SPINAL RESEARCH

fighting paralysis... and winning

6-7 September 2012

The 14th Spinal Research Network Meeting

Spinal Cord Injury
"Golden opportunity for future generations"

ABSTRACTS

ABSTRACTS

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

POSTER PRESENTATIONS

Poster session is scheduled from 5pm in Hendrix & Madonna Suite at the end of the first day, immediately after the main meeting, on Thursday, 6th September. There will also be time during the coffee and lunch breaks on Friday to view the posters.

SCIENTIFIC ORGANIZING COMMITTEE

Professor Sue Barnett PhD
University of Glasgow

Professor Robin Franklin BSc BVetMed PhD MRCVS FRCPath
University of Cambridge

Professor Ann Logan PhD
University of Birmingham

Professor Gennadij Raivich MD DSc
University College London

Table of Contents – Presentation Abstracts

Thursday 6th September

Friday 7th September

Session 1: Regulators of CNS regeneration
Chair: Joost Verhaagen

Molecular models of poor axonal regeneration

Gennadij Raivich

Perinatal Brain Repair Group, Institute for Women's Health, University College London, UK

g.raivich@ucl.ac.uk

The robustness of axonal regeneration following peripheral nerve injury – unlike the situation in the central part – has long prompted interest into what makes it more or less successful. Peripheral regeneration is not always perfect. For example, repair following chronic disconnection is associated with severe reduction in the speed of regeneration and the extent of recovery, pointing to time constraints in the presence of at least some proregenerative factors in the freshly injured nervous system. Nerve injury in the neonate also causes extensive death of the lesioned neurons with impaired functional recovery, highlighting a maturation-dependent defect in trophic support.

On molecular level, peripheral injury unleashes neural expression of a host of effector molecules including various cell adhesion glycoproteins, neuropeptides, cytokines and neurotrophins, and growth-permissive cytoskeletal adaptor molecules. So far, single effector molecule deletions produced only mild or at most moderate reduction in axonal regeneration, pointing to a multitude of complementary and compensatory pathways. However, this appears to change with direct deletion of transcription factors – c-Jun, SOX11, STAT3, cEBPbeta - which could act as master switches for the synthesis of many different effector molecules[1].

Studies into the role of c-Jun have begun to shed light on two surprisingly different models of poor regenerative response using the facial nerve axotomy paradigm. In the first model, neuronal c-Jun is a double edged sword – it is required for robust regeneration, the chromatolysis and the molecular response to axotomy. However, it also causes the moderate neuronal cell loss normally observed in this adult model of nerve injury[2]. These effects are abolished following neuron-specific deletion of c-Jun using synapsin promoter driven Cre recombinase in floxed jun mice[3], abbreviated as the syn-jun mutants.

In contrast, c-Jun expressed after injury in Schwann cells appears essential for providing trophic support required for successful regeneration[4]. Deletion of c-Jun in peripheral nerve Schwann cells (using p0 promoter driven Cre recombinase in floxed jun mice, the p0-jun mutants) increases neuronal cell death by 2-3 fold, to levels observed in neonatal injury, even though most of the cell body response is not affected. Axonal regeneration is reduced, but most of the defect in target reinnervation and functional recovery appears due to excessive neuronal cell death. This deletion of Schwann cell c-Jun interferes with local production of neurotrophins, including BDNF, LIF, GDNF and Artemin; in the same vein supplementation with exogenous GDNF and Artemin promotes neuronal survival, target reinnervation as well as functional recovery. *In vitro*, anisomycin-enhanced c-Jun phosphorylation strongly enhanced Schwann cell synthesis of GDNF and Artemin[4]. Moreover, global replacement of all 4 N-terminal c-Jun phosphorylation sites (Ser63&73, Thr91&93) with alanines (jun4A) *in vivo* also reproduced most of the phenotype observed with the p0 jun mutants.

Interestingly, further cell-type specific deletions also appear to fall into one of these 2 categories above. Thus, neuronal deletion of STAT3 completely reproduces the syn-jun phenotype; codeletion experiments make it likely that c-Jun and STAT3 act as two keys for the same, common programme. On the other hand, neuronal deletion of the beta1 integrin reproduces the changes observed with p0-jun mice, suggesting that this neuronal adhesion molecule is also critically involved in trophic signalling. Here, better understanding into the non-overlapping parts of the regeneration programme could help to improve structural defects in peripheral nerve injury, but also use this knowledge to enable long distance regeneration – perhaps up to the appropriate target – in the central nervous system.

References

1. Patodia S, Raivich G (2012) Front Mol Neurosci. 2012;5:8
2. Raivich G, Bohatschek M et al (2004) 43:57-67
3. Ruff CA, Staak N et al (2012) J Neurochem 121:607-18
4. Fontana X, Hristova M et al (2012) J Cell Biol 198:127-41

Functional roles of chondroitin sulfate glycosaminoglycans in axon regeneration

Linda C. Hsieh-Wilson

Division of Chemistry and Chemical Engineering, California Institute of Technology and the Howard Hughes Medical Institute, Pasadena, CA 91125, USA

lhw@caltech.edu

A major obstacle to functional recovery after CNS injury is the inhibitory environment encountered by regenerating axons. Chondroitin sulfate (CS) polysaccharides and their associated proteoglycans (CSPGs) are the principal inhibitory components of the glial scar, which forms after neuronal damage and acts as a barrier to axon regeneration. It is well established that the inhibitory activity of CSPGs is primarily derived from their CS chains, as chondroitinase ABC (ChABC) treatment promotes axon regeneration, sprouting, and functional recovery after injury *in vivo*. However, the mechanisms by which CS polysaccharides inhibit axon re-growth are not well understood, limiting the development of molecular approaches to counteract CSPGs. We will describe the synergistic application of organic chemistry and neurobiology to understand how CS polysaccharides contribute to neuronal growth and regeneration. By taking advantage of our ability to synthesize defined oligosaccharides, we demonstrate that a specific sugar epitope on CSPGs, chondroitin sulfate-E (CS-E), potently inhibits axon growth. CS-E functions as a protein recognition element to engage receptors, including the transmembrane protein tyrosine phosphatase PTPσ, thereby triggering downstream signaling pathways that inhibit axon growth. Masking the CS-E motif using a CS-E-specific antibody reverses the inhibitory activity of CSPGs and stimulates axon regeneration *in vivo*. Targeting specific sugar epitopes using antibodies, small molecules, or other approaches may offer a more stable, selective, and less immunogenic alternative to ChABC. Given that CS-E appears to interact with multiple receptors, strategies that block the sulfated CS-E epitope may also prove more effective at neutralizing CSPGs than targeting individual receptors or pathways.

Supported by NIH R01 GM093627-03, Roman Reed Spinal Cord Injury Research Fund, and Christopher & Dana Reeve Foundation.

Transcriptional and epigenetic regulation of neuroregeneration

Simone Di Giovanni

Hertie Institute for Clinical Brain Research, University of Tuebingen, D-72076 Tuebingen, Germany

simone.digiovanni@medizin.uni-tuebingen.de

The ability of injured neurons to mount a pro-axonal regenerative response is highly deficient in the central nervous system (CNS), in part due to a lack of a pro-regenerative gene expression program. The post-axotomy neuronal gene expression response is regulated at both the transcriptional and at the epigenetic level, including DNA methylation and histone modifications.

Importantly, transcription factors and histones share a number of post-translational modifications (PTMs) that dictate the nature of the transcriptional environment on specific promoters. Ultimately, it is the composition of these PTMs that regulates gene expression contributing to neurite outgrowth and axonal re-growth following lesions. Although several transcription factors have been shown to play a role in CNS axonal outgrowth and regeneration, the role of the epigenome is still largely obscure. In the last several years we have found a novel role for the transcription factor and tumor suppressor p53 in neurite outgrowth, axonal regeneration and sprouting in the injured facial nerve and spinal cord (Di Giovanni et al. EMBO J, 2006; Floriddia et al., J Neurosci, 2012). Importantly, we detected that active pro-neurite outgrowth p53 is acetylated and forms a transcriptional complex with the histone acetyltransferases (HAT) CBP/p300 and PCAF to occupy promoters and drive the expression of a number of pro-axonal regeneration associated genes both *in vitro* and *in vivo* (Tedeschi et al., Cell Death and Diff, 2009; Gaub et al., Cell Death and Diff, 2010). These mechanisms are responsible to drive neurite outgrowth on permissive as well as on inhibitory substrates in several neuronal populations, including cortical, dorsal root ganglia and cerebellar granule neurons (Tedeschi et al., Cell Death and Diff, 2009; Tedeschi et al., J Neurosci, 2009; Gaub et al., Cell Death and Diff, 2010). Importantly, we found that overexpression of the histone acetyltransferase p300 in retinal ganglia neurons promotes axonal regeneration of the crushed optic nerve by enhancing the expression of p53-dependent gene transcription as well as by epigenetic modifications (Gaub et al., Brain, 2011). More recently, in search for epigenetic marks that may modify the capacity of injured axons to re-grow, we discovered that select histone PTMs play a role in the capacity of DRG neurons to regenerate, while DNA methylation does not seem to be significantly regulated in injured DRG neurons. Specifically, we found that H3K9 acetylation occurs on a number of regeneration associated genes and that this is mediated by the HAT PCAF, which is activated by retrograde signaling following sciatic nerve injury but not after dorsal column lesions in the spinal cord. In fact, PCAF occupies and acetylates a number of pro-regeneration promoters driving their gene expression only when regeneration occurs such as after a sciatic nerve lesion. More importantly, viral overexpression of PCAF in DRG neurons is able by epigenetic mechanisms to drive axonal regeneration in the injured spinal cord, where regeneration is otherwise absent. These experiments have revealed novel transcriptional and epigenetic pathways relevant for axonal regeneration that may hopefully represent novel therapeutic targets to foster neurological functional recovery.

References

1. Floriddia EM, Rathore KI, Tedeschi A, Quadrato G, Wuttke A, Lueckmann M, Kigerl KA, Popovich PG, **Di Giovanni S**. P53 regulates the neuronal intrinsic and extrinsic responses affecting the recovery of motor function following spinal cord injury. J Neurosci. *In press.*
2. Gaub P, Joshi Y, Naumann U, Wuttke A, Schnichels S, Heiduschka P, **Di Giovanni S**. The histone acetyltransferase p300 promotes intrinsic axonal regeneration. Brain. 2011 134, 2134-2148.
3. Tedeschi A, Nguyen T, Gaub P, Puttagunta R, **Di Giovanni S**. A p53-CBP/p300 transcription module is required for GAP-43 expression, axon outgrowth and regeneration. Cell Death Differ. 2009 Apr;16(4):543-54.
4. Gaub P, Tedeschi A, Nguyen T, Schmandke A, Puttagunta R, **Di Giovanni S**. HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse via CBP/p300 and P/CAF-dependent p53 acetylation. Cell Death Differ. 2010 Sep;17(9):1392-408.
5. Tedeschi A, Nguyen T, Steele SU, Feil S, Naumann U, Feil R, **Di Giovanni S**. The tumour suppressor p53 transcriptionally regulates cGKI expression during neuronal maturation and is required for cGMP-dependent growth cone collapse. J Neurosci. 2009 Dec 2;29(48):15155-60.
6. **Di Giovanni S**, Knights C, Beers J, Mahadev Rao, Yakovlev A, Catania J, Avantaggiati ML, and Faden AI. The tumor suppressor protein p53 is required for neurite outgrowth and axon regeneration. EMBO J. 2006 Sep 6;25(17):4084-96.

