interaction with other cells is also a crucial consideration. Within the olfactory system, OECs together with fibroblasts form channels through which axons extend [21] and fibroblasts promote the proliferation of OECs in the olfactory bulb [22]. When OECs have been transplanted together with olfactory nerve fibroblasts they formed an effective bridge for growth of axons across an injured corticospinal tract [23] and enhanced regeneration of nigrostriatal dopaminergic axons [24]. Transplantation of OECs has also been shown to stimulate endogenous Schwann cells [10, 25]. These results highlight the importance of determining the role of OECs within a multicellular environment.

Purification of OECs for transplantation

The variations in the neuroanatomical and functional outcomes in the different neural repair models can be attributed in part to the source of the OECs and the purification methods used in the trials. Not surprisingly, each laboratory optimises their own dissection, purification and culturing protocols with the result that there are almost as many different preparations of OECs as there as models of neural injury into which the OECs are transplanted. For example, OECs can be purified from either the peripheral or the central nervous system regions of the olfactory system [26]. "Central OECs" used for transplantation therapies are often acquired from the entire nerve fibre layer of the olfactory bulb [27-29]. However, other studies have used central OECs without specifying the topographical locations from which they were obtained [24] or have used a more restricted population of central OECs such as the rostral region of the olfactory bulb [30] or the ventral olfactory bulb [31]. "Peripheral OECs" from the lamina propria underlying the olfactory epithelium lining the nasal cavity have also been used [10] particularly as they have relevance to human trials [6] due to their accessibility within the nasal cavity.

Isolating OECs from the mucosa or bulb requires separation from the many potential "contaminating" cells. However, it may be that OECs in combination with other cells provide superior outcomes. When OECs have been transplanted together with olfactory nerve fibroblasts they formed an effective bridge for growth of axons across an injured corticospinal tract [23] and enhanced regeneration of nigrostriatal dopaminergic axons [24]. Transplantation of OECs has also been shown to stimulate endogenous Schwann cells [10, 25] and the interaction of fibroblasts directly altered Schwann cell migration and organisation [32]. Thus not only do we need to consider the source of the OECs but also their interactions with other cells.

Further complicating our understanding of the results is the fact that OECs do not consist of a uniform population of cells but instead there are several subpopulations of cells each with distinct characteristics that are likely to affect axon growth in different ways. The different subpopulations of OECs that are used may explain in part the variations in outcomes of the different trials. To understand the differences amongst the subpopulations of OECs it is important to revisit their roles within the olfactory systems.

OECs promote growth, guidance and regeneration of olfactory axons

The primary olfactory system is one of the few regions within the mature vertebrate nervous system to exhibit continual turnover of neurons and a capacity for axon growth and regeneration throughout life. Throughout development and adult life, neurogenesis continues to occur in the olfactory epithelium in the nose where stem cells in the basal layer of the epithelium proliferate to generate new sensory neurons [33-35]. In normal healthy animals, when the neurons reach the end of their life-span of 1-3 months, the neurons undergo apoptosis and are replaced by new neurons originating from progenitors in the nasal mucosa. Thus, 1-3% of olfactory neurons turn over daily. In an injury model, severing the olfactory nerve leads to apoptosis of the sensory neurons in the epithelium which are then replaced quickly by new neurons arising from enhanced neurogenesis as demonstrated in many studies, including in primate [33, 36]. In both healthy animals and following widespread degeneration, the stem cells lining the basal layer of the olfactory epithelium give rise to new neurons that extend axons from the PNS into the CNS.

The remarkable ability for continual axon growth and successful targeting is attributed to the presence of the glia associated with the olfactory nerve, since the OECs accompany the axons from the epithelium through to the olfactory bulb in the brain [37]. Each neuron expresses one of ~1000 odorant receptors and as the neurons mature, they project their dendrites to the apical side of the epithelium where odorant detection

