SPINAL RESEARCH

fighting paralysis... and winning

2-3 September 2011

The 13th Spinal Research Network Meeting

ABSTRACTS

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Speakers' abstracts appear in presentation order, followed by poster abstracts in alphabetical order

POSTER PRESENTATIONS

Poster session is scheduled from 4.45pm in Hendrix and Madonna Suits at the end of the first day, immediately after the main meeting, on Friday, 2nd September. There will also be time during the coffee breaks on Saturday to view the posters.

SCIENTIFIC ORGANIZING COMMITTEE

Sue Barnett PhD
University of Glasgow

James Fawcett PhD FRCP
University of Cambridge

Steven McMahon PhD FMedSci
King's College London

Joost Verhaagen PhD
Netherlands Institute for Neuroscience

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Friday 2nd September

Saturday 3rd September

Session 1: Astrocytes as target in CNS repair – Chair: Geoffrey Raisman

Astrocytes modulators of CNS repair?

Susan C. Barnett

Institute of Infection, Immunity and Inflammation, University of Glasgow, 120 University Place, Glasgow G128TA, Scotland, UK

susan.barnett@glasgow.ac.uk

After injury and during disease astrocytes express a continuum of phenotypes from quiescent to reactive. A pathological hallmark of CNS injury is reactive astrocytes and generally is thought to prevent repair. However, it has also been suggested that astrocytes can become activated, and promote repair. We have manipulated astrocytes *in vitro* to be activated or quiescent and used them as support cells for myelinating cultures. These cultures are comprised of rat dissociated spinal cord cells which if plated on control astrocytes recapitulate many recognised stages of glial/axonal interactions over time, culminating in the ensheathment of axons and formation of myelin internodes and nodes of Ranvier. We have found that quiescent astrocytes (astrocytes plated on tenascin C) do not support myelination, whereas activated astrocytes (astrocytes plated on PLL and treated with CNTF) promote myelination. Data will be shown to demonstrate CNTF directly affects astrocytes rather than oligodendrocytes. A combination of microarray gene expression analysis and quantitative real-time-PCR identified CXCL10 as a potential candidate for the reduction in myelination in cultures on quiescent astrocytes which was confirmed using neutralising antibodies and recombinant protein. Furthermore, CXCL10 treatment of purified OPCs did not affect proliferation, differentiation or process extension, compared to untreated controls, suggesting a role in the later stage of myelination. Data will also be shown on how addition of glial cells to the cultures can affect myelination via the astrocytes target. Thus we have shown a direct correlation of astrocyte phenotype with their ability to support myelination. This observation has important implications with respect to the development of therapeutic strategies to promote CNS remyelination in demyelinating diseases.

This work was supported by the MS Society of Great Britain and Northern Ireland

Astrocytes and glial progenitors after SCI: Can the protective and growth inhibitory effects be separated and can these populations be transformed to a growth-promoting phenotype?

Lyn B. Jakeman

The Ohio State University, Department of Physiology and Cell Biology and the Center for Brain and Spinal Cord Repair*; 1645 Neil Ave., Columbus, OH 43210, USA

Jakeman.1@osu.edu

In the normal CNS, astrocytes are highly sensitive sentinels of neuronal activity and metabolic function. After traumatic injury, astrocytes and glial progenitors favor a neuroprotective role. They respond to complex signals in the local microenvironment; they proliferate and mobilize rapidly to the edge of the region of damage, and contribute to the growth-inhibitory glial scar separating spared neuropil from the invading peripheral and inflammatory cells within the lesion[1]. In contrast, astrocytes and ependymal cells in lower vertebrates and very young mammals are capable of extensive migration into the lesion site where they form a highly permissive scaffold for regenerating axons. Indeed, small remnants of similar permissive glial bridges can also be observed after injury in adult specimens following contusion or compression injuries, but these are minimal and overwhelmed by the resounding influence of local neuroinflammation and secondary injury. In recent studies, we have sought to identify mechanisms that might be exploited to drive the reparative capacity of endogenous astrocytes and their progenitors in the injured adult spinal cord[2]. As a proof of principle, we observed that intrathecal infusion of transforming growth factor alpha (TGFα) early after contusion injury altered the response of the endogenous astrocyte population[3]. We have confirmed that TGFα directly activates the EGFR on adult spinal cord astrocytes and progenitor cells *in vitro*, inducing proliferation, migration, and transformation to a radial phenotype that supports robust neurite outgrowth. Localized overexpression of TGFα *in vivo* by adeno-associated virus injection directly adjacent to the injury site enhanced early proliferation of cells near the lesion border, reduced the size of the GFAP-free lesion core and resulted in increased axon density at the rostral lesion border, but these benefits were limited with long-term administration. To determine if intrinsic EGFR activation is necessary after injury, SCI was also performed on *Velvet* (C57BL/6J-Egfr^{Vel}/J) mice, a mutant strain with defective EGFR activity. The affected mice exhibited a malformed glial border, larger lesions, and impaired recovery of function. Thus, the consequences of complex signaling by EGFR activation in astrocytes are essential for the full neuroprotective function of the glial border. In addition, by enhancing progenitor proliferation and modifying astrocyte function early after injury, controlled enhancement of EGFR activity at the lesion border may be included in future strategies to enhance endogenous cellular repair and glial bridge formation following injury. Future studies seek to understand the balance of intracellular downstream signaling pathways in these cells that would facilitate directing their function toward reparative and pro-regenerative roles while minimizing their inhibitory effects on axonal growth.

Supported by NINDS 043246 and the OSU College of Medicine-Research Investment Fund

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Additional contributors to work presented include Robin White PhD, Dana McTigue PhD, Brian Kaspar PhD, Meghan Rao BS, John Gensel PhD, Todd Lash MS, Qin Feng Yin DM, Kent Williams MS, Mariano Viapiano PhD and Paul Gruenbacher.

Functions of reactive astrocytes and glial scars

Michael V. Sofroniew

Department of Neurobiology, UCLA, Los Angeles, CA, USA

sofroniew@mednet.ucla.edu

Reactive astrocytes and glial scars are prominent features of spinal cord injury that are often been regarded as uniformly detrimental to clinical outcome. Nevertheless, the basic response of gliosis and scar formation in spinal cord injury is remarkably conserved in mammalian evolution, suggesting beneficial roles that have been poorly understood. Safely manipulating reactive astrocytes and glial scars to improve axon regeneration will require understanding and preserving their beneficial functions.

We have used transgenic mouse models that allow different types of loss of function studies, to investigate functions and dissect specific mechanisms of reactive astrocytes and glial scars after spinal cord injury and other forms of injury in the central nervous system. This presentation will examine findings from our and other laboratories indicating that reactive astrogliosis is a complex, multifaceted process comprising numerous potential cellular and molecular changes with a wide range of potential effects regulated by different signaling molecules.

It is now clear that reactive astrogliosis, including scar formation, is not a simple all-or-none phenomenon but is a finely gradated continuum of changes that occur in context dependent manners regulated by numerous different specific molecular signaling cascades. These changes range from reversible alterations in gene expression and cell hypertrophy with preservation of cellular domains and tissue structure, to long lasting scar formation with rearrangement of tissue structure.

A better understanding of specific astrocyte signaling mechanisms and the molecular mechanisms of reactive astrogliosis and scar formation has the potential to open the door to identifying molecules that might serve as novel therapeutic targets for spinal cord injury and other neurological disorders.

Supported by: NIH NS NS057624; Wings for Life; Adelson Medical Research Foundation

For recent reviews see:

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638-647.
Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7-35.

Session II: Remyelination – Chair: Wolfram Tetzlaff

Schwann Cell transplantation to support axonal repair in large animals models of SCI

F. Benavides, A. Santamaria, L. Gauda, H. Levene, J. Solano, **James Guest**

University of Miami Miller School of Medicine, 1095 NW 14th Terrace, 33136 Miami, FL, USA

jguest@med.miami.edu

Introduction. Schwann cells (SC) have been used in numerous experiments to elicit aspects of repair after spinal cord injury. SC produce trophic factors, secrete basal lamina, ensheathe or myelinate axons, stabilize damaged axons. SC do not restore the structure of normal CNS tissue and lead to the formation of fascicles within the injury region that contain fibroblastic components. Further, the types of axons whose survival and sprouting may be supported by SC has not been adequately studied but include serotonergic, propriospinal, and sensory axons. We have conducted studies in non-human primates and Yucatan minipigs in which autologous SC have been transplanted at subacute and chronic time points respectively. These studies are among those supporting a clinical trial in patients with subacute SCI. The studies have been performed in compliance with most elements of good laboratory practice (GLP) and the cells prepared under cGMP conditions.

Methods. Porcine. A T8 laminectomy was performed and a contusion created leading to a severe incomplete injury. A suitable peripheral nerve was harvested to provide starting material for SC cultures. SC were exposed to GFP lentivirus at Passage 1, and were prepared for transplantation at P2. Cells were injected stereotaxically at 50, 100, or 150 ul into the lesion epicenter under ultrasound guidance and a post-transplant MRI obtained. During surgery MEPs and SSEPs were monitored for changes. After transplant animals were assessed daily using Miami Porcine locomotor scale (MPWS) and neurological scale. At post-transplant intervals of 3d to six weeks, the animals were perfused and underwent ex vivo MRI and were then studied histologically.

Methods Primate. Animals were extensively pre-trained in hand and locomotor tasks and then underwent a radiofrequency lesion to severely damage one medullary pyramid. After lesion there was eventual recovery to a deficit on one side of the body, mainly in the hand. Deficits of thumb and finger function were permanent up to 2.5 years. Primates underwent stereotaxic autologous SC transplantation at chronic time points, survived up to six months and the tissues were extensively assessed.

Results. Porcine. Spinal cord lesions are human-like in size with a severe inflammatory response, extensive Schwannosis, and formation of cavities. Large injections lead to increased intramedullary pressures creating severe damage to intact spinal cord tissue, predominantly grey matter. Transplanted SC survive at least 30d, and migrate more extensively than in rodents. Cells outside the main nidus were detected up to 3 cm from implant site mainly within the pia, and also within some nerve roots and the central canal. Post-transplant neurological deterioration was observed only with the largest injection volumes, and damage to grey matter could be quite severe and not detected by the MPWS. This indicates that locomotor function depends on preserved white as opposed to grey matter at the thoracic level assessed. Transplanted SC ensheathed axons and formed myelin in linear bundles spanning the lesion cavity.

Results. Primate. Transplanted SC were detected at 30d, 42d, and six months after transplantation. The cells were associated with novel fibroblast lined fascicles that contained astrocytic processes, and peripheral and centrally myelinated axons. Locomotor behavioral recovery was observed.

Discussion. Autologous SC are relatively easy to prepare to large numbers and high purity. After transplantation into the spinal cord or brain stem adverse effects are not obvious in these models, although we plan more extensive studies. The cells participate in novel fascicular structures of which fibroblasts appear to be obligatory. The SC may show long term survival of a small percentage of the originally implanted cells. The mechanisms by which they contribute to locomotor recovery remain speculative.

Primate studies supported by ISRT Porcine studies supported by Miami Project Clinical Translation Grant

Stem cells and myelin regeneration

Robin J.M. Franklin

MRC Cambridge Centre for Stem Cell Biology and Regenerative Medicine and Department of Veterinary Medicine, University of Cambridge, UK

rjf1000@cam.ac.uk

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis and traumatic spinal cord injury, and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective, especially in chronic demyelinating diseases. There is now compelling evidence that ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to a failure of stem cell differentiation. This talk will review recent studies we have undertaken aimed at obtaining a detailed understanding of the mechanisms of regulating differentiation during remyelination and hence identifying novel therapeutic targets.

Cellular transplantation approaches to repair the injured and demyelinated spinal cord

Jeffery D. Kocsis

Yale University School of Medicine, Neuroscience Research Center (127A), VA Connecticut Health Care System
West Haven, CT 06516, USA

jeffery.kocsis@yale.edu

Cellular transplantation approaches are being considered for repair and protection of the injured central nervous system. Experimental cell replacement approaches for neuronal degenerative and demyelinating diseases have been carried out for decades. Several cell types have been used in experimental cell therapy studies for spinal cord injury. These include peripheral myelin-forming cells such as Schwann cells and olfactory ensheathing cells. Results from these cell transplant studies will be discussed in this presentation. One cell therapy candidate has drawn particular attention: adult bone marrow-derived mesenchymal stem cells (MSCs). MSCs are multipotent stem cells present in adults, and in culture have the ability to differentiate into a variety of lineages including neurons and glia. The systemic injection of mesenchymal stem cells (MSCs) prepared from adult bone marrow has therapeutic benefits in traumatic spinal cord injury and cerebral infarction models in rodents. Several hypotheses to account for these therapeutic benefits have been suggested, including neuroprotective effects from release or stimulation of trophic factors and cytokines, the induction of neovascularization, axonal sprouting and remyelination, and the replacement of damaged cells. This presentation will discuss experimental results of transplantation of myelin forming cells and systemic and direct delivery of MSCs in experimental spinal cord injury and stroke models.

Session III: *In vivo* imaging of CNS tissue – Chair: Claudia Wheeler-Kingshott

Genetic mouse models and imaging approaches reveal mechanisms of neuron-glia interactions *in vivo*

Frank Kirchhoff

Department of Molecular Physiology, University of Saarland, 66421 Homburg, Germany

frank.kirchhoff@uks.eu

Acute CNS injuries induce fast neuronal and glial cell damage. Secondary injury processes involve activation of different cell types like astroglia, oligodendrocytes and microglial cells. A complex and yet not understood sequence of cellular responses initiate functional recovery after the neurodegeneration process.

We have developed two types of transgenic mouse models which enable us (1) to visualize and follow cellular reactions during de-and regeneration and (2) to perform fate mapping of injury-activated glial cells.

To visualize axons and glial cells in the dorsal columns of the lumbar spinal cord, we generated triple-transgenic mice (TgN(Thy1-EYFP)xTgN(GFAP-ECFP)xTgH(CX3CR1-EGFP)) in which axons, astrocytes and microglial cells are labelled by yellow, cyan and green fluorescent proteins, respectively. The same mice were imaged before and after lesioning at the day of injury and at subsequent days for up to three months. The combination of multi-cellular labeling with multiple-time-point imaging allowed us to explore unambiguously the spatio-temporal relationship between the cellular responses in spinal cord injury.