Supported by the Hertie Foundation

Session 11: Influence of environmental factors on axonal sparing
Chair: Stephen McMahon

Lipolytic enzymes and lipid mediators in SCI

Sam David

Centre for Research in Neuroscience, The Research Institute of the McGill University Health Centre, Montreal, Quebec, H3G 1A4, Canada

sam.david@mcgill.ca

Injury-induced inflammation contributes to secondary damage after spinal cord injury (SCI). The immune responses that are triggered by injury are complex and are mediated by a variety of factors. This presentation will focus on our work on the phospholipase A_2 (PLA_2) superfamily and the downstream pathways that generate a large number of bioactive lipid mediators, some of which have pro-inflammatory and demyelinating effects, while others have anti-inflammatory and pro-resolution properties. PLA_2 enzymes act on membrane glycerophospholipids to yield a free fatty acid and lysophospholipid. Some free fatty acids such as arachidonic acid (AA) give rise to eicosanoids that promote inflammation, while others such as docosahexaenoic acid (DHA) are protective. In addition, some lysophospholipids such as lysophosphatidylcholine cause demyelination. Our work show that in a mouse model of SCI two cytosolic forms of PLA_2 [calcium-dependent PLA_2 group IVA ($cPLA_2$ GIVA) and calcium-independent PLA_2 group VIA ($iPLA_2$ GVIA)], and a secreted form [secreted PLA_2 group IIA ($sPLA_2$ GIIA)] are up-regulated. Using selective inhibitors and null mice, we found that these PLA_2s play differing roles. $cPLA_2$ GIVA mediates protection, whereas $sPLA_2$ GIIA and, to a lesser extent, $iPLA_2$ GVIA are detrimental. Furthermore, completely blocking all three PLA_2s worsens outcome, while the most beneficial effects are seen by partial inhibition of all three. The latter compound also upregulates the protective $cPLA_2$ IVA. We have also found that oral administration of fenretinide, a semi-synthetic analogue of retinoic acid, reduces arachidonic acid and increases DHA in the injured spinal cord, and reduces secondary damage and improves locomotor recovery. These studies suggest that drugs that inhibit detrimental forms of PLA_2 ($sPLA_2$ and $iPLA_2$) and upregulate the protective form ($cPLA_2$) or that alter the balance of protective versus detrimental fatty acid may be useful for targeting the inflammatory response in SCI.

A novel gene therapy approach for reprogramming intraspinal inflammation

Phillip G. Popovich

Department of Neuroscience, Wexner Medical Center at The Ohio State University, Center for Brain and Spinal Cord Repair, USA

phillip.popovich@osumc.edu

An inevitable consequence of traumatic spinal cord injury (SCI) is the accumulation of macrophages within and nearby the site of injury. These cells persist indefinitely, closely apposed to intact cells and axons. The precise role played by macrophages is controversial. Recently, we showed that most intraspinal macrophages adopt a neurotoxic "M1" phenotype[1]. M1 macrophages cause axonal "die-back" *in vitro* and *in vivo* and may also contribute to protracted cell death[1,2]. Although some newly activated macrophages become non-toxic pro-regenerative "M2" macrophages, this phenotype is not maintained by cells in/nearby the site of injury[1]. Instead, undefined factors in the injury microenvironment (or lack thereof) polarize macrophages toward an M1 phenotype[1]. A goal of our current research program is to learn how to reprogram the natural course of macrophage activation such that the ratio of M1:M2 macrophages is reduced. One way to accomplish this is to manipulate the composition of the extracellular milieu such that newly activated monocytes or microglia differentiate into M2 macrophages. Preliminary data will be presented showing that systemic post-injury injection of a novel adeno-associated virus (AAV9)[3] engineered to produce interleukin-4 (IL-4) augments M2 macrophage activation *in vivo*. Ongoing studies will determine whether functionally significant axon regeneration can be achieved after SCI by simultaneously manipulating neuron extrinsic (macrophages) and intrinsic (e.g., *Pten*) barriers to axon regeneration.

References

1. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of Two Distinct Macrophage Subsets with Divergent Effects Causing either Neurotoxicity or Regeneration in the Injured Mouse Spinal Cord. J Neurosci 29: 13435-13444.
2. Horn KP, Busch SA, Hawthorne AL, van Rooijen N, Silver J (2008) Another Barrier to Regeneration in the CNS: Activated Macrophages Induce Extensive Retraction of Dystrophic Axons through Direct Physical Interactions. J Neurosci 28: 9330-9341.
3. Foust KD, Nurre1 E, Montgomery CL, Hernandez A, Chan CM & Kaspar BK, Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes (2009) Nat Biotechnol. 27(1):59-65.

Supported in part by the International Spinal Research Trust, The Craig H. Neilsen Foundation and the Ray W. Poppleton Endowment

Collaborators: Kristina A. Kigerl, Jodie C. Hall, Brian K. Kaspar, Kevin D. Foust, Zhigang He

Transplanted NPCs instruct phagocytes to remodel the injured spinal cord tissue

Stefano Pluchino

Dept of Clinical Neurosciences, Cambridge Centre for Brain Repair and Cambridge Stem Cell Institute, University of Cambridge, E.D. Adrian Building, Forvie Site, Robinson Way, Cambridge, CB2 0PY, UK

spp24@cam.ac.uk

Compelling evidence exists that neural stem/precursor cells (NPCs) possess peculiar therapeutic plasticity. It is becoming clearer that transplanted NPCs simultaneously instruct several therapeutic mechanisms in addition to cell replacement. Here we interrogated the therapeutic plasticity of NPCs after their focal implantation in the severely contused spinal cord. We injected syngeneic NPCs at the proximal and distal ends of the contused mouse spinal cord, and performed assessment of locomotor functions, major pathological secondary events, cell fate of transplanted NPCs with endogenous cells, gene expression and inflammatory infiltration. We considered two different doses of NPCs and two treatment regimes, either subacute (7 days) or early chronic (21 days) after the induction of experimental thoracic spinal cord injury (SCI). Only the subacute transplantation of NPCs ameliorated the recovery of locomotor functions of SCI mice. Transplanted NPCs survived undifferentiated at the level of the perilesion environment and established contacts with endogenous phagocytes via cellular-junctional coupling. This was associated to significant regulation of the expression levels of major inflammatory mRNAs *in vivo*. Remarkably, transplanted NPCs reduced the proportion of inflammatory myeloid cells at the injury site – including M1-like macrophages and dendritic cells – and, in turn, led to the healing of the injured cord. We identify a precise window of opportunity for approaches to complex SCIs with therapeutically plastic somatic stem cells and suggest that NPCs posses the functional promise to correct the local environment from '*hostile*' to '*instructive*' for either the healing or the regeneration past the lesion.

Session III: Neural progenitor/stem cells to treat SCI
Chair: Sue Barnett

Long-distance growth and connectivity of neural stem cells after severe spinal cord injury

Mark H. Tuszynski, Paul Lu, Binhai Zheng, James M. Conner, Martin Marsala

University of California, Sand Diego, USA

mtuszynski@ucsd.edu

We explored the hypothesis that developing neurons exhibit activated intrinsic growth capabilities that enable axonal extension and relay formation after even severe adult spinal cord injury. Rats underwent complete T3 spinal cord transections. Two weeks later, a clinically relevant time point, neural stem cells (NSCs) expressing GFP were isolated from E14 rat spinal cord and embedded into fibrin matrices containing growth factor cocktails, and were grafted to the subacute lesion site. When examined seven weeks later, grafted cells differentiated into multiple cellular phenotypes, including neurons, which extended large numbers of axons over remarkable distances. Extending axons formed abundant synapses with host cells. Axonal growth was partially dependent on mammalian target of rapamycin (mTOR) but not Nogo signaling. Grafted neurons supported formation of electrophysiological relays across sites of complete spinal transection, resulting in BBB scores of 7 in NSC-grafted animals compared to 1.5 in controls ($P<0.01$). Electrophysiological and functional recovery were abolished by spinal cord re-transection immediately rostral to the graft implant site. Two human stem cell lines (566RSC and HUES7) embedded in growth factor-containing fibrin matrices exhibited similar axonal growth, and 566RSC cells supported functional improvement. Thus, properties intrinsic to early stage neurons can overcome the inhibitory milieu of the injured adult spinal cord to mount remarkable axonal growth resulting in formation of novel relay circuits that significantly improve function. These therapeutic properties extend across stem cell sources and species.

Respiratory dysfunction and cellular repair following mid-cervical spinal cord injury (cSCI)

Paul J. Reier[1], D.D. Fuller[2], K.-Z. Lee[2, 3], M. Sandhu[2], L. Mercier[1], M.A. Lane[1]

Departments of Neuroscience[1] (College of Medicine) and Physical Therapy[2] (College of Public Health and Health Professions), University of Florida, USA, Gainesville and Dept. Biological Sciences, National Sun Yat-sen University, Taiwan[3]

reier@mbi.ufl.edu

The majority of laboratory studies of respiratory function following cSCI to date have focused on C2 hemisection (C2Hx) lesions that primarily model interruption of bulbospinal projections to phrenic motoneurons (PhMNs). Such injuries typically result in a rapid, shallow (R-S) breathing pattern. However, despite substantial loss of ipsilateral hemidiaphragm (ipsiDIA) function, a significant increase in bilateral diaphragm activity can be elicited by brief exposure to hypercapnia or hypoxia. Meanwhile, more frequent mid-cSCIs in humans involve contusions and/or compressions at or near the phrenic nucleus (C3-C5/6) and can result in an impaired ability to respond to greater ventilatory demands. We and others have demonstrated that such respiratory insufficiency can be modeled experimentally by either lateralized or midline contusion injuries at the level of the phrenic nucleus (C3/4). R-S breathing, however, is not evident except in cases of severe white matter damage. Thus, the results of studies involving lateralized C3/4 cSCIs suggest that partial bulbospinal innervation of PhMNs caudal to the injury is sufficient for maintaining normal respiratory frequency, whereas a lasting impairment in ipsiDIA response to respiratory challenge can be attributed to a loss of PhMNs and interneurons, and/or partial denervation of PhMNs caudal to injury. With emphasis on the latter, we first wished to determine whether restoration of gray matter continuity with interneuronal progenitor-enriched transplants would result in augmented ipsiDIA responses to challenge. Midline contusions were performed on adult female Sprague-Dawley rats at a pre-determined force of 150 kilodynes (Infinite Horizon pneumatic impactor, Lexington KY). The injury epicenters were re-exposed one week post-injury and a suspension of dissociated E13-14 rat spinal cord tissue was introduced into cystic cavities. Diaphragm activity, ipsi- and contralateral to injury, was assessed terminally via EMG recordings at 5 weeks post-injury (4 weeks post-transplant) and revealed some restoration of diaphragm responses to respiratory challenges in transplant recipients. Pseudorabies virus (PRV; transynaptic retrograde tracer) was used to examine the phrenic circuitry in each experimental group and test for host-transplant integration. PRV delivered to the diaphragm resulted in phrenic motoneurons and second-order host interneuronal labeling in all animals. In addition, infection of donor neurons was observed suggesting synaptic integration with the host phrenic circuit. Anterograde tracing of electrophysiologically-identified inspiratory neurons in the ventral respiratory column and immunohistochemistry for serotonergic axons failed to show substantial growth into donor tissue. In contrast, PRV injected directly into grafts revealed transneuronal labeling of host interneurons in the cervical spinal cord, confirming synaptic host-graft integration. Our results offer functional and anatomical evidence for a host-transplant-host relay circuit that may modulate phrenic activity post-injury via “gray matter repair” and short propriospinal host-graft connectivity.