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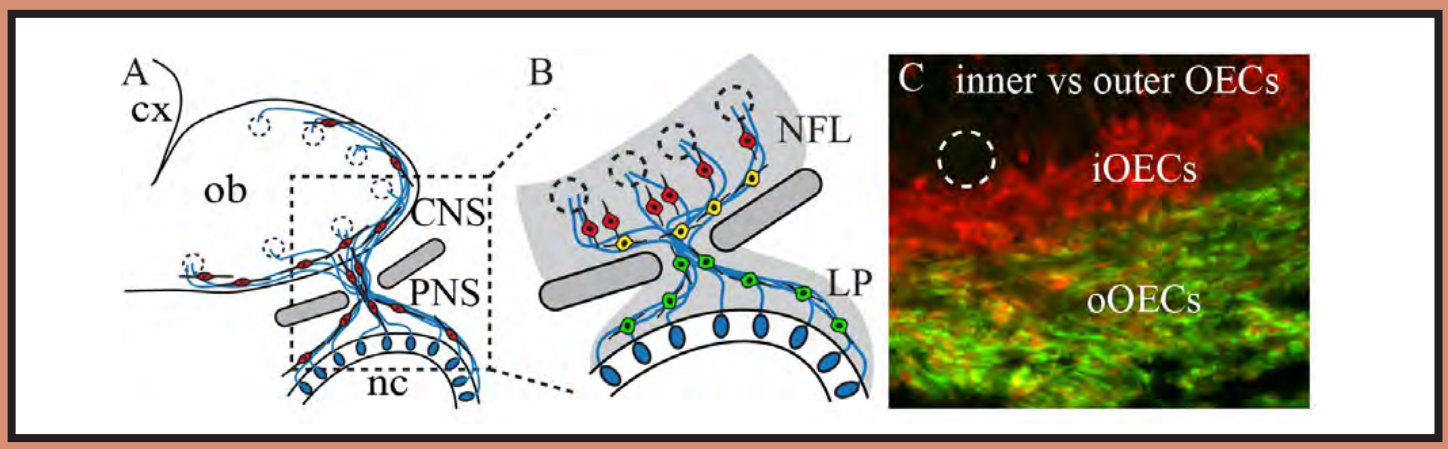

Fig. 1. Localisation of OECs in the primary olfactory nervous system.

(A) Schematic of the olfactory bulb with rostral to the right; cortex (cx), nasal cavity (nc). Olfactory neurons send their axons (blue) to glomeruli (dashed circles) in the olfactory bulb (ob) and are accompanied by OECs (red) from the PNS into the CNS. (B) Peripheral OECs (green) in the lamina propria (LP) contribute to fasciculation. In the nerve fibre layer (NFL) the central OECs consist of two further subpopulations. The outer OECs (yellow) are present where axon defasciculation occurs whereas the inner OECs (red) are present where axon refasciculation and targeting occur. (C) In S100ß-DsRed mice, the outer and inner layers of the nerve fibre layer are clearly seen. The outer OECs (oOECs) express DsRed and p75ntr (green and red), whereas the inner OECs (iOECs) express DsRed but not p75ntr.

takes place. The axons extend through the basal side of the neuroepithelium and towards the olfactory bulb in the brain. The axons form bundles that converge into larger fascicles which constitute the olfactory nerve. The fascicles consist of mixed axon populations expressing distinct types of odorant receptor, which are destined to terminate in individual glomeruli within the olfactory bulb depending on their odorant receptor profile. The fascicles enter the central nervous system by transecting the cribriform plate. When the axons reach the outermost layer of the olfactory bulb, the nerve fibre layer, they defasciculate. This region of the nerve fibre layer where the defasciculation occurs is the *outer nerve fibre layer*. The axons then extend towards their targets and sort out such that axons from neurons that express the same odorant receptor come together and refasciculate with other axons expressing the same odorant receptor type [38]. This refasciculation occurs in the *inner nerve fibre layer*. The newly formed fascicles of axons of the same odorant receptor type then project to their appropriate glomerulus in the glomerular layer, where they synapse onto second order neurons.

OECs are in close association with the olfactory sensory axons from the epithelium to the nerve fibre layer of the olfactory bulb. Within the peripheral regions of the olfactory nerve, the OECs ensheathe the fascicles of mixed primary olfactory axons that project from the olfactory epithelium to the olfactory bulb and are thought to be crucial for the growth, guidance and survival of olfactory axons as they extend towards the olfactory bulb [39]. Within the outer nerve fibre layer, the OECs are thought to contribute to the defasciculation of the mixed bundles of axons. Within the inner nerve fibre layer the OECs are thought to contribute to the sorting and refasciculation of axons of the same odorant receptor type. Thus the OECs in the peripheral region are likely to contribute to adhesion and fasciculation of the axons, whereas the OECs within the nerve fibre layer are likely to contribute to the complex sorting of axons within this layer [40].