To study the differentiation fate of activated glial cells, we generated transgenic mice with functional complementation of split Cre DNA fragments as detectors of coincident gene activities observed after injury. In these mice N- and C-terminal Cre fragments (NCre and CCre) are targeted by the glial GFAP and PLP promoter. We could demonstrate that after acute brain trauma oligodendrocyte lineage cells can differentiate into protoplasmic astrocytes.

Combination of transgenesis and *in vivo* imaging is a powerful approach to study brain function in health and disease.

Dynamics cellular interactions at play during axon regeneration

Keith Fenrich, Pascal Weber, Mélanie Hocine, Geneviève Rougon, **Franck Debarbieux**

Centre National de la Recheche Scientifique - Université de la Méditerranée - Institut de Biologie du Développement de Marseille Luminy (IBDML), UMR6216; Case 907, Parc Scientifique de Luminy 13288 Marseille Cedex 9, France

franck.debarbieux@ibdml.univmed.fr

The concomitant advances in fluorescence imaging technologies and mouse transgenesis have opened the way to dynamic investigations of single cell behaviors in their natural environment. These technological progressions represent a breakthrough for studying central nervous system trauma, since the functional regeneration of damaged axons critically depends on complex, yet unclear, cellular interactions between many cell types.

As a first step to explore the cellular basis of healing after spinal cord injury we have developed a dedicated experimental protocol to chronically image the same population of dorsal root ganglion axons and their vascular environment over 4 months in the dorsal spinal cord of adult mice. We then established a "pin-prick" lesion model of spinal cord injury and characterized the influence of post-traumatic angiogenesis on the behavior of severed axons as part of a spontaneous recovery process.

Recurrent observations of the same animals showed that injured dorsal root ganglion axons have the intrinsic ability to regenerate in the central nervous system after lesion, at a speed which correlates positively with local vascular density. At the peak of the angiogenic response, sequential or time-lapse acquisitions showed an 8-fold enhancement of sprout elongation in axons growing close to blood vessels versus axons growing distal to blood vessels.

The ability of axons sprouts to grow faster while in close proximity to vessels was unrelated to the diameter of the blood vessels, hence the rate of growth was unlikely due to increased oxygen and nutrient supplies. Rather, the perivascular extracellular matrix might have provided axons with growth promoting substrate such as perivascular laminin and/or neurotrophic factors through the recruitment of secreting immune cells.

To assess the latter point, we have recently implemented a spinal glass window protocol on multicolor fluorescent mice. These windows allow us to simultaneously visualize axons, macrophages and blood vessels without performing surgery for each imaging session. Massive and sequential recruitment of circulating macrophages have been observed after injury. These cells have often been observed to form close appositions with retracting and degenerating cut axon terminals.. The proximity between macrophage processes and axon sprouts suggest that the two immune cell populations might contribute to regulate axon retraction and regeneration.

Functional MRI: progress towards a tool for clinical assessments of spinal cord function

Patrick W. Stroman

Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada

stromanp@queensu.ca

Functional MRI of the human spinal cord (spinal fMRI) has the potential to become a valuable clinical tool for assessment of spinal cord function after trauma or disease. A significant challenge for all MRI methods applied to the spinal cord is the poor field homogeneity caused by magnetic susceptibility differences, as well as by metallic fixation devices used to stabilize the spine after trauma. A challenge for clinical applications of fMRI is to be able to develop a method that is practical in a clinical setting, both in how it can be applied and the time required, while ensuring that it provides sufficient information for diagnostic purposes.

Here, we present a novel method of stimulating multiple sensory dermatomes simultaneously, in order to map function on both the right and left sides of the cord, as well as above and below the injury level. Thermal stimuli are used because they are passive, and involve spinal cord pathways involved with sensation, pain responses, and a component of motor responses. The stimuli are applied in distinct block paradigms that are linearly independent to permit detection of distinct responses to each of the stimuli. Results are presented from a group of spinal cord injured patients to demonstrate the clinical value, and practicality.

Session IV: NRB Studentship presentations – Chair: Ann Logan

The effects of parenteral or dietary omega-3 polyunsaturated fatty acids in rat models of spinal cord injury

Jodie Hall

Center for Brain and Spinal Cord Repair, The Ohio State University, USA

jodie.hall@osumc.edu

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are omega-3 polyunsaturated fatty acids (n-3 PUFAs) with distinct anti-inflammatory properties. In addition, both have neuroprotective effects when given intravenously (i.v.) acutely after SCI, but the effects of dietary enrichment with these fatty acids in neurotrauma is less well-characterized. It is important to characterize the effect of these compounds after parenteral and also oral administration, as both regimes could be used clinically. The aims of this studentship were: i) to characterize the inflammatory response in the rat after T12 compression SCI, ii) to characterize the effects of the acute i.v. injection of DHA or EPA on inflammation after SCI, iii) to explore the effects of i.v. DHA in a rat contusion model of SCI, iv) to assess the effects of dietary enrichment with DHA or EPA before and/or after SCI. My talk will cover some of the results from aims ii) and iv).

DHA (250 nmol/kg) i.v. injected 30 min after compression SCI significantly reduced neutrophil infiltration in the injury epicentre and the level of C-reactive protein in plasma, whereas EPA (250 nmol/kg) injection had no significant effect. Tissue injury (both laminectomy and compression SCI) elicited a sustained inflammatory response in the liver, which was not reversed by the PUFAs. At 4 h after compression injury, there was a significant increase in the cytokines IL-6, KC/GRO/CINC, IL-1β and TNF-α at the injury epicentre, with a return to baseline at 24 h. Neither DHA nor EPA reversed this increase. These results indicate that the acute neuroprotective effects of n-3 PUFAs in rat compression SCI may only be partly attributed to reduction of some of the early inflammatory events occurring after injury.

In the dietary enrichment study, female adult Sprague-Dawley rats (n=6 per group) received compression SCI at vertebral thoracic level 12, followed by control or EPA-enriched diet (400 mg/kg/day) for 4 weeks. Outcome measures included locomotor function (BBB score) and bladder functional recovery, development of hind limb mechanical hypersensitivity and histological assessment. Surprisingly, the group receiving the EPA diet had a significantly worse locomotor recovery, a significantly higher retention of urine and a permanent increase in bladder width by 4 weeks. The mechanical withdrawal threshold decreased significantly from baseline in both control diet and EPA-diet groups, but there was no significant difference between the groups. The histological assessment of specific areas at the injury epicentre and 5 mm rostral to the injury site revealed no significant difference between groups in cavity size, NeuN labelled neurones in the dorsal horn, SMI32 labelled non-phosphorylated neurofilament, APC labelled oligodendrocytes, ED1 or Iba1 labelled macrophages/microglia. Therefore, the study highlighted a possible risk associated with high level dietary EPA after SCI, but the mechanisms underlying this negative effect remain to be defined.

Further studies are required to characterize in more detail the mechanisms underlying the beneficial effect of acute i.v. n-3 PUFAs on functional recovery after SCI and the possible detrimental effect of interventions such as an EPA-enriched diet provided in the aftermath of injury. Future work should focus on the impact of n-3 PUFAs on long-term inflammatory processes after SCI, in particular on macrophage and microglia function.

Supported by a Natalie Rose Barr studentship award by the ISRT

Ward *et al*., 2010; Lim *et al*., 2008; Huang *et al*. 2007; King *et al*., 2006

Gene delivery in the DRG using AAV8: opportunities and challenges

Steven J Jacques[1], Zubair Ahmed[1], Anna Forbes[1], Michael R Douglas[1,2], Martin Berry[1] and Ann Logan[1]

[1]Neuropharmacology and Neurobiology Section, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK
[2]Department of Neurology, Dudley Group of Hospitals NHS Foundation Trust, Russells Hall Hospital, Dudley, DY1 2HQ, UK

s.j.jacques@bham.ac.uk

Adeno-associated viral vectors (AAV) are increasingly used to deliver therapeutic genes to the central nervous system (CNS) where they promote transgene expression in post mitotic neurones for long periods with little or no toxicity. In adult rat dorsal root ganglia (DRG), we investigated the cellular tropism of AAV8 containing the green fluorescent protein gene (*gfp*) after either intra-lumbar (L) DRG, or intrathecal injection and showed that transduced DRG neurones (DRGN) expressed GFP irrespective of the delivery route, while non-neuronal cells were GFP^-. After intra-DRG delivery of $AAV8_{gfp}$, the mean DRGN transduction rate was 11%, while intrathecal delivery transduced a mean of 1.5% DRGN. After intra-DRG injection, 2% of small DRGN (<30µm in diameter) were GFP^+ compared with 32% of large DRGN (>60µm in diameter) after intra-DRG injection. DRGN axons were also GFP^+, and were present in some target nuclei in the spinal cord with no transduction of intra-spinal neurones. A small number of contralateral DRGN were transduced, suggesting that AAV8 diffuses from injected DRG into the spinal canal. Microglia were activated in GFP^+ DRGN axon projection areas within the cord after intra-DRG injection, with a trend towards global activation of astrocytes within the cord. This study showed strong preferential AAV8 tropism for large DRGN unassociated with cell death, but accompanied by glial activation within the spinal cord. These results open up opportunities for targeted delivery of therapeutics such as neurotrophic factors to the injured spinal cord.

Mammalian chondroitinase ABC encourages Schwann cell integration within inhibitory environments

Philippa M. Warren*; J.C. Kwok*; M. Smith*; E.M. Muir+; J. Rogers+; R. Keynes+ & J.W. Fawcett*

* Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge, CB2 0PY, UK
+ Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK

pmw44@cam.ac.uk

Schwann cells transplanted into the CNS promote axonal regeneration and remylination[1,2] thus are an effective bridging material for spinal cord injuries. However, grafted Schwann cells show little integration with host astrocytes and reduced migration from the site of transplantation[3-6]. This results in the formation of a graft-host boundary which regenerating axons fail to transgress[7,8]. The reactive astrocytes at the graft-host boundary express, among other molecules, inhibitory chondroitin sulphate proteoglycans[9,10] (CSPGs). It has been shown that the application of bacterial chondroitinase ABC (bChABC) at the graft-host boundary reduces inhibition of the CSPGs and facilitates the integration of Schwann cells and astrocytes[7]. We have developed a chondroitinase ABC construct that can be expressed and secreted from mammalian cells[11] (mChABC). Here we investigate the biochemical properties of mChABC showing that the optimum temperature, pH and substrate specificity has not been altered, while the kinetic parameters of mChABC are equivalent to, or more efficient than, the commercial bChABC. Using *in vitro* assays of cell migration and adhesion, we show that the inhibition of CSPGs on Schwann cells integration is overcome when cells secrete mChABC. The level of integration is comparable with bChABC. Finally, through the use of boundary assay, we show mChABC facilitates the integration of Schwann cells into inhibitory astrocytic environments, and this encourages the growth of neurites across the boundary. Overall, we suggest that the application of mChABC secreting Schwann cells would be an effective combination treatment strategy for spinal cord injury.

Supported by the International Spinal Research Trust

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Session V: The cell body response & retrograde signalling after traumatic injury – Chair: Gennadij Raivich

From injury signaling to length sensing in axon growth control

Mike Fainzilber

Dept. of Biological Chemistry, Weizmann Institute of Science, 76100 Rehovot, Israel

mike.fainzilber@weizmann.ac.il

Retrograde axonal injury signaling stimulates regenerative responses by the cell body in lesioned peripheral neurons. We have previously shown that a signaling complex based on importins linked to dynein functions in axonal injury signaling. My presentation will cover recent and unpublished data on new components of this mechanism, and how it might be connected to intrinsic length sensing in neurons, with implications for axonal growth control.

Deciphering the role of c-Jun in the regeneration-associated gene regulatory network

Matthew R.J. Mason[1], S. van Erp[1], K. Bossers[1], A. Behrens[3], G. Raivich[2], J. Verhaagen[1]

[1] Neuroregeneration Group, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands
[2]Perinatal Brain Repair Group, Dept. of Obstetrics and Gynaecology, Institute for Women's Health, University College London, London, UK
[3]Mammalian Genetics Laboratory, Cancer Research UK, Lincoln's Inn Fields Laboratories, London, UK

m.mason@nin.knaw.nl

Successful regeneration requires the up-regulation of a large number of regeneration-associated genes (RAGs), and a growing number of transcription factors have been identified which are activated during regeneration. Among these, c-Jun has been shown to be functionally important for axonal regeneration but its exact role is unclear. As a first step toward dissecting the gene regulatory network controlling RAGs, we have conducted gene expression profiling on regenerating motor neurons in which c-Jun is deleted. The axotomized facial nuclei of floxed-c-Jun/nestin-cre mice and cre-negative littermates were isolated by laser dissection microscopy and processed for microarray analysis.

We identify over 350 genes that are candidate c-Jun targets and have confirmed the neuronal expression of some of these by in situ hybridization and immunohistochemistry. RAG expression profiles range from entirely c-Jun independent to partially or wholly Jun-dependent, while most other transcription factors appear to be independently activated.

Gene ontology (GO) analysis of identified c-Jun-targets reveals known functions of c-Jun, such as apoptosis and cell-cycle control, and other over-represented GO classes linked to regeneration processes, such as cell motility and neuropeptide signaling. We identify a novel class of c-Jun dependent RAGs annotated to muscle specific cell compartments, suggesting a role for stretch-sensing in regeneration. Surprisingly, in the mutants, a large number of genes are upregulated after axotomy compared to the controls, suggesting activation of an alternative gene expression program. GO analysis of these genes reveals functional classes related to synaptic plasticity and axon guidance.

We have also developed promoter analysis techniques using this dataset, in which evolutionarily conserved over-represented transcription factor binding sites are identified. This analysis reveals strong over-representation of AP-1 sites in the c-Jun target genes but also delivers novel insights into the influence of c-Jun on the RAG regulatory network.

