THEME FOUR PLENARY

Spinal Cord Injury and CNS Regeneration

0 - 46  REPAIRING THE DAMAGED CORD: FROM STEM CELL TO ACTIVITY-BASED MECHANISMS OF RECOVERY
John W. McDonald III, (St. Louis, USA)

Neurological rehabilitation is expanding its scope to include restoration of function through regeneration. However, this complex task will require multi-stage interventions, some easier to achieve than others. Of repair strategies on the horizon, harnessing the potential of neural stem cells already present in the CNS and placed there by transplantation appears promising. In fact, using stem cells to remyelinate the damaged cord is an immediate clinical target. Demyelination is an important contributor to disability after spinal cord injury because it incapacitates axons that remain intact below level of injury. Thus, remyelinating bridging axons would likely improve important functions, such as, bladder and bowel control, limb movement, and respiration. I will describe our recent studies, and those of others, transplanting embryonic stem cells into the injured rodent spinal cord focusing on remyelination. In addition, I will also focus on a second strategy, to mobilize endogenous stem cells to repair the damaged spinal cord. I will put forth a hypothesis that the conditions necessary for optimal spontaneous regeneration are not present after spinal cord injury. A growing body of evidence indicates that at least one of these factors is optimization of pattern neural activity. I will discuss the role of optimized pattern neural activity in regeneration and recovery of function and suggest that recovery of function can occur many years following injury. Although, it is impossible to predict what final strategies will be used to ultimately repair the damaged spinal cord, it is possible to discuss step-wise strategies towards partial repair.

0 - 47  PLASTICITY AND REPAIR AFTER SPINAL CORD INJURY
JC. Bresnahan (Columbus, USA)

Recent advances in approaches to spinal cord injury treatments in animal models provide substantial hope that these therapies may soon be applied to human clinical situations. This presentation will review the basic features of useful animal models of SCI, and show how the biology of spinal cord injury has been studied in the context of this complex injury situation. A variety of outcome measures useful for evaluating treatments will also be presented, and a general discussion of the role of animal modeling in planning human clinical trials will be given.

0 - 48  MOLECULAR REGULATION OF AXONAL REGROWTH AND NEUROGENESIS FOLLOWING CNS TRAUMA
PF Bartlett (Brisbane, Australia)

The production of neurons in the adult brain is now well documented to occur in a number of regions and to be influenced by external and disease processes. Although a precise functional role for these neurons has not been established, mounting evidence suggests it may underpin some higher-brain functions and has the potential to restore function following disease and mental illness. Thus, finding the key molecular regulators of neuronal production in the adult brain will provide both new insights onto this process and identify drug-candidates for the treatment of degenerative, traumatic and psychiatric diseases. To identify these candidates we have purified the stem cell in the adult brain of rodents to near homogeneity (Rietze et al, Nature 2001) and analysed their expression profile by Affymetrix
gene-microarray. We have identified two receptors expressed on the surface of the stem cell, LIF receptor and the p75NT receptor, and one on the progenitor cell, Growth Hormone receptor (Turnley et al, 2002 Nature Neuroscience) which appear to be involved in the regulation of neuronal production. The role of these receptors in regulating stem cell differentiation and neuron production will be discussed in detail. In addition, the effect of inflammatory cytokines released following trauma on neuronal production, glia scarring and axonal regrowth will be explored.

SESSION 4.1: Syringomyelia

0 - 49 PATHOGENESIS AND TREATMENT OF POST-TRAUMATIC SYRINGOMYELIA
J Klekamp (Quakenbueck, Germany)

Objective: Shunting of a posttraumatic syrinx is still widely accepted as the first line of treatment. Recently, the significance of CSF flow obstructions for the pathophysiology of syringomyelia has become more recognized. Surgical strategies aiming at treating the posttraumatic arachnopathy, spinal cord tethering and reconstruction of the spinal canal have been introduced. We conducted a retrospective study for patients with posttraumatic syringomyelia treated between 1978 and 2003 to determine the influence different surgical strategies and arachnoid changes on clinical outcome.

