## Conference Report

# Third Annual Meeting of the California Spinal Cord Injury/Neural Regeneration Consortium
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Recent initiatives in the USA have increased the funding provided by several individual states into research into spinal cord injury (SCI). One such initiative came about following heroic campaigning by Don Reed, whose son Roman broke his back in a football accident, and Californians for Cure. The Californian legislature passed the Roman Reed Bill (AB750) in July 2000 to fund research into treatments for SCI within the state of California. The funds were given to the University of California system to administer, a responsibility that was passed to the Reeve-Irvine Research Center (RIRC) at the University of California, Irvine. Renewed on a year-by-year basis, funding currently stands at $1.8 million for 2002–2003. In a deliberate attempt to broaden the research base, some of the peer-reviewed projects are based in labs with no previous experience of SCI research and, in these cases, animal work is carried out by trained personnel using the facilities at the Roman Reed Core Laboratory in the RIRC.

### Limiting the damage

The functional deficit that is the inevitable consequence of SCI is determined by the location and the size of the initial, direct mechanical trauma and vascular damage. The initial damage triggers further tissue destruction in a process that extends the size of the lesion and so increases the severity of the functional deficit. Preventing this secondary destructive phase would limit the area of damage and so reduce the functional consequences of SCI.

Modulating the damage inflicted by the immune system is one of the approaches that has attracted interest from groups not previously involved in SCI research, particularly those researching the immunological basis of multiple sclerosis (MS). In addition to neuronal death, one of the consequences of secondary degeneration in SCI is the demyelination of otherwise intact neurons that survive the initial injury. CNS demyelination also features in MS, triggered by an autoimmune reaction against myelin. Because mice strains that are more susceptible to experimental autoimmune encephalitis (EAE), a model of MS, are more susceptible to nerve damage following SCI, it is possible that similar immune responses may be involved in both cases.

Techniques to damp down or redirect the immune response in SCI include antibody treatment to prevent the activity of IP-10, one of the first chemokines to be produced following injury. Such treatment reduces the accumulation of activated T cells and macrophages in the damaged area, accompanied by reduced post-traumatic tissue loss and significant improvements in functional recovery in mice (*H Kierstead*). DNA vaccines directed against antigenic myelin components and pro-inflammatory cytokines also reduce the severity of the T-cell-mediated immune response in EAE (*L Steinman* and *P Fontura*) as does selectively preventing ion-channel activity in activated T cells using a small-molecule antagonist (*M Cahalan* and *KG Chandy*). Roman Reed funding has enabled these groups to investigate whether these experimental strategies will also benefit SCI models.

Another consequence of the secondary response to injury is formation of scar tissue, which forms a physical barrier that inhibits regeneration and sprouting of remaining neurons. Using transgenic mice, *M Sofroniew* demonstrated the importance of reactive astrocytes in scar formation. Genetically altered mice that produce greatly reduced numbers of these cells form much less scar tissue, accompanied by increased local outgrowth of neurons. Because reactive astrocytes produce IP-10, the overall immune response at

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the injury site might also be reduced in these animals, adding to the beneficial effects.

Teasing apart these relative contributions requires techniques that allow fine manipulation of the systems involved. Adenoviral vectors encoding individual cytokines and chemokines could provide another way to manipulate the immune response in SCI (*M Solbrig*). Non-viral methods of gene delivery to the CNS are also being optimised using agents that transfect a high proportion of cells that line the ventricles; the challenge now is to transfer this to cells in the spinal cord (*L Hall*).

## Promoting growth

In addition to limiting the neuronal damage following injury, promoting the regeneration of surviving neurons is vital in treating SCI. In a 'back to basics' approach *D Gardiner* described how urodele amphibians regenerate a complete spinal cord following tail-amputation. Using DNA arrays to identify the genes that are active during this process could help design strategies that enhance regeneration in humans. Studying the cues that promote neuronal growth in mammalian embryos has already established roles for DHEA and PACAP during neurogenesis. In adult mice, DHEA, a neurosteroid, improves the functional outcome of moderate contusion injury in a range of tests, including voluntary bladder control, and reduces sprouting of pain-sensitive neurons in the dorsal horn (*N Compagnone*). Studying SCIs in PACAP-knockout mice will determine if this neuropeptide also has a role in adult spinal cord (*J Waschek*).