Supported by the van den Houten fund

Sustained axon regeneration induced by a synergy between mTOR and STAT3 dependent pathways

Fang Sun[1], Kevin K. Park[2], Stephane Belin1, Dongqing Wang[3], Tao Lu[4], Gang Chen[1], Cecil Yeung[1], Guoping Feng[3], Bruce A. Yankner[4], Zhigang He[1]

[1]F.M. Kirby Neurobiology Center, Children's Hospital, and Department of Neurology, Harvard Medical School, 300, Longwood Avenue, Boston, MA 02115, USA
[2]Miami Project to Cure Paralysis, Department of Neurological Surgery, Miller School of Medicine, University of Miami, Miami, FL 33136, USA
[3]McGovern Institute for Brain Research, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
[4]Department of Pathology, Harvard Medical School, Boston, MA 02115, USA

Fang.sun@childrens.harvard.edu; zhigang.he@childrens.harvard.edu

A formidable challenge in neural repair in the adult central nervous system (CNS) is the long distances that regenerating axons often need to travel in order to reconnect with their targets. Thus, a sustained capacity for axon regeneration is critical for achieving functional restoration. Although deletion of either phosphatase and tensin homolog (PTEN), a negative regulator of mammalian target of rapamycin (mTOR), or Suppressor of cytokine signaling 3 (SOCS3), a negative regulator of Jak/STAT pathway, in adult retinal ganglion cells (RGCs) individually promoted significant optic nerve regeneration, such regrowth tapered off around two weeks after the crush injury1,2. Remarkably, we now find that simultaneous deletion of both PTEN and SOCS3 enable robust and sustained axon regeneration. We further show that PTEN and SOCS3 regulate two independent pathways that act synergistically to promote enhanced axon regeneration. Mechanistically, we discover that expressions of certain genes, including axonal transport components and growth-associated transcription factors, are maintained or activated after axotomy only in RGCs with a deletion of both PTEN and SOCS3. Our results reveal concurrent activation of mTOR and STAT3 pathways as a key for sustaining long-distance axon regeneration in adult CNS, a crucial step toward functional recovery.

This study was supported by grants from Wings for Life (to FS), Miami Project to Cure Paralysis (to KKP) and NINDS (to ZH)

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Session VI: Growth cone biology and regeneration – Chair: James Fawcett

Moderate microtubule stabilization reduces scarring and causes axonal regeneration after spinal cord injury

Frank Bradke

DZNE Bonn, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany

Frank.Bradke@dzne.de

Axons do not regenerate after spinal cord injury because the axons are growth incompetent, and inhibitory factors in the CNS myelin and the scar prevent the axons from regrowing. Microtubule dynamics regulate key processes during scarring, including cell proliferation, migration and differentiation. Moderate microtubule stabilization using the FDA approved drug Taxol prevents axonal retraction and swelling of the axon tip after CNS injury, and stimulates axon growth of cultured neurons enabling them to overcome the growth inhibitory effect of CNS myelin. Moreover, we found that moderate microtubule stabilization decreased scar formation after spinal cord injury in rodents via various cellular mechanisms, including dampening of TGF-β signalling. It prevented the accumulation of chondroitin sulfate proteoglycans (CSPGs) and rendered the lesion site permissive for axon regeneration of growth competent sensory neurons. Additionally, microtubule stabilization promoted growth of CNS axons of the Raphe-spinal tract and led to functional improvement. Thus, microtubule stabilization reduces fibrotic scarring and enhances the capacity of axons to grow. Manipulation of microtubules may offer the basis for a multi-targeted therapy after spinal cord injury.

Regeneration after axotomy - a matter of rapidly turning a local cell-biological crisis into an orchestrated assembly of a growth cone

Micha E. Spira

Dept. of Neurobiology. The Life Sciences Inst. of the Hebrew University of Jerusalem, Jerusalem, Israel

spira@cc.huji.ac.il

A critical step in regeneration of transected axons is the effective transformation of the cut axonal end into a growth cone (GC). The motile GC is a specialized compartment which orchestrates in time and space the use of cell resources for growth processes and integrates extracellular signals into growth patterns. The failure of a cut axonal end to assemble a competent GC apparatus but rather to form a non growing endbulb (EB) is a major barrier for successful regeneration.

In the presentation I will describe the composed cell biological and molecular cascades that triggers, orchestrate and coordinate the assembly of a competent GC, the mechanisms leading to the formation of an EB, and potential mechanisms to rescue the growth processes once EBs are formed.

Cytoskeletal dynamics in sensory axon growth and regeneration

Laura Gumy[1], James Fawcett[2]

[1]Cell Biology, Faculty of Science, Utrecht University, Kruytgebouw, room N504, Padualaan 8, 3584CH Utrecht, The Netherlands
[2.]Centre for Brain Repair, University of Cambridge, Robinson Way, Forvie Site, CB20PY, Cambridge, UK

lauragumy@gmail.com; jwf108@cam.ac.uk

Sensory neuron axon growth and regeneration after injury rely on the specific stabilisation and reorganisation of the microtubule (MT) cytoskeleton. However, the molecular events leading to the re-structuring of the MT network under these conditions remain elusive. We previously found in sensory axons the mRNA of the neural-specific kinesin motor protein Kif3c. This led us to ask whether the Kif3c protein would play a role in axonal growth and regeneration. Kif3c specifically localises to the tip of dynamic MTs in the growth cones which are involved in the process of growth cone turning and pathfinding. By selectively silencing Kif3c, and by using sensory neurons from a Kif3c knockout mouse strain, we show that Kif3c is required for the maintenance of the MTs. The functional importance of this mechanism is represented by the failure of Kif3c knockout axons to grow or regenerate after injury. We conclude that Kif3c is required for axon growth and regeneration by contributing to the maintenance of the balance between microtubule stabilisation/destabilisation.

Session VII: Repair of chronic SCI in whole animal models – Chair: Elizabeth Bradbury

Repair and regeneration of the chronically injured spinal cord

Michael G. Fehlings

Krembil Chair in Neural Repair and Regeneration, Director Neuroscience Program, University of Toronto, Canada

Michael.Fehlings@uhn.on.ca

The chronically injured spinal cord poses unique challenges to achieving functionally significant repair and regeneration. The presence of a glial scar, development of post-traumatic cysts and expression of various inhibitory molecules in the peri-lesional environment pose significant impediments to functional repair strategies. Moreover, these challenges are compounded by the complexity of dealing with cervical lesions which can impact on upper extremity and respiratory function. This talk will focus on the use of combinatorial strategies using adult neural stem cells, derived from ES cells and non-viral IPS cells, to induce remyelination and plasticity and bioengineered approaches to reduce the inhibitory effect of the glial scar using chrondroitinase or a polymeric blend of hyaluronic acid/methylcellulose (HAMC)[1-7]. Recent efforts to assess these approaches in a novel model of compressive/contusive injury at C6 will be discussed as well.

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Chondroitinase ABC and light stimulated recovery of respiratory rhythms in chronically C2 hemisected rats also reveals dramatic plasticity of spinal cord circuitry

Warren J. Alilain[1], X. Li[2], T. E. Dick[1], S. Herlitze[3], J. Silver[1];

[1]Department of Neurosciences, Case Western Reserve Univ., CLEVELAND, OH; USA [2]Columbia Univ., New York, NY; USA [3]Department of Zoology and Neurobiology., Ruhr University-Bochum, Bochum, Germany

warren.alilain@case.edu

Spinal cord injury is often at the cervical level and can lead to breathing complications and death. Diminished respiratory capacity is usually due to impairment or paralysis of the diaphragm. To study these complications and potential therapeutic strategies we utilize the C2 hemisection model of SCI, which disrupts bulbospinal inputs to the phrenic motor nucleus and results in paralysis of the ipsilateral hemidiaphragm. While most of the SCI community is at chronic post-injury states, there are few reparative strategies that have been successful at lengthy times after injury. Previously, we have shown that expression and photostimulation of the light sensitive cation channel channelrhodopsin-2 can induce long lasting recovery of the paralyzed hemidiaphragm at acute stages after C2 injury. Additionally, following C2 hemisection there is a rapid increase in the expression of the chondroitin sulfate proteoglycan containing perineuronal net surrounding phrenic motor neurons. We hypothesized that after infecting rat spinal neurons in and around the phrenic motor pool to express ChR2, light stimulation would restore respiratory motor function in chronic C2 hemisected adult rats. Our results show that expression of ChR2 ipsilateral or contralateral to the lesion and subsequent photostimulation can lead to modest but fragmented and chaotic inspiratory activity to the diaphragm. After combining photostimulation with chondroitinase ABC (ChABC) treatment to remove plasticity inhibiting factors, there is a more profound restoration of hemidiaphragmatic activity that is ordered and rhythmic. These results strongly suggest significant remodeling of respiratory circuitry in the chronically injured animal. Additionally, there is further evidence that photostimulation and ChABC treatment can lower the threshold for the induction of respiratory circuit activity and plasticity. Overall, these sets of experiments demonstrate that light stimulation of ChR2 and ChABC treatment can be used to induce recovery in the chronically C2 hemisected animal; as well as reveal the changes which take place after injury and the capacity for plasticity at both the circuit and systems level.

Evolution of new strategies for enhancing neuromotor recovery after neural injuries

V. Reggie Edgerton

Departments of Integrative Biology and Physiology and Neurobiology., and Neurosurgerty, Brain Res. Inst., Univ. Calif. Los Angeles, USA

vre@ucla.edu

The mammalian lumbar spinal cord has the capability to generate fictive locomotion, i.e. alternating and rhythmic flexion and extension in the absence of oscillating input from either the brain or the periphery, when stimulated pharmacologically or electrically with a tonic stimulation pattern to either the dorsum of the spinal cord or dorsal roots. Although the circuitry within the lumbosacral spinal cord undoubtedly includes those neurons responsible for central pattern generation that can interpret complex proprioceptive input, this feature is not generally recognized as one of its most outstanding. Our experiments show that the spinal circuitry can accurately perceive proprioceptive input to the lumbosacral spinal cord to detect levels of load on the hindlimbs, the speed of a treadmill belt, and the direction of the movement, can serve as the source of control of locomotion in the absence of any input from the brain. Further, repetitive use of this spinal circuitry enables the spinal circuitry to relearn how to step. These observations suggest that several concepts regarding neural mechanisms of motor control in normal movements should be reassessed.

Supported by NIH NS0620091, EB007615 and Christopher Reeve Research Foundation

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Session VIII: Clinical Session: Are neurological devices more likely to improve function after SCI than biologics? – Chair: James Guest

A continuum of strategies targeted at neuroplasiticity for recovery after neurologic injury

Susan Harkema

Professor, Department of Neurological Surgery, University of Louisville, Rehabilitation Research Director, Kentucky Spinal Cord Injury Research Center, Frazier Rehab Institute, 220 Abraham Flexner Way, Louisville, KY 40202, USA

Susan.harkema@jhsmh.org

Studies in animals and humans have shown that the functionally isolated human spinal cord maintains specific properties recognized to generate locomotion in other species. These concepts now have been translated into the clinic by the Christopher and Dana Reeve NeuroRecovery Network of seven rehabilitation centers that provide standardized Locomotor Training to individuals with chronic incomplete spinal cord injury. In seven outpatient rehabilitation centers from the Christopher and Dana Reeve Foundation NeuroRecovery Network (NRN), 206 individuals ranging from 0.9 to 26 years post injury were assessed during intensive Locomotor Training, including step training using body weight support and manual facilitation on a treadmill followed by overground assessment and community integration. While significant improvement from enrollment to final evaluation was observed in balance and walking measures for AIS C and AIS D patients, the magnitude of improvement differed significantly between AIS groups for all measures. These results indicate that rehabilitation that provides intensive activity-based therapy can result in functional improvements in individuals with chronic incomplete SCI even years after injury.

In another study we hypothesized the human spinal locomotor circuitry has sufficient automaticity potential to generate postural control and rhythmic, coordinated weight bearing stepping and that we can recruit this locomotor and postural circuitry with a tonic epidural stimulation of selected lumbosacral segments. We implanted a 23 years old individual and 3.4 years post injury at the time of surgery. He was clinically assessed as an ASIA B, i.e., some sensory, but no motor function below the lesion (C7). We implanted a 5-6-5 electrode array epidurally spanning L2-S1 spinal cord segments and a neurostimulator (Medtronic) capable of stimulating any combination of the 16 electrodes in the array at intensities up to 10.5V and with frequencies ranging from 2-50 Hz. While sitting, without epidural stimulation, we observed minimal EMG activity in all leg muscles**.** While standing in a supportive system without stimulation and with assistance provided at both knee joints by a trainer, little or no observable EMG activity occurred in the leg muscles. With epidural stimulation the transition from sitting to standing was accompanied by an increase in the EMG amplitude by orders of magnitude beyond that observed in the sitting position. In addition, after several months of training he was able to voluntarily move his legs in the presence of epidural stimulation. These results demonstrate the interaction between sensory and epidural regulation of locomotor circuitry. The results also show that a physiological state can be achieved with epidural stimulation so that the sensory input can effectively control the locomotor circuitry to stand and to step.

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Neuroprosthetic approaches to the treatments of SCI

Chet T. Moritz

Departments of Rehabilitation Medicine and Physiology & Biophysics, University of Washington School of Medicine
Seattle, WA 98195, USA

ctmoritz@uw.edu

Brain-computer interfaces and neuroprosthetic technology have the potential to dramatically improve quality of life after spinal cord injury. Building on over 40 years of clinical and basic neuroscience research, we have recently demonstrated that brain activity can be used to control Functional Electrical Stimulation (FES) delivered to muscles and reanimate simple movements of an otherwise paralyzed wrist[1]. Monkeys rapidly learned to modulate the activity of individual neurons in motor areas of the brain in order to control the timing and magnitude of FES delivered to four muscle groups about the wrist.

In addition to direct muscle stimulation, another promising neuroprosthetic approach is intraspinal stimulation. This technique has shown great promise in the lumbar spinal cord with success in reanimating coordinated lower extremity movements, including weight bearing and stepping in animal models. We recently quantified the hand and arm movements evoked by cervical spinal stimulation[2]. Movements of the digits, wrist and arm were readily evoked by intraspinal stimulation in the cervical cord of sedated monkeys. Functional synergies were also commonly evoked, likely due to activation of spinal networks or diverging fibers of passage. Due to the ease of activating complex functional movements from a small number of stimulating sites, intraspinal stimulation may be an ideal method for reanimating paralyzed limbs after. This may be particularly advantageous when brain activity is used to control spinal stimulation to form a complete neuroprosthetic system for the treatment of SCI.