Methods: A total of 84 operations in 58 of 95 patients with posttraumatic syringomyelia were performed. 37 patients did not show signs of clinical progression and were observed. 42 patients underwent arachnoid dissection, untethering of the cord and duraplasty at the site of injury. 34 patients were treated by syrinx shunts, while 6 patients underwent spinal fusions for additional instabilities or degenerative changes and 2 patients underwent dorsal root entry zone (DREZ) coagulation for pain relief.

Results: There was no correlation between the severity of trauma and likelihood for developing a posttraumatic syrinx: 24% developed a syrinx after a complete para- or tetraplegia. In 40% an incomplete cord lesion preceded the syrinx while 37% developed a syrinx without initial cord trauma. There was a tremendous variability in terms of the asymptomatic interval until radiological and clinical signs of posttraumatic syringomyelia started to appear (mean 10 years + 109 months). Postoperative neurological outcome depended on the preoperative neurological status. Even though the majority of patients reported improvements for pain and sensory symptoms, motor weakness or gait ataxia only rarely improved in a functionally significant fashion. Clinical stabilization of progressive neurological symptoms were achieved for 63% of patients after arachnoid dissection and decompression compared to 7% after syrinx shunting over a period of at least 7 years (log-rank test: $p < 0.0072$).

Conclusion: Posttraumatic syringomyelia is caused by CSF flow obstruction due to posttraumatic arachnopathy and/or additional spinal stenosis and does not require injury to the spinal cord itself. Surgery for posttraumatic syringomyelia should be directed towards the spinal level of the injury. Laminectomy, arachnoid dissection, untethering of the cord, placement of a dura graft, and spinal fusion – if required – provided a significantly better long term result than any syrinx shunting modality. In order to prevent posttraumatic syringomyelia as well as other forms of myelopathy, initial management of patients with spinal cord and spine injuries should aim to provide a wide enough spinal canal and fusion of unstable spine fractures.

0 - 50 PATHOPHYSIOLOGY OF POST-TRAUMATIC SYRINGOMYELIA
MA Stoodley (Sydney, Australia)

Background: Syringomyelia develops in over a quarter of spinal injury patients and can result in progressive neurological loss. Long-term treatment success is achieved in only 50% of patients. Better treatments are unlikely without an understanding of the pathophysiology of syrinx development.

Methods: CSF dynamics were investigated in a rodent excitotoxic injury and arachnoiditis model of posttraumatic syringomyelia. Pathways of CSF flow into the spinal cord and the effects of arachnoiditis were examined using horseradish peroxidase injected into the subarachnoid space as a CSF tracer. Spinal cord blood flow was assessed using laser Doppler flowmetry. Computational fluid dynamic modelling was used to investigate the effect of arachnoiditis on flow within the subarachnoid space and the perivascular spaces within the spinal cord.

Results: Spinal cord ischaemia is secondary to syrinx formation rather than a causative factor. CSF flows from the subarachnoid space into the cord via perivascular spaces. The major pathway for fluid entry into
post-traumatic syringes is ventral perivascular spaces. Pulsations of small arteries within the cord are sufficient to create flow within the perivascular spaces against a pressure gradient. Subarachnoid pulse pressures are higher and perivascular flow is greatest adjacent to levels of arachnoiditis than at other levels of the cord.

**Conclusions:** Pulsation-driven flow within the subarachnoid space and perivascular spaces is likely to be the cause of enlargement of post-traumatic cysts. Arachnoiditis is a major contributing factor to syrinx formation. These results support the development of treatments aimed at preventing or removing CSF obstruction at the site of injury.