Supported by NINDS NS054025 (PJR), NIH 1 R21 HL104294 (DDF), and the Anne and Oscar Lackner Chair in Medicine (PJR)

Building a neuronal relay to reconnect the injured spinal cord

Joseph F. Bonner, Michel A. Lemay, **Itzhak Fischer**

Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, USA

ifischer@drexelmed.edu

Introduction. Despite progress in elucidating neural stem cell properties and advances in developing transplantation strategies, neuronal cell replacement following CNS injury remains difficult due to the challenges of survival, differentiation, and synaptic connectivity of neurons in the lesioned CNS. The spinal cord provides a therapeutically relevant and scientifically convenient system to solve these issues in a model designed to build a neuronal relay between injured dorsal column axons and the denervated dorsal column nucleus (DCN), and to restore the deficits in sensory function. Our experimental strategy has been based on a series of studies with Dr. Mahendra Rao that examined the properties of neural stem cells derived from the developing spinal cord. These studies established the advantages of using neuronal restricted progenitors (NRP) to obtain neurons *in vivo* (without instructive cues from the host), the need to include glial restricted progenitors (GRP) when cells are grafted into a lesion (to support neuronal survival and differentiation), and the ability to direct graft-derived axonal growth along a neurotrophin gradient (generated by lentivirus vectors expressing BDNF).

Results. A mixed population of NRP/GRP, derived from the embryonic spinal cord of alkaline phosphatase (AP) transgenic rats, were grafted acutely into a dorsal column lesion at C1. One week later, BDNF-expressing lentivirus was injected into the DCN to guide graft axons to the appropriate target. The presence of a functional relay was analyzed by following 1) the fate of the grafted cells (neuronal phenotype and axonal growth), 2) the regeneration of host sensory neurons (CTB tracing), 3) formation of synaptic connections (expression of synaptic proteins and presence of synaptic structures by EM), and 4) activity across the relay (expression of c-Fos following stimulation and conduction along the relay with extracellular recordings in the DCN). Our results demonstrated regeneration of anterogradely traced sensory axons into the graft and robust growth of graft-derived AP-positive axons along the neurotrophin gradient into the DCN. Immuno-EM revealed excitatory synaptic connections between regenerating host axons and graft-derived neurons at C1 as well as between graft axons and neurons in the DCN. Functional analysis by stimulus-evoked c-Fos expression and electrophysiological recording showed that host axons formed active synapses with graft neurons at the injury site with the signal propagating by graft axons to the DCN. We observed reproducible electrophysiological activity at the DCN with a temporal delay predicted by our relay model. Additional experiments, where only GRP were grafted into the dorsal column lesion, showed sensory host axons regenerating into the lesion/graft site, underscoring the contribution of GRP-derived astrocytes to the formation of the relay. *In vitro* experiments have demonstrated that NRP-derived neurons have low expression of CSPG receptors and the ability to extend axons across inhibitory CSPG borders. Preliminary experiments with delayed transplantation suggest that a similar connectivity strategy might be possible in a chronic injury, if inhibition is reduced at the injury site (scar) and target (perineuronal nets).

Discussion. These findings provide evidence for the ability of NPC to form a neuronal relay by extending active axons across the injured spinal cord to the intended target. Importantly, it focuses attention, beyond regeneration, to the challenges of functional connectivity that will be associated with any neural repair strategy, including guidance of axons to targets and establishment of functional synapses. Future studies will therefore need to include better guidance cues for axons (both attractive and repulsive), examine the role of activity in establishing stable synaptic connections (task specific training), address the changes that occur in the denervated targets that have been subject to prolonged deafferentation, and develop experimental strategies to retrain the reconnected system to correctly interpret sensory (or motor) information at a chronic injury stage.

References

1. Bonner et al., (2011) Grafted neural progenitors integrate and restore synaptic connectivity across the injured spinal cord. J. Neuroscience 31, 4675-4686.

Supported by NIH grant NS055976, Criag H. Neilsen Foundation and Shriners Hospital for Children

Session IV: Exercise and activity driven repair
Chair: Peter Ellaway

Activity induced plasticity after spinal cord injury and rehabilitation

Ronaldo M. Ichiyama

School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK

r.m.ichiyama@ leeds.ac.uk

Locomotor and exercise training following spinal cord injuries (SCI) induces changes in the spinal networks controlling stepping, which correlate with improved stepping ability and function. The underlying mechanisms associated with such functional improvements are only now beginning to be investigated in more detail. Understanding such changes will lead to better and more efficient interventions to maximize the benefits of training for rehabilitative purposes. Precisely how the spinal locomotor circuitry is altered by rehabilitation, however, has yet to be elucidated.

We have previously demonstrated that after an SCI locomotor training induces positive electrophysiological changes in motoneurone properties such as decreased afterhyperpolarization depth and increased excitatory postsynaptic potentials induced by stimulation of afferent fibres (Petruska et al., 2007). We have also demonstrated that locomotor training induces reorganization of synaptic contacts onto motoneurones significantly decreasing the ratio of inhibitory to excitatory boutons compared to spinal cord injured non-trained rats (Ichiyama et al., 2011).

Here we will discuss how the effects of locomotor training are time dependent and how an adequate amount of training is necessary to observe positive functional results. Behavioural and histological data from intact, 7 days post injury (dpi; complete transection at T9-10), 21 dpi and 67 dpi in rats trained and not trained to step will be discussed. Adult Sprague Dawley rats (250g) were trained daily to locomote bipedally under quipazine (0.3 mg/kg) and epidural stimulation (40Hz) using an upper body weight support system. In general, significant effects of locomotor training are only observed at later time points both behaviourally and histologically, strongly suggesting that changes within spinal cord circuitry are necessary for behavioural improvements.

Finally, we will discuss the role of perineuronal nets in synaptic plasticity and its role on functional recovery following SCI and rehabilitation. The deposition of perineuronal nets around certain neurones are thought to be associated with decreased synaptic plasticity (Pizzorusso et al., 2002). We demonstrate that SCI induces an increase in expression of perineuronal nets around motoneurones. Surprisingly, locomotor training significantly increased expression of perineuronal nets compared to non-trained rats. This suggests that an intervention to decrease the amount of perineuronal nets such as application of Chondroitinase ABC could potentiate the positive effects of locomotor and exercise training on synaptic plasticity and functional recovery.

References

1. Ichiyama RM, Broman J, Roy RR, Zhong H, Edgerton VR, Havton LA (2011) Locomotor training maintains normal inhibitory influence on both alpha- and gamma-motoneurons after neonatal spinal cord transection. J Neurosci 31:26-33.
2. Petruska JC, Ichiyama RM, Jindrich DL, Crown ED, Tansey KE, Roy RR, Edgerton VR, Mendell LM (2007) Changes in motoneuron properties and synaptic inputs related to step training after spinal cord transection in rats. J Neurosci 27:4460-4471.
3. Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002) Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248-1251.

Supported by the International Spinal Research Trust and Royal Society

Neuronal dysfunction in chronic spinal cord injury

Volker Dietz, Gregoire Courtine

Spinal Cord Injury Center, University Hospital Balgrist, Zürich and Center for Neuroprosthetics, Lausanne, Switzerland

During last years it became obvious that after SCI there is not only a 'positive' neuroplasticity that can be exploited in incomplete SCI subjects. In severely affected, immobilized SCI subjects a 'negative' plasticity leads to a neuronal dysfunction (for reviews cf. 1, 2). This neuronal dysfunction is reflected in an exhaustion of leg muscle EMG activity during assisted stepping in severely affected SCI subjects and develops around 1 year after immobilization (3). It is associated by a shift from an early to a late spinal reflex response to tibial nerve stimulation which can be used as a marker for neuronal functionality (4). The occurrence of neuronal dysfunction is suggested to be due to a dysbalance of spinal neuronal activity with a bias to an inhibitory drive (1). Severely affected SCI subjects will profit from regeneration inducing therapies only if neuronal function below the level of lesion is preserved. Therefore countermeasures to prevent neuronal dysfunction have to be developed.

Only recently an adequate rodent model of a severe chronic SCI became developed (Lab of Gregoire Courtine, Lausanne) which might serve for a better understanding of the mechanisms underlying the occurrence of neuronal dysfunction (5,6). All features of dysfunction described for human SCI could be reproduced in this model. On the basis of such an animal model appropriate countermeasures can be developed and be translated to the human condition.

References

1. Dietz V (2010) Behavior of spinal neurons deprived of supraspinal input. Nature Reviews Neurology 6: 167-174.
2. Dietz V (2012) Neuronal plasticity after a human spinal cord injury: Positive and negative effects. Experimental Neurology 235: 110-115.
3. Dietz V, Müller R (2004) Degradation on neuronal function after a spinal cord injury: Mechanisms and countermeasures. Brain 127: 2221-2231.
4. Dietz V, Grillner S, Trepp A, Hubli M, Bolliger M (2009) Changes in spinal reflex and locomotor activity after a complete spinal cord injury: A common mechanism? Brain 132: 2196-2205.
5. Heutschi J, van den Brand R, Barraud Q, Friedli L, Musienko P, Dietz V, Courtine C (2012) Chaotic remodeling of spinal neuronal circuitries leads to a degradation of function in the chronic state of a severe spinal cord injury. Society for Neuroscience; New Orleans, LA.
6. Van den Brand R, Heutschi J, barraud Q, Digiovanna J, Bartholdi K, Huerlimann M, Friedli L, Vollenweider I, Martin Moraud E, Duis S, Dominici N, Micera S, Musienko PE, Courtine G (2012) Restoring voluntary control of locomotion after paralyzing spinal cord injury. Science 336: 1182-1185.