In addition to directly restoring movements, brain-triggered spinal stimulation may also aid in guiding recovery and promoting regeneration after incomplete SCI. Recent work demonstrated that paring activity recorded within the brain with stimulation delivered a short distance away lead to durable increases in connectivity based on Hebbian mechanisms. We are currently testing whether synchronizing brain activity with intraspinal stimulation below an incomplete spinal lesion leads to functional recovery. Such a regenerating neuroprosthetic may have the capacity to direct synaptic strength in spared pathways, either alone or in collaboration with stem cell therapies. Thus neurological devices have great potential for replacing and perhaps even repairing damaged pathways after spinal cord injury.

Supported by a National Institutes of Health EUREKA Award (NIH/NINDS 1R01NS066357) and Ruth L. Kirschstein NRSA (NIH/NINDS F32NS5101), an American Heart & Stroke Association Scientist Development Grant (AHA 09SDG2230091), the University of Washington Royalty Research Fund (#4417), and the Bayley Family Foundation.

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Neuroprostheses and exercise therapy devices: benefits and limitations

Arthur Prochazka

Centre for Neuroscience, University of Alberta, Edmonton AB, T6G 2S2, Canada

arthur.prochazka@ualberta.ca

For several decades clinicians and biomedical engineers have designed and tested a large variety of neuroprostheses: devices that use electrical stimulation (ES) to restore neurological function after stroke or spinal cord injury. Although ES is now widely used to strengthen muscles, only a small minority of people with SCI use neuroprostheses to augment function in activities of daily life (ADLs). This is largely due to the difficulty of restoring complex functions such as dexterous hand movements, unassisted walking, bladder, bowel and sexual function. In able-bodied people these functions are all controlled by many centres in the brain and spinal cord and many peripheral nerves. Neuroprostheses are only able to activate a small number of these neural elements. Furthermore, it is generally not easy to sense the appropriate voluntary or kinematic signals required for controlling stimulation. Nonetheless, in some cases restoring simple elements of movement can make an important difference, for example hand grasp and release, foot-lift, and relaxation of the urethral sphincter. Though there have been attempts to restore more complex movements through intraspinal stimulation, the technical barriers here are formidable.

Exercise therapy clearly plays an important role in maximizing residual function. Devices have emerged over the last decade that encourage exercise in a structured and entertaining way. Cost is an important factor. The role of robotic assistance is a matter of debate. A combination of ES, passive exercise devices and tele-therapy provided in an affordable way shows promise. Regarding the central question in this session, it is clear that for the foreseeable future, devices can augment residual function in useful but limited ways. The technology and expertise that have developed in this area over the last few decades should be seen as a valuable resource in maximizing gains achieved with biological approaches in the future.

Discussion
Electro-stimulation – what's the evidence in repair? – Chair: Michael Craggs

Panel members: Reggie Edgerton, Karim Fouad, Tessa Gordon, Susan Harkema

Table of Contents – Poster Abstracts

Viral expression of GM-CSF: neuroinflammatory response and effects on regeneration

Carolina Acosta-Saltos[1,2], Roman Gonitel[1,2], Seetal Chavda[1], Alejandro Acosta-Saltos[1], Ahad Rahim[4], Adrian Thrasher[2], Patrick Anderson[3], Gennadij Raivich[1]

[1]Perinatal Brain Repair Group, Institute for Women's Health, UCL, UK
[2]Molecular Immunology Unit, Institute of Child Heath, UCL, UK
[3]Department of Anatomy and Developmental Biology, UCL, UK
[4] Gene Technology Transfer Group, UCL, UK

francia.acosta-saltos.09@ucl.ac.uk

To test the effect of microglial activation on the neuronal regenerative response after spinal cord injury, we have created a non-integrating lentiviral vector (NILV) expressing the potent microglia mitogen and activator, granulocyte macrophage colony-stimulating factor (GMCSF). The viral vector is pseudotyped to ensure predominantly neuronal expression of the viral genome and it also encodes enhanced green fluorescent protein (eGFP) to identify transfected neurons and their axons.

In vitro, supernatant from HEK-293T cells infected with GMCSF/eGFP NILV caused a dose dependent increase in cell density of the murine microglial BV-2 cell line. In vivo, stereotactic injections of GMCSF/eGFP NILV to outbred Sprague Dawley (SD) rat CNS revealed an extensive local trauma and a strong neuroinflammatory response. To determine a viral concentration that results in moderate microglia activation with little CNS damage, the motor cortex of SD rats was injected with increasing GMCSF/eGFP NILV titre of 10^3-10^7 Plaque Forming Units/ml (PFU/ml). The results showed, with increasing viral titer, a dose dependent increase in overall microglia density and in the number phagocytic microglia/macrophages which correlated with an increase in TUNEL labelling and a decrease in eGFP+ neuronal profiles. Motor cortex injections with 10^3-10^4 PFU/ml of GMCSF/eGFP NILV produced moderate microglia activation but little or no cell death and little phagocytosis. We are currently testing rats for possible trophic effects of GMCSF (10^4, $3x10^5$, 10^7 PFU/ml) on spinal cord regeneration.

Similar injections of GMCSF/eGFP NILV in outbred CD1 mouse strain revealed a variable response: the majority of animals displayed no tissue damage and little microglia activation, but some were strongly affected. To see whether this low susceptibility is general in mice, or whether there are strains which mount a clear and strong microglial response as that seen in rats, we have screened the response in six inbred mouse strains - C3HN, Balb/C, FVB, SJL, SVJ, and C57Bl6, in addition to outbred CD1 mice. 14 days after viral injection to the striatum, all strains demonstrated efficient transfection revealed by the neuronal expression of eGFP and microglia activation. However they differed in their response to GMCSF/eGFP NILV, with C57Bl/6 showing avid neutrophil recruitment, Balb/C demonstrating strong microglial activation but no phagocytosis and the C3HN strain revealing many activated brain macrophages as those seen in SD rat after GMCSF treatment.

We are currently investigating the dose response to GMCSF in C3H/N and Balb/C mice, the two strains which seem to be most suitable for the study of GMCSF mediated neuronal toxicity and neurotrophic support following spinal cord injury.

Differential localization of integrins in CNS and PNS axons in vivo

Melissa R. Andrews[1], M. R. Mason[2], J. Verhaagen[2], B. Schneider[3], J.C. Bensadoun[3], P. Aebischer[3], J.W. Fawcett[1]

[1]University of Cambridge, Centre for Brain Repair, Robinson Way, Forvie Site, Cambridge, CB2 0PY, UK
[2]Netherlands Institute for Neurosciences, Royal Netherlands Academy of Arts & Sciences, Meibergdreef 47, 1105 BA, Amsterdam, The Netherlands [3]Ecole Polytechnique Fédérale de Lausanne, Brain Mind Institute, EPFL SV BMI LEN, AI 1207, Lausanne, Switzerland

M.R. Andrews - mra36@cam.ac.uk

The regenerative ability of CNS axons decreases with age, however this ability remains largely intact in PNS axons into and throughout adulthood. Some of these differences can be correlated with the age-related silencing of proteins necessary for axon growth and elongation. In previous studies by our lab, we have shown that reintroduction of an integrin receptor that is downregulated in the adult CNS (alpha9 integrin subunit, tenascin-C receptor, $\alpha 9$) can improve neurite outgrowth and axon regeneration in a rodent model of dorsal rhizotomy or a dorsal column crush lesion of the spinal cord. A key finding in our study was the difference observed between the substantial *in vitro* effect and the modest *in vivo* result. As a result, in the present investigation we are evaluating the *in vivo* localization/transport of different integrin subunits within adult CNS axons to determine if the integrin receptors efficiently localized to the growing tips of axons. These studies were performed by ectopically expressing an eYFP-tagged integrin (alpha 6 integrin subunit, $\alpha 6$, laminin receptor, or $\alpha 9$ integrin) using lentivirus injected into uninjured adult rat sensorimotor cortex. In intact cortex, results show that $\alpha 6$-eYFP or $\alpha 9$-eYFP remain mostly in neuronal cell bodies with some observed within the immediate processes. The inclusion of a cervical spinal injury, in an attempt to stimulate transport, did not affect localization within the axon, with the tagged integrin remaining in the cell body or proximal processes. Interestingly in contrast, evaluation of developing rat cortex (P0 aged rat pups) has revealed different results. In these experiments, we observe localization of $\alpha 9$-eYFP both in the neuronal cell body as well as in axons of the corpus callosum and internal capsule. In addition, when $\alpha 9$-eYFP was ectopically expressed in neurons of the dorsal root ganglia (using an adeno-associated virus since lentivirus is unable to transduce DRGs), we found integrin localization throughout the peripheral nerve as well as in the dorsal root. Analysis is ongoing to evaluate the distance within the CNS the integrin is transported in both the postnatal and DRG groups. Together, our results suggest a correlation may exist between the regenerative capacity of axons and the ability of transmembrane receptors such as integrins to localize within axons.

Gene delivery of chondroitinase ABC promotes functional and anatomical improvements following spinal contusion injury in adult rats

Katalin Bartus[1], Nicholas D. James[1], Karen D. Bosch[1], Claudia Kathe[1], Jean-Charles Bensadoun[2], Bernard L. Schneider[2], John H. Rogers[3], Elizabeth M. Muir[3], Elizabeth J. Bradbury[1]

[1]The Wolfson Centre for Age-Related Diseases, Regeneration Group, King's College London, London, UK
[2]Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
[3]Department of Physiology, Development and neuroscience, University of Cambridge, Cambridge, UK

katalin.bartus@kcl.ac.uk

One of the main reactive changes that occur following a spinal cord injury (SCI) is the formation of a glial scar barrier. Chondroitin sulfate proteoglycans (CSPGs) are one of the main classes of inhibitory molecules that are present in the extracellular matrix of the glial scar, and are up-regulated after a CNS injury. The bacterial enzyme chondroitinase ABC (ChABC), which degrades CSPGs, is a promising treatment option for SCI and has been shown to have a number of reparative effects. However, beneficial effects of ChABC have not been fully investigated in a clinically relevant SCI model. Furthermore, previous delivery methods required for prolonged CSPG degradation (e.g. repeated injections, intrathecal catheters) are invasive and prone to infection. Recently, a bacterial chondroitinase cDNA has been engineered that allows the expression and secretion of active chondroitinase enzyme by mammalian cells (Muir et al, J Biotechnol., 2009). Gene delivery of ChABC may have a number of advantages compared to previous treatment paradigms, including sustained CSPG degradation as well as reduced invasiveness. We have evaluated the effectiveness of lentiviral vector delivery of ChABC in an animal model of spinal contusion injury, which represents the most common form of SCI in humans and, therefore, provides a clinically relevant tool for assessing the efficacy of potential therapeutic interventions. Anaesthetized adult rats received a 150kD (Infinite Horizons) contusion injury and lentiviral vector incorporating the ChABC gene or a control GFP was immediately injected rostral and caudal to the injury site. Lentiviral vector delivery of ChABC resulted in prolonged and widespread CSPG degradation in the chronically contused rat spinal cord. This was associated with functional improvements, as assessed electrophysiologically and behaviorally, and significantly reduced cavitation. Additionally, we find increased vascularization of the lesion site following ChABC lentiviral vector treatment and that tissue bridges across the contusion site contained numerous axons. Thus, we demonstrate that sustained and widespread digestion of CSPGs, which is achieved following gene delivery of ChABC, improves functional and anatomical outcomes after a spinal contusion injury.

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Muir EM et al (2010). *J Biotechnol* **145**:103-110

Promoting the survival of transplanted Schwann cells in spinal cord by blocking ATP P2X7 receptor

Juan Luo,[1,3] Yi Zhang,[1] Dongsheng Wu,[1] Sena Lee,[1] John Yeh,[1,2] **Xuenong Bo[1]**

[1]Centre for Neuroscience and Trauma, and [2]Department of Neurosurgery, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London, 4 Newark Street, Whitechapel, London E1 2AT, UK
[3]Department of Physiology, Tongji Medical College, Huazhong University of Science and Technology,Wuhan, 430030, China

x.bo@qmul.ac.uk

Schwann cells (SCs) has been well studied for their potential to repair injured spinal cord. One of the main drawbacks that may limit their clinical use is the extensive cell death after transplantation. Various strategies, including engineered expression of PSA on SCs (Luo et al., 2011), have been tested to improve their survival. However, the outcomes are still far from satisfactory as multiple factors may contribute to the cell death. In the present study, we identify that ATP P2X7 receptor (P2X7R) may contribute to the death of SCs as well. It has been known the prolonged exposure to high concentration of ATP can lead to the death of several types of cells. We showed that P2X7R is present on SCs by immunohistochemistry and PCR. Exposure of isolated SCs to 3-5 mM ATP led to the death of SCs in a concentration-dependant manner. Non-selective pore formation on SC membrane was demonstrated after activation of P2X7R. Pretreatment of SCs with oxidized ATP (oxATP), an irreversible P2X7R antagonist, blocked the ATP-induced SC death. oxATP pretreated SCs were transplanted into uninjured rat spinal cord and it was found that 35% more SCs survived than the untreated SCs one week after transplantation. Moreover, transplantation of SCs isolated from P2X7R deficient mice showed 58% more SCs survived than those from wild-type mice one week after transplantation, which further supports that P2X7R may contribute to the death of transplanted SCs. Taken together, the findings from the current study indicate that targeting P2X7R on SCs could be a potential strategy in preventing cell death after transplantation. Such strategy may be applied to other types of cells that used for transplantation.

Supported by the International Spinal Research Trust Nathalie Rose Barr studentship

Luo J, Bo X, Wu D, Yeh J, Richardson PM, Zhang Y. (2011) Promoting the survival, migration, and integration of transplanted Schwann cells by over-expressing polysialic acid. **Glia** 59: 424-434.