Further complicating our understanding of the differences of the subpopulations of OECs is the fact that the nerve fibre layer is not a uniform structure but varies considerably with the anatomical location. The rostral and ventral nerve fibre layer is relatively thick with distinct inner and outer layers, whereas the nerve fibre layer in the dorsal and caudal regions is thin and without the distinct layers observed in the rostral and ventral nerve fibre layer. Most of the defasciculation and sorting of axons occurs when the axons first enter the rostral and ventral nerve fibre layer [40-41]; axons in the dorsal and caudal nerve fibre layer are largely already sorted and are projecting to their target glomeruli [42]. Further, the olfactory bulb develops in a rostral-caudal gradient with the rostral regions developing first. For

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continued

example, P2 odorant receptor glomeruli which are located in the ventral and rostral halves of the olfactory bulb develop from embryonic day 18 onwards [38, 43], whereas the M72 odorant receptor glomeruli which are located in the dorsal and caudal halves of the olfactory bulb develop from postnatal day 3 onwards in the mouse [44]. Thus the nerve fibre layer is not a uniform structure and OECs within the different regions are likely to have different functions particularly during development.

OECs are a heterogeneous population of cells

OECs from the olfactory nerve and olfactory bulb are not identical. Mucosal OECs express several antigens not expressed by bulb OECs [45]; they differ in their ability to grow in vitro [46], and they differ in their reparative effects when transplanted into the injured spinal cord [47]. Apart from the functional differences alluded to above, the OECs within the nerve fibre layer exhibit clear heterogeneity in the expression of several markers. The OECs of the outer nerve fibre layer strongly express S100 and p75 neurotrophin receptor (p75ntr) [40], whereas the OECs of the inner nerve fibre layer express low or negligible levels of S100 and do not express p75ntr at all [40]. In addition, the OECs of the inner nerve fibre layer express neuropeptide Y (NPY) whereas the OECs of the outer nerve fibre layer do not [48].

OECs also show heterogeneity when growing in vitro [49]. It is now established that this heterogeneity is reflective of plasticity in morphology and antigen expression that can be manipulated by growth conditions [50-51]. When monitored with time-lapse imaging, cells of each morphology are seen to change into the other during the course of an hour in different growth medium [50]. The most spindle-shaped OECs migrated three times faster than the most flattened OECs [50] so morphology may reflect a different functional state. Using the fluorescent reporter transgenic mice, S100ß-DsRed mice, we have recently purified populations of OECs to assess their behaviour [52-53]. We compared peripheral OECs and central OECs (containing both iOECs and oOECs) as it is technically very difficult to separate the inner and outer nerve fiber layer. We found that OECs from the peripheral olfactory nerve predominantly adhere to each other and migrate close together (Fig. 2A), whereas OECs from the central olfactory bulb display a mix of adhesion and repulsion responses and are only loosely associated (Fig. 2B). These findings are consistent with the proposed role of OECs in vivo: cell-cell adhesion between peripheral OECs would lead to formation of conducive fascicles, whereas the mixed behaviour displayed by central OECs possibly reflects complex sorting. We also further confirmed that within the central OEC population there are subpopulations of OECs with distinct behaviour as cells derived from different parts of the bulb displayed individual characteristics [53]. The proportion of cells demonstrating these behaviours varied depending on the region of the olfactory bulb (dorsal/ventral, rostral/caudal) from which they were derived [53]. We also noted functional differences in the OECs from the olfactory bulbs of embryonic, postnatal and adult olfactory bulbs [53]. Thus it is clear that OECs are not a uniform population of cells, but instead consist of at least three subpopulations each with distinct antigenic expression profiles and behavioural differences during cell-cell contact which are consistent with the proposed roles of the subpopulations of OECs in the guidance of olfactory sensory axons.