Influence of non-invasive direct current stimulation on excitability of the spinal cord

Marc Bolliger, Michèle Hubli, Volker Dietz

SCI Research – Spinal Cord Injury Center Balgrist, University of Zurich, CH-8008 Zurich, Switzerland

mbolliger@paralab.balgrist.ch

Individuals suffering from a severe spinal cord injury (SCI) are often not able to produce locomotor activity below the level of lesion. However, if spinal neuronal centres capable of producing rhythmic locomotor activity below the level of lesion are not affected by the injury, then modulating the excitability of these networks may facilitate voluntary control of locomotion. Recently, it has been shown that epidural spinal cord stimulation (ESCS) in combination with the delivery of serotonergic agonists induces voluntary stepping in rats with severe SCI. Continuous ESCS of lumbar posterior roots can also modulate the activity of spinal neuronal networks and induce locomotor-like movements in humans. However, ESCS is an invasive method and has risks associated it and is therefore not currently used clinically. A promising non-invasive approach to modulate excitability of neuronal networks is transcranial direct current stimulation. Although this method has been studied intensively for its influence on the brain only limited information is available on the possible effects of this stimulation approach on spinal neuronal circuitries. We investigated whether transcutaneous direct current stimulation (tsDCS) has the potential to modulate spinal neuronal circuitries and locomotor output of the spinal cord. To address this question the influence of tsDCS on the polysynaptic spinal reflex, which is assumed to be involved in locomotor generating networks, was studied in non-injured and motor complete SCI subjects. In addition, we examined if tsDCS facilitates locomotor activity. The influence of tsDCS on electromyographic muscle activity was analysed during assisted treadmill walking in motor complete SCI subjects. Moreover we examined whether tsDCS facilitates locomotion in subjects with a sensorimotor incomplete SCI. The results of these studies will be presented.

This study was **supported by** Wings for Life

Session V: NRB Studentship presentations
Chair: Liz Bradbury

Imaging microstructure with diffusion MRI in the spinal cord

Torben Schneider, Daniel C. Alexander, Claudia A. M. Wheeler-Kingshott

NMR Research Unit, Department of Neuroinflammation, UCL Institute of Neurology and Centre for Medical Image Computing, Department of Computer Science, UCL, London, UK

t.schneider@ucl.ac.uk

Studying the diffusion pattern of water molecules can inform about the microscopic organisation of biological tissue. Diffusion MRI (dMRI) is sensitive to the molecule motion patterns in tissue. Hence, dMRI provides a way to infer microstructural properties of tissue on the millimetre scale of the voxel non-invasively and in-vivo. Diffusion Tensor Imaging has been used successfully to study white matter (WM) in the healthy and diseased human CNS. However, DTI is hampered by its simplistic underlying mathematical model, which makes it difficult to relate it's parameters to individual tissue changes such as axon diameter or density.

Model based dMRI approaches have been shown to infer axon diameter distributions in the rat corpus callosum in vitro1 and in-vivo2. While some work has been done to transfer the protocols to in-vivo clinical systems in the brain3, its application to the spinal cord is yet untested.

Here, we present a novel model-based dMRI method that is designed to incorporate the mainly unidirectional fibre orientation of the spinal cord. Our focus is to develop a technique that can provide markers for axonal density and diameters within a clinical feasible acquisition time.

We demonstrate the feasibility of our method in simulations and in-vitro spinal cord samples of monkey and human spinal cord. Furthermore we present in-vivo results of axon diameter and density maps derived in the corpus callosum as a model system for unidirectional fibre organisation that took under 30 minutes to acquire on a standard clinical MRI system.

References

1. Assaf, Y et al. "AxCaliber: a Method for Measuring Axon Diameter Distribution from Diffusion {MRI}." *MRM* 59.6 (2008).
2. Barazany, D et al. " *in vivo* Measurement of Axon Diameter Distribution in the Corpus Callosum of Rat Brain." *Brain* (2009)
3. Alexander, D C et al. "Orientationally Invariant Indices of Axon Diameter and Density from Diffusion {MRI}." *NeuroImage* (2010)

Supported by the International Spinal Research Trust

Measuring synaptogenesis to investigate plasticity

Karen D. Bosch, Franziska Denk, R. Michael Linden, James W. Fawcett, Stephen B. McMahon

Wolfson Centre for Age-Related Diseases, King's College London, Guy's Campus, London Bridge, SE1 1UL, UK

Karen.bosch@kcl.ac.uk

Following spinal cord injury (SCI) most patients undergo a degree of spontaneous recovery believed to be due to reorganisation of neuronal circuits residing in spared neural tissue. Many treatment strategies of experimental spinal injuries appear to promote similar plasticity. Mostly, anatomical tracers, such as biotin dextran amines, have been used to study this phenomenon, but these methods show axonal projections, not synaptic connections.

We have developed a viral vector expressing a genetically encoded fluorescent marker of presynaptic terminals for use as an anatomical marker of the number and distribution of synapses *in vivo*. Selective expression of synaptopHluorin (SpH) was achieved with an adeno-associated viral vector expressing SpH (AAV-SpH) in peripheral and central neurons. We delivered the vector to the right sensory-motor cortex of adult rats and achieved substantial transduction of the corticospinal tract (CST), four weeks later, seen as diffuse labeling in the internal capsule, pyramids and spinal CST. The number of axons in the CST in the dorsal columns at C4 averaged 2701±436. In addition SpH accumulated in large numbers of bright puncta, widely taken as a measure of presynaptic specialisations. 1956±443 GFP-labelled synapses were observed in each transverse section of the grey matter of the cervical enlargement. 5.3±1.4% of these was contralateral to the injection site.

Delivery of AAV-SpH to the sciatic nerves of naïve animals led to transduction of a small number (1-5%) of primary afferent neurons, visible as diffuse fluorescence in lumbar dorsal root ganglia. Sensory neuron synaptic puncta were visualised in the dorsal horn of the spinal cord, providing a quantifiable method to document changes in synapse number. This novel tool provides the opportunity to study synaptic number in a particular tract or system and therefore measure synaptogenesis after neuronal injury and therapeutic interventions.

Supported by the International Spinal Research Trust

Session VI: Spasticity, bladder/bowel and sexual function
Chair: Geoffrey Raisman

New perspectives for the treatment of spasticity

Laurent Vinay, Rémi Bos, Pascale Boulenguez, Hélène Bras, Cécile Brocard, Dorothée Buttigieg, Florian Gackière, Georg Haase, Sylvie Liabeuf, Karina Sadlaoud

Institut de Neurosciences de la Timone, Centre National de la Recherche Scientifique (CNRS) & Aix-Marseille Université, Marseille, France

laurent.vinay@univ-amu.fr

A decrease in synaptic inhibition - disinhibition - appears to be an important substrate in several neuronal disorders, such as spinal cord injury (SCI) and neuropathic pain (Boulenguez et al., 2009). 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 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 (Boulenguez et al., 2010). 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.

Given the critical role of KCC2 in regulating the strength and robustness of inhibition, identifying tools that may increase KCC2 function and hence restore endogenous inhibition in pathological conditions is of particular importance. We showed that 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 (reduces spasticity). More recently, we demonstrated that activation of $5\text{-}HT_{2A}$ receptors to serotonin hyperpolarizes the reversal potential of IPSPs (E_{IPSP}) in spinal motoneurons, increases the cell-membrane expression of KCC2 and both restores endogenous inhibition and reduces spasticity after SCI in rats. Upregulation of KCC2 function by targeting $5\text{-}HT_{2A}$ receptors therefore has therapeutic potential in the treatment of neurological disorders involving altered chloride homeostasis. These results open new perspectives for the development of therapeutic strategies to alleviate spasticity and chronic pain after SCI.

References

1. Boulenguez P, Vinay L (2009) Strategies to restore motor functions after spinal cord injury. *Curr Opin Neurobiol* 19:587-600.
2. Boulenguez P, et al. (2010) Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat Med* 16:302-307.

Supported by grants to L. V. from the International Spinal Research Trust (ISRT), the Christopher and Dana Reeve Foundation, the French Agence Nationale pour la Recherche, the French Institut pour la Recherche sur la Moelle épinière et l'Encéphale. Salary support: ISRT to F.G., Institut National de la Santé et de la Recherche Médicale (INSERM) to H.B. and the Association Française contre les Myopathies (Grant 13912) to K.S.

Neural repair and modulation of lower urinary tract function after Cauda Equina/Conus Medullaris forms of spinal cord injury

Leif A. Havton

Departments of Anesthesiology & Perioperative Care and Anatomy & Neurobiology, Reeve-Irvine Research Center, University of California at Irvine, Irvine, CA, 92697, USA

lhavton@uci.edu

Cauda equina (CE) and conus medullaris (CM) forms of spinal cord injury commonly results in paralysis, sensory impairments, neuropathic pain as well as bladder, bowel, and sexual dysfunctions. No treatments to reverse these neurological deficits are currently available. However, in a bilateral lumbosacral ventral root avulsion (VRA) injury model, which mimics many of the above clinical deficits, an acute surgical replantation of the avulsed ventral roots in rats exerts a neuro-protective effect, ameliorates neuropathic pain, promotes axonal regeneration and results in functional reinnervation of the lower urinary tract. Here, urodynamic studies have demonstrated recovery of both reflex bladder contractions and external urethral sphincter muscle activation. Morphological features of the end organs, e.g. the bladder, are also restored. Recent studies have demonstrated that pharmacological interventions, using a serotonergic strategy, may also improve lower urinary tract function after CE/CM injuries. Specifically, a 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), improves voiding efficiency by means of increasing maximum intra-vesicle pressure and external urethral sphincter bursting activity during the voiding phase in rats after a chronic unilateral lumbosacral VRA injury. Thus, serotonergic modulation of the 5-HT_{1A} receptor may represent a new strategy to improve lower urinary tract function after incomplete CE/CM injuries as well as to increase lower urinary tract function after surgical ventral root repair of anatomically complete CE/CM injuries in experimental studies. For pre-clinical treatment purposes, the lumbosacral VRA injury and the surgical ventral root replantation intervention have also been translated to the nonhuman primate. Here, urodynamic study methods and electromyography (EMG) protocols for the evaluation of the external urethral and anal sphincters have been developed and are presently in use for the evaluation of the long-term effects of lumbosacral ventral root replantation on lower urinary tract and pelvic floor function.