Chondroitinase ABC promotes plasticity of spinal reflexes in peripherally nerve damaged rats

Karen D. Bosch[1], Joost Verhaagen[2], James W. Fawcett[3] and Stephen B. McMahon[1]

[1]Wolfson Centre for Age-Related Diseases, King's College London, Guy's Campus, London Bridge, SE1 1UL, UK; [2]Netherlands Institute for Neuroscience, Amsterdam, Netherlands; [3]Brain Repair Centre, Cambridge University, Cambridge, CB2 0PY, UK

karen.bosch@kcl.ac.uk

Peripheral nerve transection and repair can result in an extensive disruption of central connections, which can lead to long-lasting impairments in motor and sensory function. Here we use elctrophysiological techniques to investigate the effect of intraspinal chondroitinase ABC, an experimental treatment that may promote plasticity, on the spinal flexor reflex following various nerve injuries to the rat brachial plexus. Whole nerve recordings of the response of a flexor nerve (ulnar) to either flexor (median) or extensor (radial) repetitive stimulation at supramaximal C-fibre threshold were made. During median nerve stimulation the number of spikes recorded increased with each successive stimulus, a phenomenon known as wind up. This was not observed during radial nerve stimulation. Ulnar response to median nerve stimulation was 7.3 $\pm$ 1.8 times greater than to radial nerve stimulation in naive animals. After peripheral nerve injuries ulnar nerve wind up upon median nerve stimulation was reduced to 37 + 11% of the uninjured value. Chondroitinase was delivered to the spinal cord via intraspinal injection of a lentiviral vector. Uninjured nerves of animals which had been treated displayed the same pattern and magnitude of wind up as naive animals, so chondroitinase does not affect existing, appropriate spinal reflexes. Responses of the ulnar nerve to both injured flexor and extensor stimulation, however, were significantly increased after spinal cord treatment compared to untreated animals. This was particularly true with the least severe nerve injury studied – a transection and surgical repair; in this case the median nerve wind up recovered to 136 + 23% of naive animals. These results show that a spinal reflex, which collapses after nerve injury, can recover following the application of a plasticity promoting treatment to the spinal cord. We propose that in this way inaccurate wiring in the periphery is compensated for by the amplification of desirable adaptive changes.

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Integration of microchannel neural-electrode interfaces into dorsal and ventral roots for bladder control after spinal cord injury

Daniel Chew[1], E. Delivopoulos[2], N. Granger[3], S. Mosse[4], I. Minev[2], L. Zhu[8], S. McMahon[8], M. Craggs[5], N. Jeffery[6], N. Donaldson[5], S. Lacour[7], J. Fawcett[1]

[1] Cambridge Centre for Brain Repair, Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK [2] Cambridge Nanoscience Centre, Department of Engineering, University of Cambridge, Cambridge, UK [3] The Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Cambridge, UK [4] Implanted Devices Group, Department of Medical Physics, University College, London, UK [5] Division of Surgery and Interventional Science and Spinal Research Centre, RNOH, Stanmore, London, UK [6] Department of Veterinary Clinical Sciences, Iowa State University, Iowa, USA [7] Laboratory for Soft Bioelectronic Interfaces, EPFL, Lausanne, Switzerland [8] Neurorestoration Group, Wolfson CARD, King's College London, London, UK

dc501@cam.ac.uk

One of the debilitating consequences of injury to the supra-sacral spinal cord is loss of conscious bladder control. Depending on lesion completeness, perception of bladder fullness and self-controlled micturition is lost, requiring regular catheterisation. The development of a hyper-reflexic bladder provides some relief of urine. However, the dyssynergia between the detrusor and sphincter muscle activity results in incomplete urine expression and vesicoureteral reflux; and as a consequence, bladder and kidney deterioration and urosepsis. One effective way to restore self-directed micturition in people with spinal cord injury is through implantation of a Sacral Anterior Root Stimulator (SARS), accompanied with an irreversible dorsal root rhizotomy (DRR). The significance of this surgical procedure is the enduring reliance on the stimulator for bladder expression, loss of reflexogenic erection, and the irreversible loss of bladder fullness awareness.

The ultimate project aim is to use the afferent activity from the dorsal roots during bladder filling, to drive SARS output. This is to be accomplished through the design and implantation of dorsal rootrecording electrodes contained within microchannels, through investigation of bladder sensation in rat models. When optimised, these prostheses will provide a stimulatory input to SARS in dogs suffering SCI, with the ultimate goal of clinical translation in humans. To date a newly designed SARS book electrode for the bilateral S2 ventral roots has been implanted successfully in 4 spontaneously spinal cord injured dogs, providing effective bladder expression up to 4 months post implantation using the Finetech-Brindley stimulator.

Initial work has focused on identifying bladder afferent activity in vivo at acute and chronic implantation stages, as well as implantable device design optimisation. We have electrophysiologically recorded bladder afferents after saline infusion, from anaesthetised rats and chronically spinal cord injured dogs, using a variety of microchannel devices, showing activity is modulated by pressure and volume changes of the bladder. Bladder afferent action potentials are small in amplitude compared to cutaneous and muscle spindle spikes, and the root must be 'teased' into fine filaments during recording to improve signal-noise ratio. Implantable microchannels are fabricated from polydimethylsiloxane (PDMS), and embedded nano-meter thin gold electrodes; the microchannel can host fine (100ìm diameter) teased dorsal rootlets that innervate the bladder, namely L6 and S1 in the rat, and S1 and S2 in the dog. Dorsal roots are rhizotomised centrally and fascicules are teased 3-4mm distally and placed within 100um square, 5mm long, microchannels, and contained with a lid. Results after 1 month of L6/S1 unilateral dorsal root implantation show that DRR is an inappropriate method for the implantation procedure, with respect to acute rootlet survival within the microchannel, bladder morphology and electrophysiological activity. At 12 weeks however, activity does return within the rootlets suggestive of a regenerative response, with a notably different activity pattern and threshold decrease. We have designed a surgical technique that allows PDMS microchannels to be wrapped around intact teased dorsal rootlets, which show a greatly improved axon survival. Preliminary results suggest there is also potential for a dorsal root regenerative design of microchannel interfacing. Eventually microchannel electrodes will incorporate a subcutaneously implanted bladder afferent telemeter, which will amplify, filter, and discriminate bladder spike activity.

The potential therapeutic benefit of cortical repetitive transcranial magnetic stimulation on pelvic sphincter "guarding reflex" function to promote continence in incomplete spinal cord injury: a pilot study

Michael D. Craggs, Peter H. Ellaway, Angela Gall, Sarah Knight, Natalia Vasquez

National Centre for Neurospinal Research, London Spinal Cord Injuries Centre, RNOH, Stanmore, UK

michael.craggs@ucl.ac.uk

Bladder, bowel and sexual dysfunction continue to be the main priority problems for people with spinal cord injuries (Anderson, 2004). The principal purpose of this study is to determine whether elements of cerebral control of pelvic function can be restored by the use of repetitive transcranial magnetic stimulation (rTMS) to manipulate the plasticity of the brain and spinal cord in chronic incomplete spinal cord injury (iSCI)[1]. A recent review of potential therapeutic uses for rTMS[2] suggests that, following damage to the CNS, it is likely to be of value since such stimulation increases the ability of the brain to undergo compensatory changes and these have been related to functional benefits.

In a study funded by the ISRT, we showed that at bladder end fill volume, the volume at which detrusor hyperreflexia (neurogenic detrusor overactivity) occurs, the guarding reflex is absent or weak in most patients with a neurologically defined complete supra-sacral spinal cord lesion3. In incomplete lesions, we have shown that the reflex is often preserved but very variable. In contrast to healthy volunteers, who have a very low level guarding reflex consistent with unobstructed voiding, we have also confirmed that for most patients with a supra-sacral lesion the reflex is abnormally present as expected during detrusor-sphincter dyssynergia.

In incomplete SCI with severely compromised voluntary control of bladder and bowel function, the aberrant "guarding reflexes" can be monitored by recording the pudendo-anal reflex (PAR - a neurophysiological measure of the guarding reflex). We have been able to modulate the reflex in control and SCI subjects by single pulses of TMS4. In two control subjects the facilitation of the reflex effected by single pulse TMS was enhanced by a period of 5Hz rTMS5. In a preliminary study we have been able to demonstrate that there may be potential for plasticity in the surviving neural pathways to effect functional recovery.

In this study we have established a short protocol, suitable for testing SCI patients, for revealing the degree to which single pulse TMS facilitates the PAR despite the progressive adaptation that occurs to repeated reflexes. The protocol allows testing facilitation of the PAR at the optimal interval (30ms) of conditioning (TMS) and test (dorsal penile nerve) stimulation. The protocol will be employed in a randomised cross-over trial of rTMS and sham stimulation with male, stable incomplete spinal cord injury (iSCI) subjects.

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Electrical perceptual threshold and monofilaments: towards validation for a quantitative test of cutaneous sensation in spinal cord injury

Peter Ellaway, Maria Catley

Centre for Clinical Neuroscience, Imperial College, London W6 8RP, UK

p.ellaway@imperial.ac.uk

The ability to detect physiological change associated with novel rehabilitation procedures or treatments to effect regeneration in spinal cord injury (SCI) will be challenging using the widely employed American Spinal Injuries Association (ASIA) impairment scales (AIS) for sensory and motor function performed according to the International Standards for Neurological Classification of Spinal Cord Injury. Despite many revisions to the AIS standard neurological assessment, there remains a perceived need for more sensitive, quantitative and objective outcome measures.

The electrical perceptual threshold (EPT) method developed during the ISRT Clinical Initiative[1] uses weak electrical pulses applied to the skin to test cutaneous sensibility. The strength of stimulation is raised until the subject reports skin sensation. Each dermatome can be tested and a quantitative map drawn up showing the sensitivity, impaired or otherwise, in relation to the clinical neurological assessment of the SCI. The EPT is proving to be more sensitive than the AIS sensory assessment[2] and is highly repeatable[3] giving confidence that the test has good reliability. Additionally, evidence suggests that EPT tests the light touch pathway to the brain (dorsal columns) pathway rather than the anterolateral spinothalamic tract for pain and temperature[4,5].

The current project attempts to establish further validity for the EPT test by comparing its sensitivity and reliability with that of Semmes-Weinstein monofilaments, a well-established mechanical test of skin sensitivity that has recently been incorporated into the GRASSP assessment of upper limb function in SCI[6]. Different diameter filaments are applied to the skin and the sensitivity of each dermatome gauged by the stiffness of the filament needed to evoke a sensation of touch.

We have compared the threshold sensitivity of the EPT and monofilaments in control subjects with normal skin sensitivity. Four dermatomes (C4, T1, T8 & L4) were tested bilaterally by the same rater on two occasions separated by approximately one week. Interim analysis revealed significant but poor correlations ($r^2<0.1$, $P<0.05$) between EPT and force (monofilament) thresholds only for C4, T1, & L4 dermatomes. Intra-class correlations between the first and second assessments ranged from 0.48-0.65 for the monofilaments and 0.73-0.81for the EPT, indicating fair reliability for the monofilaments compared to moderately good reliability for the EPT.

The EPT and monofilaments may not test precisely the same modalities of cutaneous sensation: a combination of the two may be advisable for assessment of impairment in SCI. The study has revealed greater reliability for the EPT compared to monofilament testing.

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In vivo model of brachial plexus ventral root avulsion in the rat

Karen Gladwin, Pratipal Kalsi, David Choi

University College London, Institute of Neurology, Department of Brain Repair and Rehabilitation, Queen Square, London, UK

k.gladwin@ucl.ac.uk

Central spinal root avulsion is a longitudinal spinal cord injury which occurs when the spinal nerve root is torn away from the spinal cord at the PNS/CNS transition zone [1]. Avulsions of spinal roots in brachial plexus injuries are commonly caused by trauma such as motorcycle road traffic accidents [1]. The complete avulsion of brachial plexus spinal roots results in intractable pain, and the total loss of motor and sensory function in the affected arm, which is called "flail arm". Motoneurons in the ventral horn of the spinal cord are disconnected from their targets resulting in atrophy and retrograde cell death [1-3]. Before the 1980's the repair of brachial plexus ventral root avulsion and restoration of arm function was not technically possible. However, surgical experiments involving the re-implantation of avulsed ventral roots demonstrated that a large proportion of ventral horn motoneurons survived, were able to extend axons into the re-implanted ventral root and re-innervate peripheral targets [2]. This technique is now used in humans but functional repair has been limited, most likely due to loss of motoneurons available for axonal regeneration and the long distances that axons are require to traverse [1-4]. In an attempt to improve functional outcomes recent research has focused on adjunctive therapies to root re-implantation such as growth factor treatment, cellular therapies or drugs such as riluzole [5]. Here we present the development of a model of ventral root repair in the C8 nerve of the rat which will be used in future studies to investigate the effect of regenerative cell therapies on axonal regeneration. The development of this model included optimization of techniques to minimize bleeding during surgery, an atraumatic method to acquire and re-implant the ventral root, and a decalcifying and cryosectioning protocol to preserve spinal cord and nerve root morphology for immunohistological analysis which we describe below.

Female albino swiss rats were anaesthetised with Isofluorane and placed in a prone position in a custom built stereotactic frame. Using a dorsal approach and the spinous process of T2 as a landmark, the vertebra C7 and T1 were exposed and a hemi-laminectomy performed. The dura was opened exposing the C8 nerve root of which the dorsal root was transected and retracted to allow access to the ventral root underneath. Using a specially adapted blunt hook instrument the ventral root was avulsed and re-implanted by gentle positioning over a small incision in the pia on the ventrolateral surface of the spinal cord. The ventral root was glued in place with Tisseal (Baxter) and the wound closed in anatomical layers. Haemostasis was achieved using Floseal (Baxter) and biopolar coagulation. Two weeks post surgery rats were terminally anaesthetised, perfused transcardially with 4% paraformaldehyde (PFA) and the cervical-thoracic vertebral column dissected and placed in 4% PFA for 24-48 hrs. The spinal column was then dissected free of muscle tissue except the region of surgical implantation and submerged in decalcifying solution for 18 hrs. The tissue was then placed into 20-30% sucrose, soaked in OCT compound for 30 mins with gentle agitation, frozen on dry ice and 20 μm transverse sections cut. Sections were stained with either Haematoxylin and Eosin or antibody labeled for Glial fibrillary acidic protein and neurofilaments. Immunohistological analysis showed the successful avulsion and re-implantation of the C8 ventral root with degeneration of axons in the ventral root.

Results from this protocol demonstrate a reproducible model for studying the efficacy of adjunctive treatments for brachial plexus repair such as cellular based therapies. This model may also be applicable to the study of motoneuron apoptosis, which could be related to other neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) [5].