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**RESPONSES OF ENDOGENOUS NEURAL STEM CELLS IN A MODEL OF POST-TRAUMATIC SYRINGOMYELIA**

J Liao, J Tu, A Cunningham, M Stoodley (Sydney, Australia)

It was traditionally believed that adult spinal cord lacked regenerative capacity. Recent studies have demonstrated endogenous neural stem cells (NSCs) in adult spinal cord and their proliferation in various animal models of injury. NSCs have not been examined in post-traumatic syringomyelia (PTS) and this study investigated their responses in an adult rat model of PTS. PTS was induced by parenchymal injection of quisqualic acid and subarachnoid injection of kaolin at C7 and C8 of spinal cord in 4 animals. Controls were animals injected with normal saline and unoperated animals. Dividing cells were identified by intraperitoneal injection of BrdU and animals sacrificed after 2, 4, 6 or 12 weeks. Frozen-sections of spinal cord were analyzed for expression of BrdU as well as a panel of oligodendrocytic, astrocytic or neuronal markers, including NG2, MBP, GFAP, NST and NCAM, by double-labeling immunohistochemistry. Newly generated cells, identified by BrdU labeling, were demonstrated in control animals but were present in greater numbers in the PTS group. In PTS, 82% of BrdU-positive cells were in white matter, 16% in grey matter and 2% surrounded the central canal. A significant proportion of BrdU-positive cells co-expressed the immature oligodendrocyte marker, NG2, with cells co-expressing astrocytic or neuronal markers also in evidence. These findings suggest that endogenous NSCs respond to this injury by production of new glial and neuronal cells. These studies will further extend our knowledge of the pathogenic mechanisms in PTS and raise the potential for cellular repair of damaged spinal cord by harnessing its endogenous regenerative potential.

**SESSION 4.2:** Regenerative Responses of Damaged Axons

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**NEW APPROACHES TO PROMOTE THE REGENERATION OF INJURED ADULT RETINAL GANGLION CELL AXONS**

AR Harvey, K Park, Y Hu, SG Leaver, GW Plant, J Verhaagen, Q Cui (Perth, Australia; Amsterdam, The Netherlands)

Intraocular neurotrophin injections only temporarily maintain injured retinal ganglion cell (RGC) viability. An alternative strategy is to use viral vectors to introduce additional copies of growth factor genes into compromised RGCs. When injected intravitreally, adeno-associated viral (AAV) vectors efficiently transduce adult RGCs [1]. We are using bicistronic AAV vectors encoding neurotrophic genes and green fluorescent protein (GFP), and comparing the regenerative response of transduced versus non-transduced adult rat RGCs after optic nerve (ON) crush or ON section and peripheral nerve (PN) transplantation. Preliminary results show that AAV.BDNF.GFP increases RGC survival after ON crush, but does not increase axonal sprouting distal to the injury. Intravitreal injections of ciliary neurotrophic factor (CNTF) increase adult RGC survival and axonal regeneration into PN grafts, an effect enhanced by co-injection with a cell-permeant analogue of cAMP (CPT-cAMP) [2] Co-injection of CPT-cAMP and NT-4/5 also increases RGC viability, but there is minimal regeneration into PN grafts due to intraretinal sprouting induced by this neurotrophin [2]. Pharmacological inhibition of PKA, PI3-kinase or MAPK (ERK) reduces CNTF and CPT-cAMP induced neuroprotection of axotomized RGCs and blocks their axonal growth promoting effects. Regeneration of RGC axons is also enhanced by increasing CNTF production in the PN bridges themselves. Schwann cells are transduced ex vivo with a lentiviral (LV)-CNTF vector and incorporated into freeze-thawed PN sheaths [3]. Compared to PN grafts containing LV-GFP-infected Schwann cells, PN reconstituted with CNTF-transduced Schwann cells doubles RGC viability and induces an 8-fold increase in the number of regrowing RGC axons.
References


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GLIAL SCAR AND REGENERATION IN THE MAMMALIAN CNS: LESSONS FROM MICE KO FOR GFAP AND VIMENTIN
A Privat, M G Ribotta, V Menet (Montpellier, France)