Supported by: the National Institutes of Health (NS42719), California Institute for Regenerative Medicine (TR2-01785), a Translational Research Partnership Grant from the Department of Defense Congressionally Directed Medical Research Program on Spinal Cord Injury (SC090273), and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

Functional regeneration beyond the glial scar

Jerry Silver

Department of Neurosciences, Case Western Reserve University, School of Medicine, 10900 Euclid Ave. Cleveland, Ohio, USA

jxs10@case.edu

The ultimate goal of the Silver lab is to understand the basic biology that underlies regeneration failure in the adult spinal cord and then use this knowledge to develop strategies to maximally overcome axonal dieback and regeneration failure after cord injury in order to promote functional repair. In 2009 and 2011, nearly 2 decades after Chondroitin Sulfate Proteoglycans (CSPGs) had first been implicated in regeneration failure, we have identified the first neuronal receptors for CSPGs that bind specifically to the GAG chains of proteoglycans and induce a newly appreciated entrapment phenomenon by which sulfated proteoglycans mediate their inhibitory activity on cell migration or axonal elongation. Indeed, two members of the pro-synaptic, LAR family of receptor protein tyrosine phosphatases which up-regulate in adult (but not immature growth cones) when they encounter proteoglycans are the major receptors on neurons that are involved with the overly adhesive properties of this family of ECM inhibitors. Our lab has generated small peptides, administered simply by sub-cutaneous injection after cord injury (and recently with the use of specially designed, lesion homing nanoparticles by IV injection), that block these receptors on neurons in the contused spinal cord. We have generated *in vitro* evidence that the peptides may be even more highly effective than chondroitinase in releasing axons from proteoglycan mediated entrapment. Behavioral recovery particularly after acute administration of the peptide that blocks the sigma (σ) member of this receptor family was especially impressive. This novel, easily injectable, small peptide inhibitor offers a potential new avenue of treatment for paralysis. Another especially exciting development is their recent demonstration that combining the classical use of segments of autologous peripheral nerves as “bridges” to bypass a hemisection lesion of the adult rat spinal cord combined with inhibitory matrix modification via chondroitinase at the PNS/CNS interfaces, allows regenerating axons to exit the bridge, form functional synapses, and restore useful movement to the once paralyzed forelimb as well as remarkably robust functional recovery to the diaphragm. The lab is now showing for the first time that a novel modification of this strategy in a complete thoracic transection injury model and with the further addition of the trophic factor FGF can allow for an unprecedented amount of regeneration of certain brainstem neurons well past the graft site all the way to lumbo-sacral levels with restoration of near normal bladder control. These various research strategies show clearly that long distance regeneration, with appropriate re-formation of functional connections, can be achieved in the adult after catastrophic spinal cord injury providing real hope that we are now entering an era where strategies for providing functional benefit in animal models of spinal cord injury are sufficiently robust that there should be optimism for translational success. Continuing work in the lab is now being strongly focused on chronic spinal cord injury in order to bring these therapeutic strategies to the multitude that already suffer with paralysis.

This work was **supported by** grants from the International Spinal Research Trust and NINDS. We also offer thanks to the Brumagin Memorial Fund and the Ellen Becker Neuroscience Regenerative Medicine Research Fund

Session VII: Exploring intermediate models for translation
Chair: Robin Franklin

A porcine model of traumatic SCI – opportunities for translation in an intermediate model of injury

Brian Kwon

Department of Orthopaedics, International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Room 6196, Blusson Spinal Cord Centre, 818 West 10th Avenue, Vancouver, BC, V5Z 1M9, Canada

Brian.Kwon@vch.ca

In the "bench to bedside" paradigm of translational research for spinal cord injury, it is recognized that there may be important differences between traditional rodent SCI models and human SCI patients. Hence, there is interest in the development of large animal 'intermediary' models of SCI. As a 'testing ground' for novel treatments, a large animal model may offer the opportunity to demonstrate the robustness of therapeutic effect prior to human clinical trials. Additionally, a spinal cord of a more similar size and anatomy to the human cord opens the possibility of translating "bedside back to bench" and addressing questions of clinical relevance in an animal setting.

We have developed a model of contusive thoracic SCI using 20-25 kg Yucatan mini-pigs. After a weight-drop injury to the T10 region, recovery of hindlimb function is graded using a newly developed Porcine Thoracic Injury Behavioral Scale (PTIBS). In this model, the biomechanical severity of injury correlates well with PTIBS recovery over 12 weeks, which in turn is closely correlated with histologic damage through the injury site. Importantly, the spinal cord in the Yucatant mini-pig is surrounded by a prominent layer of cerebrospinal fluid (CSF) which makes it possible to instrument the intrathecal space for monitoring post-injury pressure and for taking serial CSF samples. This lecture will describe the development of this large animal SCI model and the utilization of it for addressing questions of translational relevance.

Strengths and limitations of the Yucatan minipig thoracic spinal cord injury model

James Guest

The Miami Project to Cure Paralysis and Neurological Surgery, University of Miami, Miami, USA

Jguest@med.miami.edu

For several decades the majority of spinal cord injury (SCI) research has been performed with rodents which are relatively inexpensive and practical. However, there is a persuasive argument that large non-rodent animal models are important to address questions in translation of experimental therapeutics that cannot be suitably studied in rodents. Recently, the Yucatan minipig has emerged as a translational SCI research model. Our research group has now created injuries in more than 75 minipigs and here we discuss our perception of the strengths and limitations of the model.

Strengths	**Limitations**
Spinal cord size useful to test clinical doses and equipment	Pigs are not adult when injured
Human-like CSF spaces	Continual weight gain
Robust survival after SCI	Anatomy of spinal tracts has not been defined
Trainable	Difficult to achieve injury magnitude most suited to therapeutics testing
Suitable for treadmill weight-supported training	Ungulates lacking dexterity of phalanges
Physiologic similarity to humans	Sensory testing is challenging
Lumbar spinal cord is responsive to epidural stimulation	Yucatans are genetically similar unlike primates

The use of minipigs as an experimental model requires further elucidation. In particular, it has not been shown that neuroprotective strategies that show efficacy in rodents show efficacy in minipigs where the body central of gravity, weight gain, and ungulate hoof structure make weight-support more difficult. The minipig is very useful for the emulation of procedures that will be translated to humans

Blinded randomized controlled trial of olfactory ensheathing cells for clinical canine spinal cord injury

Nick Jeffery

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA

njeffery@iastate.edu

A great number of interventions have now been shown to ameliorate the functional deficits in experimental SCI in laboratory animals. In contrast, although there have been advances in rehabilitative therapy, the ability to effectively improve outcome after severe SCI in human patients remains limited. There is therefore a clear need to determine whether successful laboratory therapies can be translated into effective medical interventions.

In translation of SCI treatments between species, the most important differences between experimental animals and human patients are increased lesion heterogeneity and the need for the intervention to provide a beneficial effect on patients' quality of life (in addition to a statistically demonstrable difference in outcome). Both of these aspects are difficult to address in traditional experimental animal models, and especially so for interventions that are more likely to be given during the chronic phase of injury, such as cell transplants. Domestic (pet) dogs form a convenient model in which success in translating from laboratory to clinic cane be evaluated because they have a high rate of 'naturally-occurring' SCI, have an inherent (but restricted) variability of lesion character between individuals and are highly amenable to analysis of outcome over prolonged periods of time.

This study was designed to determine whether intraspinal transplantation of olfactory ensheathing cells to dogs with severe SCI could provide sufficient benefit to be of value to clinical cases. Dogs with severe, chronic, thoracolumbar spinal cord injuries (equivalent to ASIA grade 'A' human patients at ~12 months after injury) were entered into a randomized, double-blinded, clinical trial in which they were allocated to receive either intraspinal autologous cells derived from olfactory mucosal cultures or injection of cell transport medium alone. The primary outcome measure was coordination between fore and hind limb stepping during treadmill locomotion; a series of secondary outcome measures were used to quantify spinal cord long tract function, including variation in paw placement in the lateral plane, transcranial magnetic motor evoked potentials, somatosensory evoked potentials and urinary bladder compliance.

Recipients of olfactory mucosal cell transplants gained significantly better fore-hind coordination than those dogs receiving cell transport medium alone, but there were no significant differences in outcome between treatment groups in measures of long tract function. None of the dogs regained the ability to walk unsupported. We conclude that intraspinal olfactory mucosal cell transplantation improves communication across the damaged region of the injured spinal cord, even in chronically injured individuals. However, there is no evidence for concomitant improvement in long tract function, which would limit the impact of OEC transplantation to provide useful benefit to human patients.

This work was **supported by** grants from the Medical Research Council of Great Britain, The Newton Trust (University of Cambridge) and the Frank Litchfield Charitable Trust

Session VIII: Learning from the wider field of regenerative medicine
Chair: James Guest

Retinal repair through transplantation of photoreceptors

Emma West, Anai Gonzalez-Cordero, Matteo Rizzi, Jane Sowden, Rachael Pearson, **Robin Ali**

University College London, London, UK

r.ali@ucl.ac.uk

Retinal degeneration is a leading cause of untreatable blindness in the developed world. Despite different aetiologies, age-related macular degeneration and most inherited retinal disorders culminate in the same final common pathway, the loss of photoreceptors. There are few effective treatments and none reverse the loss of vision. We are thus developing a novel therapeutic approach that aims to restore sight by transplanting new photoreceptors. We have established previously that photoreceptor precursors at the correct ontogenetic stage are able to migrate and functionally integrate into the degenerate adult retina (MacLaren et al., 2006). We have recently also demonstrated restoration of vision following rod-photoreceptor transplantation into a mouse model of stationary night-blindness (Pearson et al., 2012). To translate this therapeutic approach we need to establish a renewable source of correctly staged photoreceptor precursors. We have therefore investigated ES cell-derived retinal differentiation and the integration competence of ES cell-derived photoreceptor precursors. Our initial investigations optimised the generation of photoreceptor precursors by stepwise treatment of ES cells with defined factors. However, this 2-dimensional culture system generated photoreceptor cell populations that failed to integrate following subretinal transplantation to the adult retina (West et al., 2012). Following the recent development of a 3-dimensional retinal differentiation culture (Eiraku et al., 2011) we have optimized this culture system for the purposes of photoreceptor transplantation. We have efficiently generated photoreceptor precursors at a stage equivalent to the early postnatal retina and following the transplantation of these cells we have observed, for the first time, successful ES cell-derived photoreceptor integration into the adult retina.