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Tolerance of three volumes of injected Schwann cells into the contused porcine spinal cord – maximum tolerated dose study

James Guest

Neurological Surgery and the Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Lois Pope LIFE Center, 1095 NW 14th Terrace, 33136 Miami, FL, USA

jguest@med.miami.edu

We are conducting translational studies of Schwann cell (SC) transplantation into porcine thoracic spinal cord contusion injuries. This study determines the maximum tolerated injection volume of cells placed directly into the injury epicenter after moderately severe T8 contusion. Methods. Juvenile Yucatan minipigs underwent peripheral nerve harvest, T7/8 laminectomy, spinal cord contusion, and MRI. Animals were evaluated daily according to the Miami Porcine Walking Scale and Neurological Score. SC were cultured from the harvested nerves. At 14-21d post-injury the pigs underwent reopening of the surgical site, durotomy, and single injections of 50, 100 or 150 μl of Schwann cells (220,000 cells/μl) into the lesion epicenter. Cells were pressure injected using a stereotaxic device during apnea to reduce spinal cord motion. Injection duration ranged from 3-5 minutes+1 minute for dwell and needle removal. Visible cord swelling and cell extrusion were recorded. Motor, sensory and cross epicentre conduction before and after injection was recorded. Post-injection MRI was obtained. Walking and neurological scores were compared before and after the transplant surgery, at approximately 14d animals were perfused, underwent ex-vivo MRI, and histological examination. Results. The 50μl volume was tolerated without altered cord potentials when these could be obtained prior to injection and was not associated with adverse changes in locomotor scores. Furthermore, this volume was well tolerated within the spinal cord cavity with localized graft survival and SC myelin formation. The 150μl volume, however, was associated with transient or permanent reductions in motor scores, loss of intra-procedural monitored potentials in some animals, lengthy rostral-caudal T2 signal changes and ex-vivo MRI and histology showed cellular extrusion and gray matter dissection rostral and caudal to the injection site. Conclusion. In this porcine thoracic contusion model 50μl SC injections were tolerated without adverse changes whereas 150μl were not.

Differential activation of brainstem and spinal locomotor circuits between trained and non-trained rats after a complete spinal cord transaction

Barnaby Marsh[1], Roland R. Roy[2], V. Reggie Edgerton[2] , **Ronaldo M. Ichiyama[1]**

[1] Institute of Membrane and Systems Biology, University of Leeds, Leeds, UK
[2] Brain Research Institute, UCLA, USA

r.m.ichiyama@leeds.ac.uk

Daily locomotor training has been demonstrated to change electrophysiological, morphological and pharmacological properties of spinal motoneurones after a complete spinal cord transection. However, information about changes in brainstem and spinal interneuronal circuits controlling locomotion after injury and rehabilitation is scarce. Forty rats received a complete midthoracic spinal cord transection and were implanted with epidural stimulation (ES) electrodes on L2 and S1. Starting one week after surgery, ten rats were trained daily (5 days/week) 30min/day for 8 weeks to step bipedally over a treadmill belt under ES and quipazine (5-HT2 agonist) administration (i.p., 0.3 mg/kg) using an upper body weight support apparatus. The remaining rats were not trained and either received quipazine only (n=10), ES only (n=10), no treatment (n=5) or no ES implant (n=5). At the end of the training period all rats were tested for their locomotor capacity. In order to induce FOS activation, rats stepped for 60min under ES and quipazine and returned to their cage for another 60min before transcardiac perfusion (4% paraformaldehyde). Brainstem and spinal cords (L1 to L6) were prepared for FOS-immunohistochemistry (IHC) double labelled for Choline Acetyl Transferase, Calbindin or Parvalbumin. Our preliminary results demonstrate that step trained rats showed significant improvements in locomotor behaviour, with greater step height and length, better coordination and consistency of stepping than either of the non-trained groups. Within the spinal cord, FOS IHC revealed that locomotor training selectively activates a subset of spinal interneurones in order to perform a locomotor task. There were higher numbers of FOS positive partition neurones in trained rats compared to non-trained, although there was no difference in activation in lamina IX motoneurones. Moreover, there were no differences in activation of Renshaw cells or Ia-Inhibitory interneurones. Our preliminary results also suggest that during locomotion brainstem activity increases in trained animals compared to non trained transected animals in the cuneate nuclei, the peri-aqueductal grey (PAG), the pontine locomotor nuclei, the pontodorsal tegmental group, the A5 adrenergic region and the cuneiform nuclei. These data demonstrate that reciprocal and recurrent inhibitory pathways in spinal lamina IX are not affected by injury or training. The increased activation of partition interneurones in trained rats suggests one possible intraspinal adaptation to training conducive to improved locomotor performance. Finally, the increased activity of locomotor circuits in the brainstem in trained rats is surprising given that these rats were completely transected.

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Reduced intrinsic regeneration ability of chronically injured sensory axons

Ken Kadoya[1], Ramsey Samara[1], Jiun Do[1], Giovanni Coppola[3], Dan Geschwind[3], Mark H. Tuszynski[1,2]

[1]Department of Neurosciences, University of California – San Diego, CA, USA
[2]Veterans Administration Medical Center, La Jolla, CA, USA
[3] Department of Neurology, University of California, Los Angeles, CA, USA

kkadoya@ucsd.edu

Previous studies showed a marked reduction in regenerative capacity of chronically injured spinal cord axons compared to acutely injured axons, an effect likely due to a combination of intrinsic and extrinsic factors. To induce meaningful axon regeneration in chronic spinal cord injury (SCI), treatments need to address these factors, but they are not clearly understood. To study chronically injured spinal cord axons, we characterized a cervical dorsal column lesion (DCL) and dorsal column sensory axons as a model system, and we examined the intrinsic regenerative ability of chronically injured sensory axons.

Adult rats were subject to C3 DCL, followed by sacrifice 1 week, 6 weeks, 6 months, and 15 months later. Immunohistochemistry for CSPGs and GFAP of lesion sites showed that glial scars surrounded lesion sites 6 weeks after injury and persisted 15 months after injury. CTB immunolabeling exhibited that retraction of injured sensory axons still occurred between 6 months and 15 months after SCI. These findings suggest that glial scar and axonal retraction still hamper axonal growth prolonged time after injury. Regarding to cell bodies of injured axons, no death or atrophy was detected in lumbar DRGs 15 months after injury. In vitro neurite outgrowth assay also demonstrated that lumbar DRG neurons 15 months after injury had an equivalent ability of neurite outgrowth and sensitivity to sciatic nerve conditioning lesions (CL), compared to naïve neurons. Microarray analysis of lumbar DRGs revealed that gene expression profiles 7 months after C3 DCL were extensively overlapped with naïve DRGs and that CLs applied 7 months after C3 DCL could initiate quite similar gene expression profiles to CLs applied to naïve subjects. These findings indicate that the regenerative ability in cell bodies of chronically injured sensory axons was maintained at prolonged time after C3DCLs.

Next, we investigated how the extent of axon regeneration changed when treatments were delayed. Treatments consisted of the bone marrow stromal cell graft into lesion sites and CLs. CLs were performed 1 week prior to cell grafts. When treatments were delayed at 6 weeks, 4 months, and 15 months after injury, there were 61%, 87%, and 93% reduction in the number of axons in the graft, compared to acute treatment. To determine the relative contribution of glial scars, axonal retraction, and other possible extrinsic factors to reduced axon regeneration in chronic SCI, we retransected dorsal column sensory axons at C7 at various time points after initial C3 DCL, and the same treatments were applied acutely. In this fresh C7 lesion site, there was no established glial scar and axonal retraction. Notably, when these C7 retransection and treatments were performed 6 weeks after initial C3 injury, the number of axons in the graft was completely restored, that was equal to the acute treatment. However, when these C7 retransection and treatments were applied 4 months and 15 months after initial C3 injury, there were still 69% and 64% reduction in the numbers of axons in the graft, compared to acute treatment. These findings suggest that the intrinsic regeneration ability of chronically injured sensory axons significantly reduces 4 months after injury, even though the regeneration ability of neuronal cell bodies is still maintained. To explore mechanisms underlying this reduced intrinsic regeneration ability, cellular and ultra-structural studies are ongoing.

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Promoting neurological recovery by maximising sensory-motor activation during stepping and walking: development and assessment of robotics-assisted delivery platform

Danial Kahani, Bernie Conway

University of Strathclyde, Bioengineering department, Glasgow , G4 0NW UK

Danial.Kahani@strath.ac.uk

In recent years there has been increasing recognition that intensive rehabilitation can promote better functional outcomes for patients with incomplete spinal cord injury. The scientific justification behind this relates to the ability of the human nervous system to remodel itself in response to changing demands. This is termed neuro-plasticity and it is the natural process behind skill acquisition and learning throughout life. It is also believed to be the key process that leads to recovery of neurology following traumatic spinal cord injury. Accordingly, successful rehabilitation is closely related to the ability of a therapy programme to influence neuroplastic processes. Once we recognize that neuroplasticity is a process that influences outcome, it becomes imperative to ensure that rehabilitation programmes are delivered in a way that targets the key neurophysiological processes. In Spinal Cord Injury intensive weight assisted treadmill training has been demonstrated to have positive effects on over ground walking in recovering incomplete patients (Galen et al 2011; Ellaway et al 2011). The therapy requires treadmill training of 1 hour per day, for up to 8 weeks during which time the patient is assisted to walk on a treadmill by therapists or by robotic devices that move the legs to simulate realistic stepping movements. As the patient attempts to walk the assistance provided by the robot helps to create a patterned sensory feedback from the moving limbs that promotes neuroplastic actions which re-engage the neural pathways that control stepping. The most common robotic locomotor training system for use in spinal cord injury is the Hocoma Lokomat. This training system comprises a robotic gait orthosis that supports reciprocal hip and knee motion to recreate the kinematics of the step cycle. The current Lokomat device does not incorporate any control of the ankle joint. In this device, the ankle joint is passively restrained by elastic straps. Critically, new research has demonstrated that control over the ankle joint is vital for effective stepping and recent research highlights that this control is associated with corticospinal pathways (Barthélemy et al 2010). We therefore believe that successful recovery of walking requires these pathways to be operational and that targeting therapy to promote plasticity in pathways that participate in the control the ankle joint during walking will lead to improved voluntary stepping in patients.

This project is investigating methods we believe will target pathways that support corticospinal control of the ankle and which can be integrated into devices like the Lokomat to ultimately improve their effectiveness as rehabilitation aids.

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Respiratory function following cervical contusion injury in rat spinal cord

Michael A. Lane, S. Hussey, K. Salazar, L.M. Mercier, B.E. O'Steen, D.D. Fuller[1], P.J. Reier

Departments of Neuroscience (College of Medicine) and Physical Therapy[1] (College of Public Health and Health Professions), University of Florida, Gainesville, USA

michael.lane.@mbi.ufl.edu

High cervical spinal cord injury (SCI) often results in compromise of the phrenic motor system, diaphragm dysfunction and, in severe cases, ventilator dependency. Respiratory impairments and associated complications are the leading cause of morbidity and mortality in people with cervical SCIs. While experimental studies have revealed some spontaneous recovery following lateral C2 hemisection (selective white matter lesion), less is known about the plasticity potential following more clinically relevant injuries. As is the case with human SCI, experimental contusion injury results in combined white and grey matter damage. Given the experimental and translational advantages such a model offers, the aim of the present study was to determine the extent of respiratory compromise that occurs following mid-cervical contusion and assess the potential for plasticity and therapeutic enhancement of recovery.

Adult female Sprague-Dawley rats received either midline or lateral cervical contusion injuries at C3/4 (Infinite Horizon; 150-250 kilodynes). Plethysmography was used to assess ventilation (e.g. breathing frequency and tidal volume) both prior to injury and on a weekly basis thereafter. Ventilatory measurements were made under baseline (breathing normoxic, normocapnic air) and hypercapnic (normoxic gas containing 7% CO_2) conditions. All animals were then left to recover for 1-12wks post-injury. At the end of the study, animals were anesthetized for terminal measurements of diaphragm activity. The diaphragm was exposed and bilateral EMG recordings made in spontaneously breathing animals under baseline and hypercapnic conditions. In a subset of animals, telemetric electrodes were implanted in the diaphragm to obtain repeated EMG recordings Anterograde (biotin dextran amine) and transynaptic retrograde tracing (pseudorabies virus) were used to examine the changes in phrenic circuitry following injury.

All contusion injuries resulted in substantial gray matter compromise, extending into the ventral horns. While there was extensive white matter sparing, anterograde tracing revealed that some projections from inspiratory neurons in the medulla were compromised by contusion. There was a significant impairment in the diaphragm response to respiratory challenge (hypercapnia) 1 week post-injury which persisted for at least 3 months post-injury. Unexpectedly, ventilatory function remained relatively unaffected. It is therefore likely that compensatory plasticity accounts for deficits in diaphragm function and contributes to maintenance of ventilation post-injury. Relative to what has been reported following C2 hemisection, this also suggests that the deficits in diaphragm activity are associated with predominant grey matter loss. Transneuronal tracing results showed increased interneuron labeling rostral to the lesion epicenter, one week post-injury. While requiring further investigation the altered interneuronal labeling may reflect anatomical plasticity post-contusion. Experiments are underway to test the therapeutic efficacy of neural progenitor transplantation in this injury model, to enhance repair and improve diaphragm function.