Central neurons have long been considered intrinsically unable to regenerate their axons. Since the work of Aguayo, the absence of regeneration has been shifted from the neurons to their environment. Any injury in the CNS triggers the formation of a glial scar, which is not permissive for regeneration. Astrocytes are one of the main components of the scar, and reactive astrocytes are characterized by the up-regulation of GFAP and the re-expression of vimentin. In order to overcome this obstacle, we have raised mice KO for the genes of GFAP and vimentin, and performed on those mice as well as on control lateral hemisections of the spinal cord at lower thoracic level. Unlike controls, KO mice do not constitute astrocytic scars, grow axons from the intact side of the cord, which contact their normal targets on the opposite side below the level of the lesion, and recover partially the function of their paralyzed hindlimb. We conclude that reactive astrocytes are one of the major impediments to axonal regeneration, and that the present findings pave the way for new therapeutic strategies in spinal cord injury.

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EFFECT OF TISSEEL® ON AXONAL REGENERATION IN THE DORSAL SPINAL COLUMN CRUSH IN THE ADULT LONG EVANS SHAKER RATS.
R Avram, C Hai, J Tang, K Neil, W Tawil, J Bain, JM Kwiecien (Ontario, Canada; Deerfield, USA)

Although transection of axons in normally myelinated CNS axons such as in the spinal cord injury is permanent and cut axons do not regenerate, axonal plasticity remains considerable in severely dysmyelinated CNS of adult Long Evans Shaker (LES) rat. In a previous work it has been shown that in a dorsal column crush LES rat axons stop abruptly at the caudal margin of the cystic lesion. In this study we sought to test capacity of Tisseel® (Baxter International) to provide a functional bridge for re-growing axons to cross the site of the lesion. We hypothesized that once dysmyelinated axons cross the crush site, they will continue to elongate in the dorsal column rostral to the site of the injury since inhibition of axonal plasticity is not evident in the LES rat. Crush injury was created in anaesthetized rats with fine forceps at T8 level and fast polymerizing Tisseel® injected into the crush site. Rats without paraplegia and paralysis of urinary bladder were maintained for a total of 2 weeks. Cholera toxin subunit B (CTB) tracer conjugated with an AlexaFluor dye was microinjected into both sciatic nerves 5 days prior to the perfusion. Numerous AlexaFluor-positive axons were observed in the dorsal column caudal and up to \( > 1 \) cm rostral to the site of injury treated with Tisseel®. We consider the adult LES rat a suitable model for studies of axonal regeneration after spinal cord injury. Tisseel® appears to be conducive for axonal regeneration across the site of the spinal cord injury.
GUIDANCE MOLECULE EXPRESSION IN THE REGENERATING AND DEGENERATING VISUAL SYSTEM  
CE King, J Rodger, LD Beazley, SA Dunlop (Perth, Australia)

The guidance molecules, Eph receptor tyrosine kinases and their ephrin ligands, are involved in establishing topographic connections within the developing CNS. We have compared expression patterns in species with differing capacities for adult optic nerve regeneration, after severing all retinal ganglion cell (RGC) axons. In normal goldfish, retinal EphA3/EphA5 expression is uniform whereas tectal ephrin-A2 forms a shallow ascending rostro-caudal gradient. RGC axons regenerate to the main visual centre, the optic tectum and, coincident with restoration of topography, retinal EphA3/EphA5 and tectal ephrin-A2 form, respectively, significant ascending naso-temporal and rostro-caudal gradients. Furthermore, if EphA/ephrin-A interactions are blocked in the tectum, topography is abnormal. In the lizard Ctenophorus ornatus, RGC axons regenerate to the optic tectum, but topography is not restored and animals remain blind via the experimental eye. Unlike goldfish, an ascending naso-temporal EphA5 gradient in normal retina becomes uniform following nerve section and tectal ephrin-A2 form, respectively, significant ascending naso-temporal and rostro-caudal gradients. In mammals, such as rat, severed RGC axons show only limited spontaneous regeneration and the superior colliculus (SC) remains permanently denervated. Similar to lizard, the shallow ascending naso-temporal EphA5 gradient in normal retina becomes uniform after optic nerve injury, whereas SC ephrin-A2 forms a persisting rostro-caudal gradient. The results suggest that inappropriate Eph/ephrin expression profiles may be responsible for failure to form topographic maps in lizards. Furthermore, the similarity between expression in rat and lizards suggests that induction of goldfish-like expression profiles in mammals may be a prerequisite for restoration of topography once regeneration has been achieved.