It is established that several growth factors induce some neuronal growth and functional recovery in rodents, even in the presence of inhibitory scar tissue. Implanting autologous fibroblasts that have been retrovirally transformed to produce either neurotrophin 4/5 (NT-4/5) or glial-cell-line-derived growth factor (GDNF) into rats increases the density of cells, including neurons, at the site of a complete T7 transection (*A Blesch*). However, there are many types of neurons within the spinal cord and not all respond to every growth factor. That different factors selectively stimulate the neuronal growth is apparent from the selective increase in the number of noradrenaline-containing axons (rubrospinal tract) at the lesion site following treatment with NT-4/5 but not with GDNF.

Bone marrow stem cells (BMSCs) that are genetically modified to produce growth factors might have advantages compared to fibroblasts in stimulating neuronal regrowth (*A Blesch*). Because collecting BMSCs from individual patients is relatively simple, these cells might form a convenient source of autologous stem cells for cell-based therapies. They also migrate through the brain after injection into sites of traumatic brain injury (*R Vuillet*). Similar migration by BMSCs modified to produce growth factors might result in 'trails' of growth factors that draw regrowing neurons into and through the damaged region.

As an alternative to cell-based approaches, two groups are investigating using synthetic polymers (*S Stokols* and *M Sofroniew*). These polymers are well tolerated by the body and could provide a depot for the continuous release of growth factors as well as physical support for the growing axons.

In most studies, the effects of treatment are assessed by carefully observing the movement and behaviour of the animal. Although this provides a good indication of gross recovery, it gives little detailed information about fine limb movement and locomotion. Traditionally, these are assessed using kinematics, which is feasible in few labs. Roman Reed funding is being used to develop two robotic devices, one for mice (*VR Edgerton* and one for rats (*D Reinkensmeyer*), that will provide quantitative, objective information about limb movement following injury and treatment.

## Synaptic plasticity

Animals (and humans with incomplete injuries) can learn to stand and walk again after long-term repetitive training in these specific motor tasks, presumably due to synaptic plasticity within the spinal cord. Injecting markers into hindlimb muscles should identify the motor neurones and groups of interneurons involved (*L Havton*). A fascinating explanation for training-induced changes in synaptic connectivity comes from studies of exercise training in rats (*G Twiss* and *F Gomez-Pinilla*). Physiological activity increases the expression of brain-derived neurotrophic factor (BDNF) and NT-3 in dorsal root ganglia and motor neurons in the lumbar spinal cord, accompanied by increased growth of neurons from dorsal root ganglia in culture.

Taking a neuroengineering approach, a tiny array of 96 microelectrodes implanted into the spinal cord can record and stimulate activity or groups of neurons (*C Yang* and *J Judy*). The detailed spatiotemporal analyses of the activation patterns in the spinal cord during normal movement will help develop stimulation paradigms that initiate simple, coordinated movements following injury. This device should also lead to a better understanding of the changing contributions of different neuronal populations during exercise training.

Neuronal plasticity is most often studied in the context of motor function. However, *C Darian-Smith* described changes in sensory innervation that occur in macaques following lesion of the dorsal rootlets that contain sensory input from the index finger and thumb. Although no neurons regrow, dexterity does improve after injury, probably because the few residual afferents that were not damaged by the injury become dominant.

The changes that accompany SCI provide the focus of a collaborative project to develop a model to study neuronal plasticity and regeneration in primates (*M Tuszynski, VR Edgerton, J Hodgson, L Havton, B Dobkin, R Roy, H McKay, J Roberts, H Yang, A*

*Blesch* and *O Steward*). Some approaches to treating SCI are beneficial in rodent models of SCI but, before being used in humans, the benefits and safety of any potential treatments need to be confirmed in a second animal model. Because of similarities in the size, anatomy, locomotion and immune systems, many feel that primates provide the best model and *M Tuszynski* outlined preliminary collaborative studies that utilise the expertise of numerous groups to gain the maximum information from each animal. Initial studies have established that the pattern of changes following unilateral lesion of the corticospinal tract at T10 is similar to that seen in rodents. Increases in the number of neurons and Schwann cells as well as growth-inhibitory factors, such as chondroitin sulphate proteolgycans, in the lesion area following injection of autologous NT-3-secreting are also similar. The group is now to expand these studies to include cervical injuries in which the functional consequences of small increases in neuronal 'recovery' will be more apparent.

The diversity of the projects described, from established groups as well as those new to SCI research, illustrate the startling expansion in skills and knowledge that can be achieved with modest funding in a relatively short time. Hopefully, some of the approaches outlined here will provide the groundwork for potential future treatments.