To study the behaviour of olfactory neurons in the presence of different subpopulations of OECs, we developed a mouse model in which primary olfactory neurons express the fluorescent protein ZsGreen (OMP-ZsGreen mice) [54]. We found that the presence of OECs is essential for growth of primary olfactory neurons in vitro [55]. Importantly, we showed that olfactory neurons behaved differently when co-cultured with peripheral and central OECs. In the presence of peripheral OECs, axons formed fascicle-like structure (Fig. 2A). In contrast, when co-cultured with central OECs, axons extended in a dispersed manner (Fig. 2B) [53]. Again, these results support the proposed in vivo roles of peripheral OECs mediating fasciculation and central OECs mediating sorting/defasciculation.

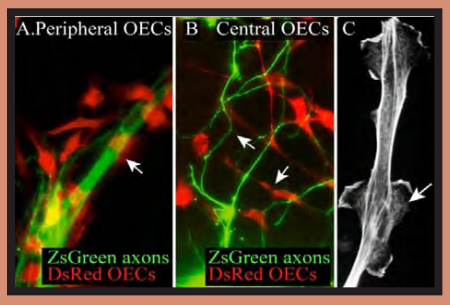

Fig. 2. Peripheral and central OECs migrate and regulate axonal growth differently. (A) OECs (red) from the peripheral nerve migrate in close association with each other. Olfactory axons (green) that grow on the peripheral OECs form tight fascicles. (B) OECs from the olfactory bulb are loosely associated and axons that grow on these central OECs are more dispersed. (C) Lamellipodial waves (arrow) are crucial for mediating cell-cell contact.

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Organised OEC migration dramatically enhances axon regeneration.

In experimental models of spinal cord injury, implanted OECs have been shown to migrate considerable distances from the site of injection [56-57]. Thus, OECs display clearly unique migratory properties, but what are the mechanisms by which OECs migrate and how can the migration of OECs be optimised for transplant therapies? During development of the olfactory system, OECs have been reported to migrate slightly ahead of the primary olfactory axons en route to the olfactory bulb [58]. This is in contrast to Schwann cells which migrate along already defined axonal pathways during development of the peripheral nervous system [59]. The OECs are thought to promote axon growth by providing a cellular substrate containing molecules that facilitate axonal adhesion and extension and by expressing growth-promoting agents such brain derived neurotrophic factor, glia-derived nexin and nerve growth factor [7, 60-64]. These properties also make OECs good candidates for use in neural repair therapies.

We have investigated how the migration of OECs influences axon extension during regeneration. When unilateral bulbectomy was performed followed by administration of methimazole to delay axon regeneration, the OECs migrated into the injury site ahead of the axons [65]. This resulted in the formation of a permissive glial environment that not only dramatically enhanced the growth of olfactory axons, but also resulted in axons projecting in well-formed fascicles and terminating together in distinct well-formed structures. In comparison, in bulbectomized animals where axonal growth was not delayed, there was limited growth of axons and while the

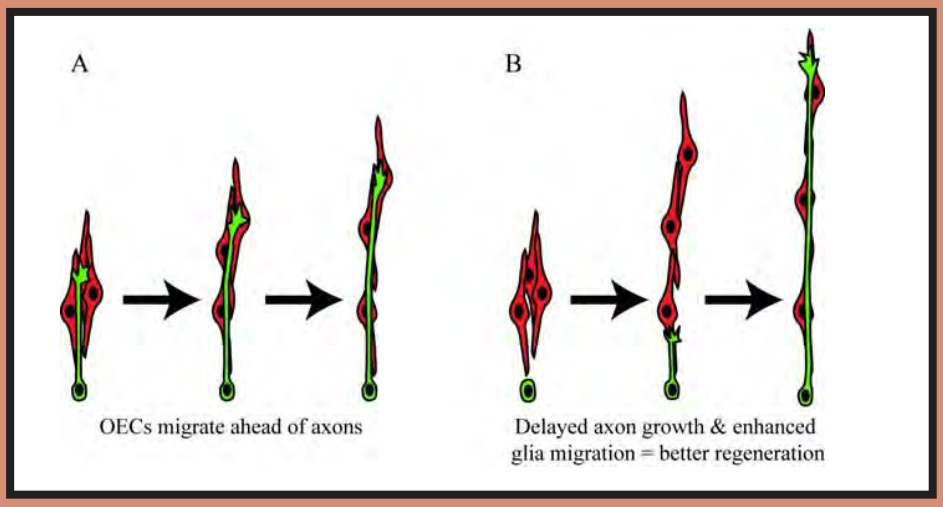

Fig. 3. *(A) OECs (red) migrate ahead of axons (green) during normal regeneration. (B) By delaying axon growth, the migration of glia is enhanced leading to a subsequent dramatic increase in axon extension (Chehrehasa et al., 2010).*

axons did form some fascicles they were less distinct. Thus, enhancing OEC migration subsequently resulted in superior axon growth and fascicle formation (Fig. 3).