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Viral vectors generate active chondroitinase in vivo and promote sprouting of corticospinal axons in spinal cord injury

João Nuno Alves[1,2], Rong-Rong Zhao[1], Melissa R. Andrews[1], Joost Verhaagen[3], Elizabeth Moloney[3], Roger J. Keynes[2], James W. Fawcett[1], John H. Rogers[2], **Elizabeth M. Muir[2]**

[1]Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge CB2 0PY,U.K
[2]Department of Physiology Development and Neuroscience, University of Cambridge, Tennis Court Rd, Cambridge CB2 3EG, UK
[3]Laboratory for Neuroregeneration, Netherlands Institute for Neuroscience, Meibergdreef 47,1105BA Amsterdam, The Netherlands

emm1@mole.bio.cam.ac.uk

The enzyme chondroitinase ABC has been shown to promote axon regeneration in spinal cord injury and is therefore a potential component of treatment. Because of the risks attending prolonged or repeated infusions, gene therapy could be a desirable route of administration. We have modified the bacterial chondroitinase gene so that it can direct efficient secretion of active chondroitinase from mammalian cells, and inserted it into lentiviral and AAV vectors, to allow long-term expression by glia and/or neurons in the CNS. Whereas lentiviral vectors transduce neurons and glia locally, AAV vectors can also be retrogradely transported up axons so as to transduce neurons that project to the site of injection. Lentiviral and AAV chondroitinase vectors were injected into adult rat cortex. Both produce widespread chondroitinase activity, as assessed by 'stub' antibody staining, including activity in the corpus callosum and contralateral cortex, indicating secretion from long-range axonal projections. To test the effect in spinal cord injury, lentiviral chondroitinase vector was injected adjacent to a dorsal column crush lesion at level C4. The corticospinal tract was then traced by anterograde labelling with BDA, and the animals were perfused 4 weeks post-lesion. Corticospinal axons showed reduced retraction, enhanced regrowth along the edge of the lesion cavity, and enhanced lateral sprouting proximal to the lesion. These results show that viral vectors can deliver chondroitinase efficiently to the adult CNS, and that it has biological activity comparable to direct injection of the enzyme.

Potential pitfalls of fMRI in the spinal cord

Moreno Pasin, Ahmed Toosy, Claudia Wheeler-Kingshott

Department of Neuroinflammation, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, UK

moreno.pasin.10@ucl.ac.uk

Neural activity can be inferred and quantified non-invasively using functional MRI (fMRI). Although much progress has been made with brain fMRI in recent years, there is increasing interest in applying fMRI to the spinal cord. This is challenging mostly due to issues concerning physiological motion artefacts, lower signal-to-noise, susceptibility change between tissue and bone, lack of specific analysis software.

There are methods to reduce the physiological noise that rely on cardiac and respiration triggering (RETROICOR) but better localization of the activations in spine will result from further work on the acquisition and image analysis technique. Moreover there is need to develop further registration and noise reduction methods. The challenge is greater at high magnetic field strengths (3T and above) because of the greater geometrical distortions of Gradient Echo (GE) Echo Planar Imaging (EPI) methods used for BOLD fMRI.

These distortions have been partially overcome by employing parallel imaging methods in the cervical cord, but cannot be employed to study thoracic and lumbar sections, especially in the transverse plane, because of fold-over artefacts from the surrounding body. However, using a localization technique, such as the ZOnally Oblique Multislice (ZOOM) sequence it should be possible to investigate all levels of the spinal cord. Recent work has also addressed the problem of spinal cord motion (macroscopic and physiological) and its effect on fMRI activation when acquiring longitudinal (sagittal) non-EPI images or cervical transverse EPI images. Registering subsequently acquired volumes of transverse spinal cord EPI images is not so obvious because of the lack of anatomical markers (especially in the superior-inferior direction).

We are working to implement a fMRI sequence (ZOOM) for transverse imaging of the spinal cord that is sensitive to BOLD changes during functional activation, using a reduced field-of-view (FOV) excitation to minimise geometrical distortions, no fold-over artefacts in the phase encoding direction (hence applicability to the entire cord) and a better signal-to-noise ratio properties for robust fMRI activation detection.

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Transcription factor STAT3 is required for efficient regeneration after peripheral nerve injury

Smriti Patodia[1], M. Makwana[1], P. Anderson[2], A. Behrens[3], P. Rumajogee[1], M. Hristova[1], G. Raivich[1]

[1]Centre for Perinatal Brain Protection and Repair, Institute for Women's Health, UCL, 86-96 Chenies Mews, WC1E 6HX, UK
[2]Cell and Developmental Biology, Anatomy Building, UCL, Gower Street, WC1E 6BT, UK
[3]Mammalian Genetics Laboratory, Cancer Research UK, London, WC2A3PX, UK

s.patodia@ucl.ac.uk

Unlike CNS, sensory and motor axons normally do regenerate after peripheral nerve injury. These regenerating neurons strongly upregulate several molecules including transcription factors, cell adhesion molecules, cytoskeleton-regulatory proteins, neuropeptides, and cytokines, and these molecules are suggested to play a key role in successful nerve regeneration and functional recovery.
Signal Transducers and Activators of Transcription (STATs) comprise a family of transcription factors that mediate a wide variety of biological functions in the central and peripheral nervous systems; *in vitro* STAT3 promotes neurite growth from primary sensory neurons. Here, we explored the *in vivo* neuronal function of STAT3 in axonal regeneration after facial nerve axotomy using Cre/loxP with the homozygous floxed STAT3 gene deleted by cre recombinase expressed under the neuron-specific synapsin promoter; littermates without cre served as controls.

Following transection of right facial nerve, STAT3 deletion caused profound defects in the usual retrograde response, with a severe early and persistent reduction by 75-90% in microglial activation and recruitment of lymphocytes, and 2 weeks after injury in perineuronal sprouting of the CGRP+ and galanin+ facial motoneurons. At 30 days, axotomized STAT3-deficient motoneurons appeared shrunk by 50-60% in size (from 19.5±0.9 to 11.2±0.5um, n=7) but displayed no cell death ((#co-#ax)/#co: -9±7%, n=7). This was unlike their control counterparts which underwent chromatolytic enlargement by 10-20% (from 19.2±0.5 to 22.1±0.8um, n=7) and revealed a significant level of neuronal cell death (27±4%, n=7). Regeneration and functional recovery was dramatically reduced and the mutant animals showed 80% less target muscle reinnervation. As with neuronal c-Jun deletion which interferes with axonal regeneration, microglial activation and lymphocyte recruitment (Raivich et al Neuron 2004), expression of regeneration-associated molecules CD44, α7β1 integrin and the nuclear translocation of ATF3 molecules was also greatly impaired or abolished in the STAT3 deficient motoneurons. However, the expression of c-Jun itself was not affected, suggesting that deletion of either transcription factor – c-Jun or STAT3 – will produce a very similar regeneration-deficient phenotype.

The inflammatory effect of poly-Inosinic: poly-Cytidylic acid (poly I:C) on rat motor cortex- is this a conducive environment for cortico-spinal axon regeneration?

Catherine Pringle, Carolina Acosta-Saltos, Gennadij Raivich, Patrick N. Anderson

Department of Cell and Developmental Biology, University College London, Gower Street, London, WC1E 6BT, UK

cjpringle@doctors.org.uk

Peripheral nervous system (PNS) axons regenerate spontaneously after injury, accompanied by a local inflammatory response. Central nervous system (CNS) axons however, lack both this spontaneous regenerative capacity and accompanying inflammation, with CNS injury causing debilitating clinical symptoms. We explored whether generating a 'safe' level of inflammation within motor cortex, using poly-inosinic: poly-cytidylic acid (poly I:C) a pro-inflammatory toll-like receptor 3 (TLR3) agonist, produces an environment conducive for cortico-spinal axon regeneration.

Poly I:C was stereotacticaly injected into rat motor cortex against a control injection of phosphate buffer solution (PBS) in the contra-lateral hemisphere. To establish a non-toxic concentration we tested three concentrations of poly I:C - at 0.1mg/ml, 1.0 mg/ml and 5.6mg/ml. Three days post treatment; we found a dose dependent increase of microglia and astrocyte density. At the highest poly I:C concentration there was visible tissue damage, but at 1mg/ml there was moderate microglia activation with no necrosis. To investigate the neuroinflamatory changes with the non-necrotic dose of poly I:C, we explored the effects of the 1mg/ml dose at day 3, 7, and 14 post poly I:C at 1mg/ml concentration, with PBS injection as control. In the presence of poly I:C, microglia and astrocyte numbers reached a peak at day 3 and decreased by 14 days. Moreover, single injection of poly I:C strongly enhanced functional recovery (Whisker hair movement) following facial nerve cut.

We are currently examining these potentially pro-regenerative effects of Poly I:C treatment on speed of outgrowth and quantitative reinnervation of target tissue after facial axotomy, and on effects on CNS regeneration after spinal cord injury.

Reliability of tract-specific q-space imaging metrics in healthy spinal cord

Torben Schneider, Olga Ciccarelli, Carolina Kachramanoglou, David L. Thomas, Claudia A.M. Wheeler-Kingshott

NMR Research Unit, Department of Neuroinflammation, UCL Institute of Neurology, London, UK

t.schneider@ucl.ac.uk

Introduction: We investigate accuracy and sensitivity of tract-specific q-space imaging (QSI) metrics in healthy controls. The clinical potential of q-space metrics in the assessment of white matter diseases has been shown previously in both the brain and spinal cord. However, most clinical QSI studies only focused on a small number of patients and failed to demonstrate the reliability of QSI. The aim of this study is to report reproducibility of QSI metrics in the cervical spinal cord on a standard 3T clinical MRI scanner. We also assessed QSI measures both in-plane (XY) and parallel to the main spinal cord axis (Z), not presented before. We compare QSI measures derived in gray matter and different ascending and descending white tracts of the cervical spinal cord in healthy subjects and investigate associations between QSI parameters and conventional apparent diffusion coefficient (ADC) measures, both in plane and along the cord.

Imaging protocol: We recruited 10 healthy subjects to be scanned on a 3T Philips Achieva MRI scanner. Five subjects were recalled for a second scan on a different day to assess intra-subject reproducibility of QSI derived parameters. We performed cardiac-gated high b-value axial DWI over three different DWI directions: two directions perpendicular (XY) and one parallel (Z) to the main spinal cord axis. From the signal we compute the displacement probability density function (DPDF) in each voxel. Maps of the full width at half maximum (FWHM) and zero displacement probability (P0) were derived for XY and Z. For comparison we also computed the apparent diffusion coefficient (ADC) from the monoexponential part of the decay curve ($b \leq 1100 s/mm^2$) for both XY and Z directions.

ROI analysis: We semi-automatically delineate the whole cervical spinal cord area (SCA) between levels C1 and C3 on the b=0 images. In addition, four regions of interest (ROI) were manually placed in specific white matter tracts and one ROI was positioned in the gray matter on all slices between level C1 and C3. The four white matter regions comprised the left and right tracts running in the lateral columns and the anterior and posterior tracts.

Statistical processing: We report reproducibility as the intra-subject coefficient of variation (COV) for the five scan/rescan subjects and the inter-subject COV among all ten subjects. Further, we compare significant differences in the group mean values of the ADC parameters (ADCxy, ADCz) and QSI metrics (P0xy, P0z, FWHMxy, FWHMz) between tracts by performing the Hotellings-T^2 test. To investigate the relevance of measurements in the Z direction, we compute the same significance test of XY-only QSI parameters (P0xy, FWHMxy).

Results: Reproducibility: In both intra-subject scan/rescan experiments and among subjects we observe a consistently lower COV in QSI metrics compared to ADC measurements. In particular, ADCxy shows the largest intra- and inter-subject variation (>25%) in most tracts. In contrast, tract-specific QSI measurements vary less, and the majority of observed CoVs are between 5-10%.

Tract-specific differences: We find significant group differences in QSI and ADC parameters between different white matter tracts. Most significant differences were found in the parallel direction ($p<0.01$) while no significant differences were found between any tracts using perpendicular measurements. In addition, the combination of XY&Z QSI and ADC metrics discriminated between lateral and posterior and anterior tracts with high significance ($p<0.001$).

Discussion & Conclusion: QSI metrics obtained without sequence development, using standard DWI protocol available on a 3T clinical scanner, show a good reproducibility that is superior to simple ADC analysis. We observe tract-specific correlations between ADC and QSI parameters. We further demonstrate that QSI parameters provide complementary metrics that allow discrimination of white matter tracts in healthy controls in conjunction with ADC. Our findings also suggest that the Z direction provides additional information to perpendicular measurements.

Skin-derived precursors differentiated into Schwann cells (SKP-SCs) transplanted eight weeks post thoracic spinal cord contusion improve recovery of motor function and bladder pathology

Assinck, Peggy[1], Dworski, Shaalee[2], Sparling, Joseph[3], Duncan, Greg J.[3], Wu, Di L.[3], Jiang, Yuan[3], Liu, Jie[3], Lam, Clarrie K.[3], Kwon, Brian K.[1], Miller, Freda[2], **Wolfram Tetzlaff[3]**

[1]ICORD, UBC, Vancouver, BC, Canada, [2]Hospital for Sick Children/University of Toronto, Toronto, ON, Canada, [3]ICORD, UBC/ICORD, Vancouver BC, BC, Canada

tetzlaff@icord.org

Cell transplantation has emerged as a promising candidate therapy for spinal cord injury. However, the best candidate cell for a transplantation-based treatment of SCI remains a matter of intense investigation. Our laboratories have previously shown that Schwann cells differentiated from skin-derived precursors (SKP-SCs), when transplanted 7 days after contusion injury, promote histological repair and functional recovery in rats (Biernaskie et al. 2007). SKPs are potentially suitable for autologous transplantation; however this would require several weeks time to grow up sufficient SKP-SC from a patient, and that SKP-SCs are still effective in the (sub-)chronic spinal cord injury environment. Here, we transplanted one million SKP-SC cells from GFP-expressing transgenic Sprague Dawley rats into the lesion site of adult female SD-rats at 8 weeks post T9/T10 contusion injury and allowed survival until week 29. Immunosuppression with CyclosporineA was used to prevent rejection. Behavioral analysis shows that SKP-SC transplantation prevented the decline of forelimb and hindlimb stride length (Catwalk) and elicited higher BBB scores, which reached significance at week 19-21 post injury. However, there was a general decline in health and function during weeks 25-29 in both rat groups which we presently attribute to the chronic immunosuppression. By histology, we found surviving SKP-SC in all transplanted rats; and between 70,000-120.000 SKP-SC we represent in 7 of our 15 rats ("top 7") at 21 weeks post transplantation. Ki67 immunoreactivity indicated very little (<0.03-0.05%) proliferative activity in this chronic stage. Cellular bands of SKP-SCs bridged the lesion sites in predominantly rostro-caudal orientation and showed good integration into the host spinal cord. This was also reflected by significantly less GFAP immunoreactiviy at the lesion transplant interface in rats with good transplant survival. SKP-SC and astrocyte processes interdigitated extensively at this host transplant interface which was crossed by numerous axons; and these SKP-SC bridges were massively filled with neurofilament positive axons (of a wide caliber range) running predominantly in rostro-caudal orientation. Immunoreactivity for serotonin transporter (SERT) or tyrosine hydroxylase revealed many axons growing into and through these SKP-SC bridges with some crossing the distal interface, indicative of true regeneration. Fewer axons were positive for CGRP or SP, markers of sensory fibers from the periphery. About 70% of the transplanted SKP-SCs produced P0-positive myelin and there was a significant contribution by endogenous Schwann cells also myelinating axons in these bridges as well as in the spared host rim of the spinal cord. Hence, the number of P0-positive fibers were significantly higher in SKP-SC transplanted rats, indicating a stimulation of an endogenous Schwann cell repair response by SKP-SC transplantation. Interestingly, in a post hoc analysis, the media control treated animals show a significant increase in bladder wall thickness as compared to the SKP-SC treated animals, suggesting improved bladder function in the transplanted group. In summary, our results highlight the potential of SKP-SCs as a possible cell for autologous transplantation in the subchronic state of spinal cord injury. We are now testing the effects of SKP-SC after chronic cervical hemicontusion injuries with cells from isogenic donors – as the next milestone on the road to translation.