OLFACTORY ENSHEATHING CELLS AND SPINAL CORD REPAIR  
A Mackay-Sim (Brisbane, Australia)

The olfactory ensheathing cell is a glial cell which normally ensheaths and guides the olfactory sensory axons as they course from the nose to the brain. Previous studies showed that olfactory ensheathing cells taken from the olfactory nerves in the cranium would assist spinal cord repair. Our aim was to investigate whether these cells taken from the nose could assist in promoting recovery after spinal cord injury. Spinal cords of adult rats were transected at T10. Pieces of olfactory tissue or cultured olfactory ensheathing cells were transplanted into the injury site at the time of injury or one month later. Recovery was measured behaviourally, anatomically and physiologically for 10 weeks. Transplantation at the time of spinal cord transection (Lu et al, 2001, Brain Res 889:344) and transplantation one month later (Lu et al, 2002, Brain, 125:14) resulted in significant recovery of locomotor behaviour. There was anatomical and physiological evidence for reconnection of brainstem motor pathways across the site of spinal cord injury. We then developed new methods for the culture and purification of olfactory ensheathing cells from human olfactory mucosa (Bianco et al, 2004, Glia, 45:111). Small olfactory biopsies of the olfactory mucosa were dissected and the cells dissociated and grown in a serum-free medium containing neurotrophin-3. This produces pure populations of olfactory ensheathing cells that can be generated in large numbers. A Phase I clinical trial has commenced where autologous olfactory ensheathing cells are transplanted into the injured spinal cord of paraplegic humans with complete injuries at T4-T10.
Remyelination of demyelinated injured axons can optimize the axonal function of the spared white matter in injured spinal cord. This is particularly relevant since relatively small changes in neuroanatomical integrity in the central nervous system (CNS) can impact substantially on clinical neurological recovery. In present study, we transplanted neural stem cells generated from the subventricular zone of forebrain of transgenic mice expressing Green Fluorescent Protein (GFP) into subacutely injured spinal cord of rat (23 g/1 min at T7). Three and six weeks posttransplantation, a substantial number of transplanted GFP-positive NSCs survived in the injured spinal cords of the rats which had received a combination of growth factors (EGF and bFGF) intrathecally using osmotic minipumps. The grafted GFP-NSCs well integrated along white matter tissue surrounding the lesion site and showed a close contact with the host cellular profiles including the axons and glial cells. Six weeks after transplantation, these cells had migrated within host injured spinal cord tissue away from the implanted sites. Moreover, some grafted GFP-NSCs displayed the antigenic properties of astrocytes and mature oligodendrocytes, but not neurons, which was in contrast to our in vitro observations showing their multipotential capacity to differentiate into both neurons and glial cells. Our results showing the ability of adult NSCs to survive, integrate and migrate along axonal tracts and to differentiate into oligodendrocytes in injured spinal cord represent an important requirement for potential remyelination after injuries to adult CNS or demyelinating diseases. Financial support: CIHR, Krembil Chair in Neural Repair and Regeneration (MGF).

A NEWLY DEVELOPED ORIENTATED COLLAGEN TYPE I SCAFFOLD FOR USE IN ACUTE SPINAL CORD INJURY - CYTOCOMPATIBILITY AND ORIENTATED NERVE REGENERATION
S Moellers, I Heschel, J Noth, G Brook (Aachen, Herzogenrath, Germany)