In spinal injury transplant therapies, OECs have been introduced as cell suspensions or in matrices into the injury site where they disperse and integrate with the host tissue [7, 66-67]. However, unfortunately they do not maintain a high degree of cell-cell contact with each other and therefore do not form a continuous uniform mass. Instead the OECs tend to be interspersed amongst other cells and remain in close association with axons [67-68]. Our results have demonstrated that in the absence of axons the OECs rapidly formed an extensive uniform mass and that the presentation of OECs in this format resulted in subsequent superior axon growth [65]. It would therefore be of interest to examine whether the delivery of OECs that would encourage their migration as a uniform mass within spinal transplant models would result in improved axon growth. To achieve this we need to better understand the mechanisms that regulate OEC migration.

Traditionally, cell migration has thus far been attributed mainly to the activity of the leading edge searching its environment, establishing adhesion followed by movement of the cell body and retraction of the cell rear [56, 69-70]. Using S100•DsRed OECs which fluoresce brightly red, we have studied the behaviour of the cells using high-resolution time-lapse microscopy. We made a break-through discovery in finding that OECs display highly motile lamellipodial protrusions along the shaft and cell body (Fig. 2C), which significantly increase the migration of peripheral OECs in vitro (Fig. 2b) [52]. These lamellipodial waves primarily induced cell-cell adhesion between peripheral OECs, which did not occur without initial wave contact. Thus, it appears that lamellipodial waves act to induce contact-stimulated migration of OECs. It has previously been shown that GDNF can stimulate OEC migration [71]. Significantly, we found that GDNF enhanced both the size and number of

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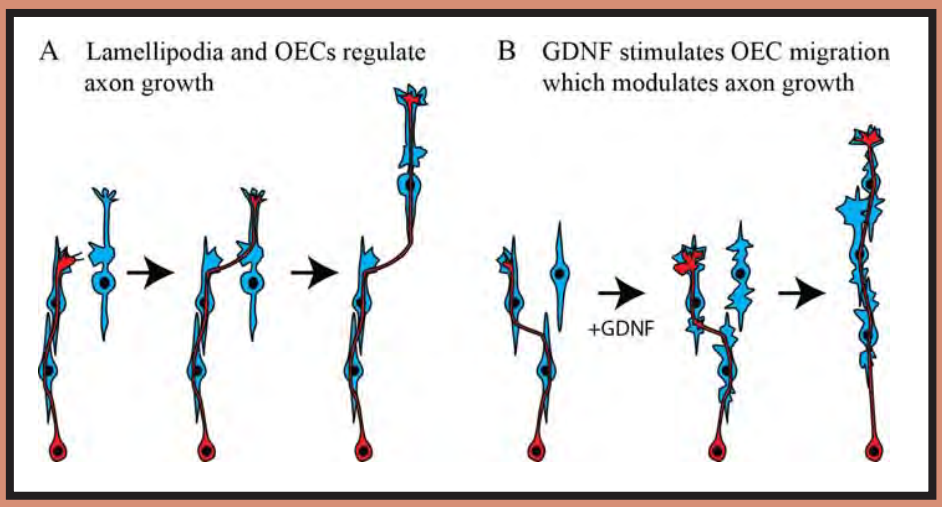

Figure 4. *(A) Olfactory axons (red) extend along the surface of OECs (blue). Growth cones contact lamellipodial waves on OECs and then further extend along the new OEC. The migration of the OECs strongly regulates the motility of the axon. (B) GDNF stimulates the lamellipodial waves on OECs which results in close adhesion and contact-mediated migration of the OECs. The axons and their growth cones mirror the movement of the OECs.*

lamellipodial waves (Fig. 4), resulting in a 2-fold increase in migration rate [52]. We also showed that specific inhibition of Mek, which acts down-stream of GDNF, blocked wave activity while leaving the leading edge intact, demonstrating that the activity of peripheral waves is regulated by different pathways than the leading edge [52-53]. These findings suggest that in OECs, both the leading edge and peripheral lamellipodial waves are involved in initiating cell-cell contact and promote migration.