References

1. Eiraku, M., N. Takata, H. Ishibashi, M. Kawada, E. Sakakura, S. Okuda, K. Sekiguchi, T. Adachi, and Y. Sasai, 2011, Self-organizing optic-cup morphogenesis in three-dimensional culture: Nature., v. 472, no. 7341, p. 51-56.
2. MacLaren, R. E., R. A. Pearson, A. MacNeil, R. H. Douglas, T. E. Salt, M. Akimoto, A. Swaroop, J. C. Sowden, and R. R. Ali, 2006, Retinal repair by transplantation of photoreceptor precursors: Nature., v. 444, no. 7116, p. 203-207.
3. Pearson, R.A., A.C. Barber, M.Rizzi, C. Hippert, T. Xue, T, E.L. West, Y. Duran, Y, A.J. Smith, J.Z. Chuang, S.A. Azam, U.F. Luhmann, A. Benucci, C.H. SungJ, J.W. Bainbridge, M. Carandini, K-W Yau, J.C. Sowden, R.R. Ali. Restoration of vision after transplantation of photoreceptors. Nature. 2012 v 485, no 7396, p 99-103.
4. West, E. L., A. Gonzalez-Cordero, C. Hippert, F. Osakada, J. P. Martinez-Barbera, R. A. Pearson, J. C. Sowden, M. Takahashi, and R. R. Ali, 2012, Defining the integration capacity of embryonic stem cell-derived photoreceptor precursors: Stem Cells., v. 30, no. 7, p. 1424-1435.

Adult stem cells for peripheral nerve regeneration

Giorgio Terenghi

Blond McIndoe Laboratories, Regenerative Medicine, University of Manchester, UK

giorgio.terenghi@manchester.ac.uk

Currently peripheral nerve gap injuries are repaired with an autologous nerve graft, which provides regrowing axons with natural guidance channels, and cellular and molecular supporting elements. The harvesting of the nerve for grafting causes further morbidity to the patient, and a bioengineered artificial nerve could provide a suitable alternative for nerve reconstruction. Biocompatible nerve conduits offer a suitable surface micro-geometry which, in combination with transplanted cells, facilitates improved nerve regeneration. The addition of Schwann cells within the nerve conduit is crucial for nerve regeneration, and we have demonstrated their active participation in the regenerative process. However, these cells are difficult to source and culture, and as an alternative we have been able to differentiate adult adipose-derived stem cells towards expressing phenotypic and functional characteristics of Schwann cells. The differentiated stem cells secrete neurotrophic factors such as NGF and BDNF, express myelin proteins and have the potential to myelinate regrowing axons during regeneration. We have transplanted differentiated stem cells into bioengineered nerve conduits, and long-term *in vivo* experiments have shown that they have beneficial effects in promoting enhanced nerve regeneration. Tests have also indicated that differentiated stem cells retain their neurotrophic and regenerative potential irrespective of the age of the cell donor. Differentiated human adult stem cells have similar characteristics to rodent differentiated stem cells, confirming their capability to promote nerve regeneration. Overall our results show the promising potential of adult stem cells for clinical application, and indicate that an artificial bioengineered nerve is becoming closer to clinical reality.

References

1. Di Summa PG, Kalbermatten DF, Pralong E, Raffoul W, Kingham PJ, Terenghi G – Long-term *in vivo* regeneration of peripheral nerves through bioengineered nerve grafts. *Neurosci* 2011, 181: 278-291
2. Mantovani C, Raimondo S, Haneef MS, Geuna S, Terenghi G, Shawcross SG, Wiberg M – Morphological, molecular and functional differences of adult bone marrow- and adipose-derived stem cells isolated from rats of different ages. *Exp Cell Res* 2012, 318:2034-2048
3. Tomita K, Madura T, Mantovani C, Terenghi G – Differentiated adipose-derived stem cells promote myelination and enhance functional recovery in a rat model of chronic denervation. *J Neurosci Res* 2012, 90: 1392-1402

Circuit repair in neurodegeneration: using Huntington's disease as a model disease

Anne Rosser

Cardiff University Brain Repair Group, Neuroscience and Mental Health Institute, Schools of Biosciences and Medicine, Museum Av, Cardiff CF10 3AX, UK

rosserae@cf.ac.uk

Neural transplantation as a therapeutic strategy in neurodegenerative disorders offers to replace cells lost during the disease process, with the potential to reconstruct dysfunctional circuitry and thus alleviating associated disease symptoms. Huntington's disease (HD) is an inherited degenerative condition of the basal ganglia, and is increasingly recognized, not only as an important target in itself, but also as a powerful model system within which to assess and refine this potential therapeutic strategy. This is in part due to the relatively focal loss of medium-sized spiny neurons (MSN) in the striatum, thus providing a clear therapeutic target. In addition, there are a variety of animal models of the condition and it and provides a stringent test of the capacity of transplanted cells to repair neural circuits. Preclinical studies in animal models, and preliminary feasibility safety and efficacy studies in individuals with HD suggest that transplantation can provide functional benefit, provided certain biological parameters are respected. In parallel, rapid advances are being made towards generating neurons for transplantation from stem cells and establishing protocols for their reliable differentiation into specific neuronal phenotypes, with the prospect of translating these novel sources to cell therapy for patients in new clinical trials.

Session IX: Clinical session
Chair: David Allan

Current trends in the treatment of infertility in men with spinal cord injury

Nancy L. Brackett

The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, 1095 NW 14th Terrace, Miami, Florida, 33136, USA

nbrackett@miami.edu

In countries throughout the world, men with spinal cord injury (SCI) outnumber women with SCI, often by as much as 4:1. Because the most common causes of injury include motor vehicle accidents, violence, falls, and sports-related injuries, it has been assumed that this gender disparity is due to more men than women engaging in risk-taking behaviors, however, this assumption has not been confirmed. The majority of new spinal cord injuries occur to persons between 18 and 35 years, i.e, the prime parenting years.

Following SCI, women can conceive via sexual intercourse and deliver children with nearly the same success rate as women without disability. In contrast, most men with SCI are infertile due to a combination of erectile dysfunction, ejaculatory dysfunction, and semen abnormalities. Treatments that are effective for erectile dysfunction in the general population are also effective in men with SCI, including phosphodiesterase type 5 inhibitors, intracavernous injections of alprostadil, penile prostheses, and vacuum constriction devices.

In anejaculatory patients who wish to father children, semen retrieval is necessary. Penile vibratory stimulation (PVS) is recommended as the first line of treatment. Patients who fail PVS can be referred for electroejaculation. Surgical sperm retrieval should be considered as a last resort when other methods fail. Most men with SCI have a unique semen profile characterized by normal sperm concentration but abnormally low sperm motility. This problem does not seem to be due to simple lifestyle factors, such as elevated scrotal temperature from sitting in a wheelchair, infrequency of ejaculation, or the method of bladder management. Longitudinal and cross-sectional studies have found no progressive decline in semen quality with the ensuing years post-injury. Endocrinopathies may be present, but are not the sole cause of abnormal semen quality in these men.

Evidence suggests that a toxic seminal plasma environment contributes to the problem. For example, a significant increase in semen concentrations of leukocytes is present in most men with SCI. Cytofluorographic analysis of leukocyte subtypes revealed that most were T lymphocytes, known to produce cytokines that are toxic to sperm. Seminal plasma from men with SCI contains elevated concentrations of different cytokines, including proinflammatory cytokines. Inactivation of these cytokines improves sperm motility. Recent evidence suggests that the inflammasome may play a role in elevating semen cytokines in men with SCI.

Despite these semen abnormalities, men with SCI can father children. The choice of assisted conception method is currently controversial. Some practitioners proceed directly to surgical sperm retrieval without examining the ejaculate for sperm. The downside to this approach is that surgical sperm retrieval rarely yields enough motile sperm for any assisted conception procedure other than intracytoplasmic sperm injection (ICSI), which is currently the most invasive and expensive option. Ejaculates can be obtained by PVS or electroejaculation from 97% of men with SCI. Reasonable pregnancy rates have been obtained with intrauterine insemination or even intravaginal insemination of sperm obtained by PVS or electroejaculation of male partners with SCI, and it is therefore recommended that these less-costly, less-invasive options be considered before proceeding to surgical sperm retrieval and ICSI.

References

1. Brackett NL, Lynne CM, Ibrahim DA, Sonksen J. Treatment of infertility in men with spinal cord injury. Nat Rev Urol. 2010;7:162-72.
2. Brackett NL, Ibrahim E, Iremashvili V, Aballa TC, Lynne CM. Treatment for ejaculatory dysfunction in men with spinal cord injury: an 18-year single center experience. J Urol. 2010;183:2304-8.
3. Kafetsoulis A, Brackett NL, Ibrahim E, Attia GR, Lynne CM. Current trends in the treatment of infertility in men with spinal cord injury. Fertil Steril. 2006;86:781-9.
4. Kathiresan AS, Ibrahim E, Aballa TC, Attia GR, Lynne CM, Brackett NL. Pregnancy outcomes by intravaginal and intrauterine insemination in 82 couples with male factor infertility due to spinal cord injuries. Fertil Steril. 2011;96:328-31.

Investigating structure-function integrity in SCI using MRI

Claudia A.M. Wheeler-Kingshott

NMR Research Unit, Queen Square MS Centre, Department of Neuroinflammation, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

c.wheeler-kingshott@ucl.ac.uk

Introduction

Magnetic Resonance Imaging (MRI) can reveal details of the underlying tissue structure and therefore be sensitive to damage and repair. Spinal cord injury is debilitating and the patient's clinical evaluation would greatly benefit from a routinely increase in the use of quantitative MRI techniques to help assessing damage, adding possible prognostic value and contributing to the patient's management, perhaps even in terms of treatment strategies.

Methods and Results

This review talk will focus on how we can use MRI to investigate several aspects of spinal cord damage after trauma, beyond the visual inspection of the affected and distal areas, using conventional MRI methods. A broad view of all available techniques to quantify spinal cord function, compression, axonal damage, demyelination, gliosis and scar tissue creation will be presented.

There are several methods that can be used to quantitatively assess the spinal cord integrity and that are available on clinical scanners or at least on most research scanners through collaborations between sites or through research agreements with manufacturers [Stroman et al., 2012]. Methods that will be presented and discussed will include techniques such as spinal cord compression assessment to predict recovery [Wilson and Fehlings, 2011], diffusion weighted imaging (DWI) to investigate tissue structural integrity [Cohen-Adad et al, 2011], magnetization transfer imaging (MTI) in particular for detecting myelin changes [Smith et al., 2010], magnetic resonance spectroscopy (MRS) for assessing a number of metabolites linked to axonal damage, mitochondrial function and gliosis [Kachramanoglou et al, 2012], functional MRI of the spinal cord for examining functional integrity [Cadotte et al, 2012].

Discussion and Conclusion

The talk will conclude with a critical assessment of the field to gather information on unmet needs. Wherever possible examples of clinical studies (mainly pilots) using these techniques will be given to demonstrate where the state of the art and clinical use of quantitative MRI lays, together with a critical appraisal of how to promote their adoption by a wider user base.