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Post-injury ketogenic diet improves gross and skilled forelimb motor function after cervical SCI in rats

Streijger, Femke[1]; Plunet, Ward T.[1]; Lee, Jae HT[1]; Liu, Jie[1]; Lam, Clarrie K.[1]; Park, Sophia[1]; Assinck, Peggy[1]; Kwon, Brian K.[2]; **Wolfram Tetzlaff[1]**

[1]University of British Columbia, International Collaboration on Repair Discoveries (ICORD), Vancouver, BC, Canada
[2]Department of Orthopaedics, University of British Columbia, Combined Neurosurgical and Orthopaedic Spine Program (CNOSP), Vancouver, BC, Canada

tetzlaff@icord.org

We discovered previously that dietary restriction in rats (every-other-day fasting, EODF) initiated *after* injury, improved outcomes from SCI. However, an intermittent fasting regimen is understandably met with little enthusiasm by clinicians, whose patients lose up to 40% of their (paralyzed) muscle mass in the first 2 months post-injury. Hence, we have shifted our focus to the ketogenic diet (KD), a diet high in fat and very low in carbohydrates, that mimics certain aspects of fasting including the production of neuroprotective ketone bodies *without* severe calorie restriction. KD is known to be beneficial in a wide range of neurodegenerative disease models, including traumatic brain injury and stroke, yet the mechanisms are poorly understood. Of note, KD is successfully used for drug-resistant epilepsy in humans.

Here, we investigated the high fat, low carbohydrate ketogenic diet (KD) as a possible treatment for acute cervical SCI in rats. Four hours following C5 hemi-contusion injury, adult male rats were allowed access to either a standard carbohydrate-based diet or KD. Forelimb function was evaluated for 14 weeks. Following SCI, KD-treated rats showed improved usage and motor control of the affected paw during rearing and grooming behavior. In addition, KD regimen improved pellet-retrieval of the ipsilateral limb and recovery of wrist and digit movements. Importantly, after returning to a standard carbohydrate-based diet after 12 weeks of KD treatment, the beneficial effects on forelimb function remained stable for at least 6 weeks. No effects on body weight gain, cholesterol levels or bone morphology/density were observed. Histology revealed a significant gray matter but not white matter sparing effect, a trend to less inflammatory infiltration and significantly increased expression of the glucose and monocarboxylate transporters (GLUT1 and MCT1) in the lesion penumbra.

Taken together, KD treatment not only enhanced gross forelimb movement, but also improved recovery in fine-motor skills in two reaching tasks as observed in two independent experiments which emphasizes the robust nature of this diet-induced effect. Thus, we conclude that a short term KD regimen enhances functional recovery from SCI in rats and has lasting therapeutic effects without apparent side effects.

From a translational perspective, our results mandate the reconsideration of standard clinical practices, which have traditionally promoted high carbohydrate nutritional content in acute SCI patients. The optimization of such aspects of patient care is highly desirable, particularly when the influence of nutritional status on secondary injury becomes apparent in animal studies. We encourage opening further dialogue on this important aspect of patient care in acute SCI, given the limited interventions currently available for this devastating injury.

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Modulation of chloride homeostasis as a new target to treat spasticity after SCI

Laurent Vinay, Bos R., Boulenguez P., Bras H., Brocard C., Liabeuf S., Sadlaoud K.

Laboratoire Plasticité et Physio-Pathologie de la Motricité, Centre National de la Recherche Scientifique (CNRS) & Aix-Marseille Université, CNRS, 31 chemin Joseph Aiguier, F-13402cx 20, Marseille, France

vinay@dpm.cnrs-mrs.fr

A decrease in synaptic inhibition –disinhibition- appears to be an important substrate in several neuronal disorders, such as spinal cord injury (SCI), neuropathic pain... Glycine and GABA are the major inhibitory transmitters in the spinal cord. An important emerging mechanism by which the strength of inhibitory synaptic transmission can be controlled is via modification of the intracellular concentration of chloride ions ([Cl-]i) to which receptors to GABA/glycine are permeable. Briefly, a low [Cl-]i is a pre-requisite for inhibition to occur and is maintained in healthy neurons by cation-chloride co-transporters (KCC2) in the plasma membrane, which extrude Cl-.

We showed recently (Boulenguez et al., Nature Medicine, 2010) that KCC2 is down-regulated following SCI in rats, particularly in motoneuron membranes, thereby depolarizing the Cl^- equilibrium potential and reducing the strength of postsynaptic inhibition. This result can account for the hyperexcitability of spinal reflexes and reduced inhibition which are commonly associated with spasticity after SCI. Blocking KCC2 in intact animals by intra-thecal injection of DIOA reduces the rate-dependent depression (RDD) of the Hoffmann reflex as observed in spasticity. RDD is also decreased in KCC2-deficient mice and in intact rats after intrathecal injection of BDNF which down-regulates KCC2. The early decrease in KCC2 after SCI is prevented by pre-treatment with the BDNF-sequestering TrkB/Fc chimera protein. Conversely, two weeks after SCI, BDNF up-regulates KCC2 and restores RDD. Our results open new perspectives for the development of therapeutic strategies to alleviate spasticity and chronic pain after SCI.

Mammalian chondroitinase ABC encourages Schwann cell integration within inhibitory environments

Philippa M. Warren*; J.C. Kwok*; M. Smith*; E.M. Muir[+]; J. Rogers[+]; R. Keynes[+] & J.W. Fawcett*

* Cambridge Centre for Brain Repair, Forvie Site, Cambridge, CB2 0PY, UK
+ Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3DY, UK

pmw44@cam.ac.uk

Schwann cells transplanted into the CNS promote axonal regeneration and remylination[1,2] thus are an effective bridging material for spinal cord injuries. However, grafted Schwann cells show little integration with host astrocytes and reduced migration from the site of transplantation[3-6]. This results in the formation of a graft-host boundary which regenerating axons fail to transgress[7,8]. The reactive astrocytes at the graft-host boundary express, among other molecules, inhibitory chondroitin sulphate proteoglycans[9,10] (CSPGs). It has been shown that the application of bacterial chondroitinase ABC (bChABC) at the graft-host boundary reduces inhibition of the CSPGs and facilitates the integration of Schwann cells and astrocytes[7]. We have developed a chondroitinase ABC construct that can be expressed and secreted from mammalian cells[11] (mChABC). Here we investigate the biochemical properties of mChABC showing that the optimum temperature, pH and substrate specificity has not been altered, while the kinetic parameters of mChABC are equivalent to, or more efficient than, the commercial bChABC. Using *in vitro* assays of cell migration and adhesion, we show that the inhibition of CSPGs on Schwann cells integration is overcome when cells secrete mChABC. The level of integration is comparable with bChABC. Finally, through the use of boundary assay, we show mChABC facilitates the integration of Schwann cells into inhibitory astrocytic environments, and this encourages the growth of neurites across the boundary. Overall, we suggest that the application of mChABC secreting Schwann cells would be an effective combination treatment strategy for spinal cord injury.

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Delegates

First Name	Last Name	Location	Email Address
Carolina	Acosta-Saltos	London	francia.acosta-saltos.09@ucl.ac.uk
Warren	Alilain	Cleveland	wja4@case.edu
David	Allan	Glasgow	spinalunit@ggc.scot.nhs.uk
Melissa	Andrews	Cambridge	mra36@cam.ac.uk
Mark	Bacon	Bramley	mark@spinal-research.org
Susan	Barnett	Glasgow	susan.barnett@glasgow.ac.uk
Katalin	Bartus	London	katalin.bartus@kcl.ac.uk
Armin	Blesch	Heidelberg	Armin.Blesch@med.uni-heidelberg.de
Xuenong	Bo	London	x.bo@qmul.ac.uk
Karen	Bosch	London	karen.bosch@kcl.ac.uk
Elizabeth	Bradbury	London	elizabeth.bradbury@kcl.ac.uk
Frank	Bradke	Bonn	Frank.Bradke@dzne.de
Thomas	Carlstedt	Stanmore	carlstedt.thomas@googlemail.com
Daniel	Chew	Cambridge	dc501@cam.ac.uk
David	Choi	London	d.choi@ion.ucl.ac.uk
Michael	Craggs	Stanmore	michael.craggs@ucl.ac.uk
Franck	Debarbieux	Marseille	franck.debarbieux@ibdml.univmed.fr
Reggie	Edgerton	Los Angeles	vre@ucla.edu
Wagih	El Masry	Oswestry	wagih.elmasri@rjah.nhs.uk
Peter	Ellaway	London	p.ellaway@imperial.ac.uk
Mike	Fainzilber	Rehovot	mike.fainzilber@weizmann.ac.il
James	Fawcett	Cambridge	jf108@cam.ac.uk
Michael	Fehlings	Toronto	Michael.Fehlings@uhn.on.ca
Karim	Fouad	Edmonton	karim.fouad@ualberta.ca
Robin	Franklin	Cambridge	rjf1000@cam.ac.uk
Sujay	Galen	Glasgow	sujay.galen@strath.ac.uk
Karen	Gladwin	London	k.gladwin@ucl.ac.uk
Tessa	Gordon	Toronto	tessat.gordon@gmail.com
Nicolas	Granger	Cambridge	ngranger@oncovet.net
James	Guest	Miami	jguest@med.miami.edu
Laura	Gumy	Utrecht	lauragumy@gmail.com
Jodie	Hall	Columbus	jodiehall@hotmail.com
Susan	Harkema	Louisville	susan.harkema@jhsmh.org
John	Hick	London	jwahick@waitrose.com
Stephen	Hunt	London	hunt@ucl.ac.uk
Nazakat	Hussain	Sheffield	nazakat.hussain@sth.nhs.uk
Ronaldo	Ichiyama	Leeds	r.m.ichiyama@leeds.ac.uk
Steve	Jacques	Wolverhampton	sjj894@bham.ac.uk
Lyn	Jakeman	Columbus	Jakeman.1@osu.edu
Nicholas	James	London	nicholas.n.james@kcl.ac.uk
Nick	Jeffery	Ames	njeffery@iastate.edu
Ken	Kadoya	La Jolla	kkadoya@ucsd.edu
Danial	Kahani	Glasgow	danial.kahani@strath.ac.uk
Pratipal	Kalsi	London	p.kalsi@ucl.ac.uk
Frank	Kirchhoff	Homburg	Frank.Kirchhoff@uniklinikum-saarland.de
Peter	Kirkwood	London	peter.kirkwood@ucl.ac.uk

Naomi	Kleitman	Bethesda	KleitmaN@ninds.nih.gov
Jeffrey	Kocsis	West Haven	jeffery.kocsis@yale.edu
Timea	Konya	Bramley	timea@spinal-research.org
Michael	Lane	Gainesville	michael.lane@mbi.ufl.edu
Ann	Logan	Birmingham	a.logan@bham.ac.uk
Daljeet	Mahay	Birmingham	d.mahay@bham.ac.uk
Milan	Makwana	London	milanmakwana@hotmail.com
Matthew	Mason	Amsterdam	m.mason@nin.knaw.nl
Kidangalil	Mathew	Sheffield	kidangalil.mathew@sth.nhs.uk
Stephen	McMahon	London	stephen.mcmahon@kcl.ac.uk
Adina	Michael-Titus	London	a.t.michael-titus@qmul.ac.uk
Chet	Moritz	Seattle	ctmoritz@uw.edu
Pouria	Moshayedi	Cambridge	pm406@hermes.cam.ac.uk
Elizabeth	Muir	Cambridge	emm1@mole.bio.cam.ac.uk
Moreno	Pasin	London	moreno.pasin01@libero.it
Smriti	Patodia	London	smriti_17@hotmail.com
John	Priestley	London	j.v.priestley@qmul.ac.uk
Catherine	Pringle	London	cjpringle@doctors.org.uk
Arthur	Prochazka	Edmonton	arthur.prochazka@ualberta.ca
Geoffrey	Raisman	London	G.Raisman@ion.ucl.ac.uk
Matthew	Ramer	Vancouver	ramer@icord.org
Leanne	Ramer	Vancouver	Leanne@ICORD.org
John	Riddell	Glasgow	j.riddell@bio.gla.ac.uk
John	Rogers	Cambridge	jhr11@cam.ac.uk
Jane	Roskams	Vancouver	roskams@zoology.ubc.ca
Torben	Schneider	London	t.schneider@ion.ucl.ac.uk
Michael	Sofroniew	Los Angeles	Sofroniew@mednet.ucla.edu
Micha	Spira	Jerusalem	spira@cc.huji.ac.il
Patrick	Stroman	Kingston	stromanp@queensu.ca
Fang	Sun	Boston	Fang.Sun@childrens.harvard.edu
Wolfram	Tetzlaff	Vancouver	tetzlaff@icord.org
Natalia	Vasquez	London	nativasquezp@hotmail.com
Joost	Verhaagen	Amsterdam	J.Verhaagen@nin.knaw.nl
Laurent	Vinay	Marseille	vinay@dpm.cnrs-mrs.fr
Philippa	Warren	Cambridge	pmw44@cam.ac.uk
Claudia	Wheeler-Kingshott	London	c.wheeler-kingshott@ion.ucl.ac.uk
Hiromi	Yamada	Fujisawa	gr10306509y@st.brs.nihon-u.ac.jp
Binhai	Zheng	La Jolla	binhai@ucsd.edu