The next step that needs to be undertaken is to determine how the different subpopulations of OECs influence the growth and fasciculation of axons at the cellular level and to determine the molecules that regulate the interactions between the different OECs and axons.

The way forward

In summary, the use of OECs for repair of nerve injuries is a promising approach but it would be wise not to assume all authors are using the same cells. It is clear that subpopulations of OECs exist with demonstrated heterogeneity in morphology, antigen expression and function of OECs which vary depending of the different regions of the olfactory system, the developmental stages and the different culture conditions. These are all variables that will confound any comparison of outcomes of transplanting olfactory ensheathing cells into the injured spinal cord. What is now needed is a systematic analysis to determine the differences in the molecular and cellular characteristics of the various subpopulations of OECs. This will provide increased understanding of the role of OECs and pave the way for improving the use of these cells for transplantation therapies ■

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NEWS

Local Responsibility Grows on Smelly Issues

The laws on air pollution are clear and impose strict penalties on polluters. Pervasive odours are considered a breach of air pollution laws and Local Governments are increasingly responsible for their enforcement.

Disputes over unclean air favour the complainant. Polluters are requested to stop stinking before penalties are imposed. They may be unaware they stink because they have adapted to the odour.

Odour is pervasive if it breaches the emitter's property boundary. Complainants can become intensely agitated when they feel ignored.

Local governments are in the middle as constituents defend their commercial interests, lifestyles, property values and health. Fair resolution calls for an objective measurement of the offending odour and an unemotional response.

There are three options available to Local Government, when assessing the complaint:

Employ a certified nose, to visit the emitter's and complainant sites to perceive strength and type of smells. This is a subjective process. Smells may be transient and don't always coincide with a visit by a professional sniffer.

Secondly, employ an environmental consultant to take bagged air samples back to a laboratory for analysis. Again, the transient nature of odours can be uncooperative, samples degrade with time and lab work is expensive.

Thirdly, use an instrumental electronic nose (e-nose) to measure the smell intensity and quality, continuously and in real time at the sites. This is an inexpensive, objective solution to air pollution issues. E-Nose Pty Ltd supplies e-noses for purchase or rent, and offers training and analytical services ■

13th Scientific Meeting of the Australasian Association for ChemoSensory Science (AACSS)

7-9 December 2011
Ascension Wine Estate, Matakana, New Zealand
(*one hour's drive north of Auckland*)

Save the Dates!

The 13th meeting of the AACSS will be held north of Auckland in wonderful Matakana close to wineries and beautiful beaches. The program will cover all aspects of chemosensory science in both invertebrate and mammalian systems. Please lock in those dates.

Please watch the AACSS website for more information including the programme, invited speakers, registration and abstract submission dates and details of accommodation: http://www.aacss.org/

Any queries regarding the meeting can be directed to Richard Newcomb,
Richard.Newcomb@plantandfood.co.nz

Upcoming Events

24-29 July 2011	**Summer School on Human Olfaction** Dresden, Germany thummel@mail.zih.tu-dresden.de
31 July to 2 August 2011	**20th International Clean Air and Environment Conference** CASANZ (change of date from 5-8 July) Auckland (Change of venue from Christchurch) New Zealand www.casanz.org.au
4-8 September 2011	**9th Pangborn Sensory Science Symposium** Sheraton Center, Toronto Canada www.pangborn2011.com
12-16 November 2011	**Neuroscience 2011** Washington DC, USA www.sfn.org/am2011/
7-9 December 2011	**Australasian Association for ChemoSensory Science (AACSS)** Annual Scientific Meeting Matakana, Auckland, New Zealand www.aacss.org
29 January – 1 February 2012	**Australian Neuroscience Society (ANS)** Annual Conference Gold Coast, Queensland
7-9 March 2012	**EcoForum** Conference and Exhibition Australian Technology Park, Sydney www.ecoforum.net.au

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