References

1. Cadotte DW, Stroman P, Bosma R, Mikulis DJ, Nugaeva N, Fehlings MG. (2012). Visualizing plasticity and altered neuronal signaling in the injured human spinal cord with FMRI. Neurosurgery. 71(2):E558.
2. Cohen-Adad J, Leblond H, Delivet-Mongrain H, Martinez M, Benali H, Rossignol S. (2011). Wallerian degeneration after spinal cord lesions in cats detected with diffusion tensor imaging. Neuroimage. 1;57(3):1068-76.
3. Kachramanoglou C., De Vita E, Thomas DL, Wheeler-Kingshott CAM, Balteau E, Carlstedt T, Choi D, Thompson AJ, and Ciccarelli O (2012). Metabolic changes in the spinal cord after brachial plexus root re-implantation. Neurorehabilitation and Neural Repair, in press.
4. Smith SA, Jones CK, Gifford A, Belegu V, Chodkowski B, Farrell JA, Landman BA, Reich DS, Calabresi PA, McDonald JW, van Zijl PC. (2010). Reproducibility of tract-specific magnetization transfer and diffusion tensor imaging in the cervical spinal cord at 3 tesla. NMR Biomed. 23(2):207-17.
5. Stroman PW, Bosma RL, Kornelsen J, Lawrence-Dewar J, Wheeler-Kingshott C, Cadotte D, Fehlings MG (2012). Advanced MR imaging techniques and characterization of residual anatomy. Clin Neurol Neurosurg. 114(5):460-70.
6. Wilson JR and Fehlings MG (2011). Emerging approaches to the surgical management of acute traumatic spinal cord injury. Neurotherapeutics. 8(2):187-94.

Acknowledgements: The speaker thanks the MS Society of Great Britain and Northern Ireland for supporting the group with programme grants and scanner maintenance, the International Spinal Research Trust for supporting specific spinal cord MRI development, the EPSRC for funding a project grant to look at microstructural imaging biomarkers, and the UCLH CBRC for their support to staff.

Measuring and optimizing spinal cord perfusion pressure after spinal cord injury

Marios C. Papadopoulos

Academic Neurosurgery Unit, St. George's, University of London, Cranmer Terrace, London SW17 0RE, UK

mpapadop@sgul.ac.uk

After severe brain injury, patients are urgently transferred to a neuro-intensive care unit for intracranial pressure monitoring. Neurosurgical treatment aims to optimize cerebral perfusion pressure [1]. There is no method to measure spinal cord pressure at the injury site. Therefore, the neurosurgical management of acute severe spinal cord injury differs from the management of acute severe brain injury and is focused on realigning and fixing the fractured spine without monitoring spinal cord perfusion [2].

Here I will describe a novel way to manage acute spinal cord injury, which aims to optimize spinal cord perfusion at the injury site. Following our studies in mice [3], we developed a novel method to measure spinal cord pressure in humans, by inserting a probe intradurally at the injury site. Patients are admitted within 72 h of the injury to measure cord pressure for up to a week. We compute spinal cord perfusion pressure (arterial blood pressure minus cord pressure) and the autoregulation coefficient PRx. So far we monitored spinal cord perfusion pressure in 14 patients with severe spinal cord injury (ASIA A – C).

Our data indicate that even after realigning the fractured spine, spinal cord pressure is high at the injury site. In some patients cord pressure is high on admission and gradually normalizes, in some the elevated cord pressure persists for >1 week, and in some the cord pressure is low on admission and gradually rises. Spinal cord pressure is higher at the injury site than above or below, consistent with focal cord swelling as evident on MRI. Bony decompression (laminectomy) does not effectively reduce spinal cord pressure, because the swollen cord remains compressed against the surrounding dura. Laminectomy is potentially detrimental by allowing external forces applied to the skin to be transmitted to the injured cord thus generating high cord pressures, up to 80 mmHg. Changes in arterial pCO_2 (4 – 6 kPa) and sevoflurane concentration (MAC 1.0 – 1.5), or iv administration of 20 % mannitol have little effect on spinal cord pressure. When spinal cord pressure rises above 20 mmHg, vasoreactivity becomes impaired (PRx >1). Increasing spinal cord perfusion pressure after incomplete spinal cord injury improves outcome as evidenced by increased amplitude in motor evoked potentials recorded from below the level of injury. Increasing spinal cord perfusion pressure after complete spinal cord injury also improves outcome as evidenced by increased amplitude in motor evoked potentials recorded from just above the level of injury.

Based on these findings, we conclude that measuring spinal cord pressure after severe spinal cord injury allows us to optimize spinal cord perfusion, avoid secondary insults and improve motor outcome.

References

1. Czosnyka M, Pickard JD (2004). Monitoring and interpretation of intracranial pressure. *J Neurol Neurosurg Psychiatry* 75:813-21.
2. Fehlings MG, Cadotte DW, Fehlings LN (2011). A series of systematic reviews on the treatment of acute spinal cord injury: a foundation for best medical practice. *J Neurotrauma* 28:1329-33.
3. Saadoun S, Bell BA, Verkman AS, Papadopoulos MC (2008). Greatly improved neurological outcome after spinal cord compression injury in AQP4-deficient mice. *Brain* 131:1087-98.

Acknowledgements

I thank Ms. M.C. Werndle (research fellow) and Dr. A. Zoumprouli (neuro-anaesthetist) of St. George's Hospital as well as Dr. M. Czosnyka (Reader in brain physics) of Cambridge University. Supported by grants from the U.K. Spinal Cord Injuries Research Network, the Neurosciences Research Foundation, the London Deanery, and the Royal College of Surgeons of England.

Table of Contents – Poster Abstracts

Mouse strain dependent neuroinflammatory response to lentiviral GMCSF: Modelling pro-regenerative and toxic effects of microglia activation

Carolina Acosta-Saltos [1,3], Roman Gonitel[2], Ainkaran Santhirasekaram[1], Elisabeth Thubron[1], Alejandro Acosta-Saltos[1], Conrad Vink[3], Patrick Anderson[4], Adrian Thrasher[3], Simon Waddington[5], Gennadij Raivich[1]

[1]Centre for Perinatal Brain Protection and Repair, University College London, UK
[2]Imperial College School of Medicine, Imperial College, UK
[3]Institute of Child Health, University College London, UK
[4]Cell and Developmental Biology, University College London, UK
[5]Gene Transfer Technology Group, University College London, UK

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

In contrast to the PNS, the CNS displays limited axonal regeneration after injury, which is associated with a lack of perineuronal inflammation. Stimulation of microglia around the cell bodies of axotomised neurons has been demonstrated to promote CNS regeneration. In our experiments, stimulation of microglia with granulocyte macrophage colony stimulating factor (GMCSF) produces an increase in axonal sprouting in the injured spinal cord. However, the effect is strongest with intermediate levels of microglial mitogen and decrease at high levels thus, stimulation of microglia may not only increase regeneration but also lead to neurotoxicity. The current study used integrase deficient lentiviral vector encoding proinflammatory GMCSF to explore the degenerative role of microglia. First, adult inbred and outbred mouse strains received a striatal injection of GMCSF/eGFP or eGFP-only control lentivirus, which revealed a strain dependent response. The inbred strains 129/SvJ had a weak neuroinflammatory response, in BALB/c it was moderate but wide spread throughout the hemisphere, and in FVB it was strong. A dose response study in 129/SvJ, BALB/c and FVB revealed that GMCSF virus produces a neurotoxic/phagocytic microglia response in the high responder FVB strain but not in low responder 129/SvJ.

Undue perinatal activation of periventricular phagocytes, also known as fountains of microgllia, has been suggested to result in white matter damage, causing persistent motor disabilities up to adulthood. Here we have used 129/SvJ and FVB mouse stain to model potential neurotoxic effects of GMSCF. E14 mice received eGFP-only or GMCSF/eGFP via trans-uterine injections to the brain and treated pups were collected at P0, P3 and P7.So far, at P3, injection of GMCSF lentivirus was associated with a considerably higher number of IBA1+ phagocytic microglia at the periventricular part of the external capsule compared to eGFP injected mice. In conclusion, the neurotoxic response to GMCSF in the FVB strain may help us study the neuroinflammation associated with motor conditions such as cerebral palsy and multiple sclerosis. On the other hand, the presence of a nontoxic microglial response could also allow us to harness their positive effect on spinal cord regeneration.

Supported by the International Spinal Research Trust

Promotion of neuroplasticity by modifying perineuronal nets using polysialic acid

Louise Adams, Yi Zhang, John Priestley, Xuenong Bo

Centre for Neuroscience and Trauma, Blizard Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, 4 Newark Street, London, E1 2AT, UK

L.Adams@qmul.ac.uk

In the central nervous system (CNS), certain populations of neurons are surrounded by a specialised form of extracellular matrix called the perineuronal net (PNN). Formed of a network of proteins, including a high concentration of chondroitin sulphate proteoglycans (CSPGs), the PNN is anchored to the cell membrane by the neural cell adhesion molecule (NCAM) and acts to limit neuroplasticity in the adult CNS.

Unpublished data from our lab suggests there is a negative correlation between PNN formation and the expression of polysialic acid (PSA) during early postnatal development of the rat spinal cord. PSA, a homopolymer of α2-8-linked *N*-acetylneuraminic acid is attached to NCAM by the enzyme polysialyltransferase (PST). Predominantly found during postnatal development, PSA is thought to decrease the adhesive force of NCAM and has been shown to increase intercellular spacing by up to 15 nm. We believe that in addition to this, PSA can also modify the neuronal extracellular matrix, in particular the PNN.

We have developed a lentiviral vector carrying the PST transgene (LV/PST) that has previously been shown to overexpress PSA *in vivo*, when compared with a control vector carrying the transgene for green fluorescent protein (LV/GFP). In this study, we injected either LV/PST or LV/GFP into the lumbar spinal cord of adult rats. After 6 weeks, animals were sacrificed by transcardial perfusion and spinal cords were prepared for double labeled immunohistochemistry, using antibodies against PSA and components of the PNN.

Preliminary results demonstrate that immunoreactivity for two markers of the PNN, Wisteria floribunda agglutinin and cartilage link protein 1, were diminished in the regions of PSA overexpression. Although it appears the PNNs have been degraded at these regions, further immunohistochemistry using antibodies directed against individual CSPG core proteins is required before we can form any firm conclusions.