Acute spinal cord injury results in disruption of nerve fibre tracts that are important for locomotors control. Spontaneous axonal regeneration shortly after this event is hindered by secondary events, including fibrous and glial scar formation as well as the presence of myelin-associated axon-molecules. Here we present a newly developed, highly orientated collagen type I sponge which may be combined with growth promoting olfactory nerve ensheathing cells (ONECs). Cyto-compatibility assays included cell seeding, cell viability/cytotoxicity tests, proliferation and migration analyses as well as different culture conditions on a variety of neural cell types. Cells seeded onto the sponge showed highly orientated growth in all cases, as well as migration and proliferation deep within the material. Explants of dorsal root ganglia revealed orientated Schwann cell migration that was accompanied by nerve fibre outgrowth after 2–4 weeks. Non-seeded sponges or sponges seeded with enriched ONECs were implanted into acutely injured rat spinal cord at level C2. Both types of sponge displayed good vascularization as early as 14 days after implantation and a relatively minor macrophage response, indicating good integration with host tissue. ONEC containing sponges promoted a strong and directed axonal regeneration. Immunohistochemistry revealed that both CGRP and Serotonin positive axons contributed to this regenerative response.

TRAUMATIC SPINAL CORD INJURY AND GENE THERAPY: SCHWANN CELL AND OLFACTORY ENSHEATHING GLIA TRANSPLANTS
G Plant, M Ruitenberg, AR Harvey, D Levison, J Verhaagen (Perth, Australia; Netherlands)

Cell transplantation has been widely investigated to encourage axonal regeneration following spinal cord injury (SCI). Two glial cells have been intensively studied in translational neuroscience -- these are Olfactory ensheathing glia (OEG) and Schwann cells (SCs) isolated from the olfactory bulbs and sciatic nerve of adult rats. Both cell types have been shown to improve behavioural recovery following SCI in rats (Plant et al., 2003; Takami et al., 2002). The authors examined the effects of OEG and SCs transplantation following a seven day delay after contusion injury. In the combined studies presented here, we have looked at the viral mediated engineering of both OEG and SCs into SCI models. Lentiviral-mediated labelling of both glial cells (using Green Fluorescent Protein or DSRED-2 reporter genes) has shown dissimilar integration of OEG and SCs with the astrocytic environment and dispersal/migration of the glial cells after transplantation. Adenoviral-mediated transduction of OEG and transplantation to the hemisected adult rat spinal
cord showed increased sparing of spinal cord tissue by approximately 10%, compared to lesion only controls. Improved behavioural outcomes were also achieved when adenoviral transduced glial cells secreting neurotrophic factors (BDNF/NT-3) were used along with increased axonal regrowth. OEG are seen to associate with regrowing axons within the lesion site and express p75 phenotype, but little myelination profiles in OEG transplants were apparent. The combined use of viral mediated gene transfer and transplantation of SCs and OEG provides an important new avenue of research and treatment of acute and chronic traumatic SCI.

HEMATOPOIETIC STEM CELLS DERIVED FROM HUMAN UMBILICAL CORD BLOOD TRANSPLANTATION FOR SPINAL CORD INJURY

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Objectives: The use of hUCB (human Umbilical Cord Blood) cells has recently been reported to improve behavioral consequences of central nervous system injuries such as stroke, traumatic brain injury and spinal cord injury. We previously reported that transplanted hematopoietic stem cells from bone marrow differentiate into neural lineage cells and promote functional recovery after spinal cord injury in mice. We investigated the effect of transplanted hematopoietic stem/progenitor cells derived from hUCB in rat spinal cord injury.

Materials and Methods: We used adult male Wistar rats in this study. CD34-positive hematopoietic stem cells were enriched using Magnetic Activated Cell Sorting (MACS, Milteny Biotech). One week after injury, enriched CD34-positive cell fraction was injected into lesion cavity with glass micro-pipette (2–3 × 10⁵ cells/animal: hUCB group). Vehicle was injected into lesion cavity as a control (control group). Motor functional recovery was assessed with BBB locomotor scale between two groups. Immunohistochemistry was performed to detect survival and differentiation of transplanted cells.

Results and Conclusion: hUCB group rats showed better recovery in BBB locomotor scale than control group. Immunohistochemistry revealed that transplanted CD34-positive fraction survived in injected spinal cord. These results suggest that transplantation of CD34-positive fraction from hUCB may have therapeutic effect for spinal cord injury.