Supported by the International Spinal Research Trust

Chondroitinase ABC treatment at super chronic injury states promotes respiratory motor recovery following C2 hemisection

Warren J. Alilain[1,2] , Jerry Silver[1]

[1]Department of Neurosciences, Case Western Reserve University School of Medicine, [2]Department of Neurosciences, Rammelkamp Center for Education and Research/Metrohealth Medical Center, Cleveland, OH, USA

warren.alilain@case.edu

Most spinal cord injuries (SCI) are at the cervical level. This can result in disruption of bulbospinal inputs to phrenic motor neurons which innervate the diaphragm. This oftentimes results in the inability to breathe and the need for mechanical ventilation, severely diminishing the quality of life of those afflicted with the injury. In our laboratory we utilize a lateral C2 hemisection model of SCI which results in paralysis of the ipsilateral hemidiaphragm to study the anatomical and physiological changes which take place after cervical injury and explore strategies to restore function. A majority of studies have investigated treatments at acute stages, within 1 week of injury, to restore respiratory motor function. Since a majority of the human SC injured population is at chronic stages, it is necessary to investigate if these same strategies are still effective long after the initial injury. One cannot assume that treatments that are effective at acute stages will be equally as effective later on since a variety of untoward changes may occur over time. One strategy that has a well characterized positive regenerative/sprouting effects at acute stages is treatment with chondroitinase ABC (ChABC). ChABC breaks down inhibitory chondroitin sulfate proteoglycans (CSPGs) in the lesion scar and the perineuronal net (PNN) – which are upregulated immediately following injury and potently inhibit plasticity and regeneration. In the current study we found that ChABC treatment alone at “super” chronic stages – 1 and 1.5 years post injury – can, indeed, restore hemidiaphragmatic function. Additionally, our data suggests that the restored function is greater than that which returns after acute treatment. Overall, this data suggests that at super-chronic injury states CSPGs and the PNN are still present and inhibitory to endogenous mechanisms to restore function. However, when enzymatically removed with ChABC alone, there is an enhanced response compared to acute treatment, further suggesting that precisely targeting CSPGs may be an important priority even in chronic SCI patients.

Supported by the International Spinal Research Trust Grant STR106 (WJA) and NINDS NS060767(JS)

Therapeutic strategies in a novel *in vitro* model of spinal cord injury

S.D. Boomkamp, M.D. Houslay[1], M.O. Riehle[2], M.F. Olson[3], **Susan C. Barnett**

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK
[1]Molecular Pharmacology Group, Wolfson Link and Davidson Buildings, Institute of Neuroscience and Psychology, College of Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
[2]Centre for Cell Engineering, Institute of Molecular-, Cell- and Systems-Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
[3]CRUK Beatson Institute, Garscube Estate, Glasgow G61 1BD, UK

Susan.Barnett@glasgow.ac.uk

Spinal cord injury (SCI) often results in paralysis, which in the majority of cases is permanent due to the limited capacity of the axons within the central nervous system (CNS) to regenerate. The need to study the mechanisms underlying inhibition of regeneration in SCI to harness repair has given rise to various experimental models. To our knowledge, the existing *in vitro* models are often limited in that they do not comprise the complete repertoire of cells that reside in the complex CNS. In the present study, we have devised an *in vitro* model of SCI using our previously described myelinating cultures. This culture system comprises dissociated embryonic spinal cord cells plated onto a monolayer of neurosphere-derived astrocytes, which form myelinated fibres interspaced with nodes of Ranvier. After creating a lesion across the culture, an initial cell-free area appears with lack of neurite outgrowth, demyelination, the presence of microglia and reactive astrocytes, besides apoptosis coincident with changes in the immune cells and their response. These findings are in agreement with *in vivo* findings. Furthermore, this model of SCI can be used to screen compounds alone and in multiple combinations for their effects on CNS repair. A cell-permeable form of the C. botulinum-derived Rho antagonist, C3 ADP-ribosyltransferase (C3), which promotes axonal regeneration *in vivo* and *in vitro*, induced neurite outgrowth in these cultures with a concomitant reduction in CSPG and phosphomyosin levels. However, remyelination was only enhanced when C3 was introduced together with ROCK inhibitor Y-27632 or PDE4 inhibitor rolipram. These results demonstrate the validity of this novel *in vitro* model as a tool to examine the effect of a pharmacological and/or biological approach on not only axonal outgrowth but also other CNS cell types that would be affected after SCI.

Gene delivery of chondroitinase ABC reduces secondary pathology and alters inflammatory events following spinal cord injury

Katalin Bartus, Athanasios Didangelos, Nicholas D. James, Karen D. Bosch, John H. Rogers, Bernard L. Schneider, Elizabeth M. Muir, Elizabeth J. Bradbury

King's College London, Regeneration Group, The Wolfson Centre for Age-Related Diseases, London SE1 1UL, UK

katalin.bartus@kcl.ac.uk

Chondroitin sulfate proteoglycans (CSPGs) are inhibitory molecules present in the extracellular matrix that become up-regulated following spinal cord injury (SCI) and are inhibitory to growth and repair. The bacterial enzyme chondroitinase ABC (ChABC) removes CSPG glycosaminoglycans, rendering the SCI environment more permissive to growth, and is a promising treatment option for SCI. However, despite the known effects on promoting repair and recovery of function, the mechanisms that underlie the observed improvements are poorly understood. Here we have carried out a detailed characterization of pathological and biochemical changes that occur in response to spinal contusion (a traumatic injury model that closely resembles the pathology and disease progression that occurs in a human SCI) and treatment with ChABC. We optimized the therapy by using genetically modified ChABC delivered via a lentiviral vector (LV-ChABC), since this produces long-lasting and widespread CSPG degradation and leads to improved functional outcome following spinal contusion. Adult rats received a moderate severity contusion injury (level T10) and were immediately injected with 1 ul LV-ChABC or control LV-GFP. At different post-injury time points (from 1 day - 12 weeks post-injury) secondary injury pathology was assessed. Histological assessments revealed significantly reduced cavitation, enhanced neuronal survival and sparing of spinal axons, increased vascularisation throughout the injury site and a marked change in the nature of reactive gliosis and the inflammatory response around the injury epicenter and cavity borders following LV-ChABC treatment compared to LV-GFP controls. To investigate the mechanism of neuroprotection in more detail, biochemical analysis was used to characterize LV-ChABC-mediated cellular and extracellular changes in injured spinal cords. LV-ChABC specifically modulated the levels of lysosomal ED1, a marker of phagocytic macrophages/microglia and increased the expression of CD206, the key marker of alternative (M2) macrophage polarization. The differential accumulation of aggrecan, versican and brevican was also affected by over-expression of ChABC. These findings demonstrate that a single administration of LV-ChABC prevents much of the secondary degenerative pathology that develops following contusion injury not only by altering the glycosylation of CSPGs but also by shifting the macrophage/microglia population towards the more reparatory M2 phenotype.

Supported by The U.K. Medical Research Council and the International Spinal Research Trust

Combined treatment by cotransplantation of mesenchymal stem cells and neural progenitors with exercise/enriched environment in mouse spinal cord injury

Marina Boido[1], De Amicis E.[1], Niapour A.[2], Salehi H.[3], Vercelli A.[1]

[1]Neuroscience Institute of Cavalieri Ottolenghi, Neuroscience Institute of Turin, Italy
[2]Dept. of Anatomy, Ardebil University of medical sciences, Ardebil, Iran
[3]Dept. of Anatomy, Isfahan University of medical sciences, Isfahan, Iran

marina.boido@unito.it

Stem cells give rise to undifferentiated stem cells, differentiate into committed mature cells, support regeneration after an insult, exert an anti-inflammatory potential and interact with the host immune system: therefore, they are good candidates in regenerative medicine of the CNS, which otherwise has a limited capacity for self-repair.

We used a mouse experimental model of spinal cord injury (SCI), reproducing the damage to neurons and axons fibers observed in patients. We previously tested the effect of transplantation of either mesenchymal stem cells (MSCs) or neural precursors (NPs): here, we tested the synergistic effect obtained by grafting both stem cells and enriched housing. In fact, two weeks after SCI, we injected a cell cocktail into the lesion cavity: immediately after, mice were housed in enriched environments in order to stimulate their locomotor activity. Injured mice without graft served as controls: some housed in enriched cages, the others in conventional ones. To evaluate functional recovery, mice underwent a battery of motor tasks. Three weeks after graft/saline, animals were perfused and their spinal cords analyzed. Grafted cells survived well, especially MSCs, but expressed neural markers only rarely.

Exercise elicited by enriched environment enhances recovery compared to normal environment after stem cell transplantation: moreover, the glial cyst volume, measured with Neurolucida software, appears affected by exercise [lesion volume reduction of 41.1% (transplanted mice vs controls) and 37.9% (enrichment housed mice vs controls)]. These results correlate with the behavioural improvement shown by the motor/sensory tests (BMS, foot-fault test, hindlimb flexion).

Therefore we propose that stem cells can secrete neurotrophic and immunomodulatory molecules, modulating the neuroinhibitory environment of the injured spinal: the combination of exercise with stem cell graft further improves its efficacy, both on histological grounds and in terms of functional recovery.

Supported by Girotondo Onlus, FAIP and AIM Foundations grants to AV

Spinal cord response to stepwise and block presentation of thermal stimuli

Rachel L. Bosma, Patrick W. Stroman

Queen's University, Kingston, ON, Canada

rachaelbosma@gmail.com

Spinal cord functional magnetic resonance imaging (fMRI) can be used to produce maps of neural function and therefore enables non-invasive visualization of spinal cord responses to peripheral thermal sensory stimuli. Given the limited temporal resolution of this technique, long stimulation paradigms are required to obtain enough data to accurately characterize the spinal cord response. However, repeated stimulation of unmyelinated C-polymodal fibers and thermoreceptors results in response depression, and these receptors quickly acclimate to an adaptation temperature. Therefore, careful selection of the stimulus paradigm is required to maximize the spinal cord response. The purpose of this study is to evaluate the characteristics of the spinal cord and brainstem response to peripheral stimulation in which temperature is raised in a stepwise fashion, in order to enhance receptor responses, compared to the conventional block design.

Functional MRI studies of the spinal cord and brainstem were carried out in healthy volunteers in a 3T Siemens Magnetom Trio. An MR-compatible Peltier thermode (Medoc) was placed on the right hand of participants and provided thermal stimuli during the experiments. Two sensory stimulation paradigms were employed; a stepwise design and a block design. In the stepwise condition the temperature was raised from 32 ^{0}C (baseline) to 37 ^{0}C, held for 13 seconds and raised to 42 ^{0}C, held for 14 seconds and raised a final time to 45 ^{0}C for 20 seconds. In the block design condition the temperature was raised from baseline to 45 ^{0}C and sustained at that temperature for 47 seconds. The ramp rate was 2 ^{0}C/second for all conditions.

Spinal cord data were analyzed using custom software (written in MatLab). A time series analysis was carried out by means of a general linear model (based on SPM) to identify areas responding to the thermal stimulation. Connectivity analyses were then conducted to identify the sensory network and determine interregional spinal cord and brainstem activity correlations during thermal stimulation.

Robust ipsilateral dorsal horn activity was evident in the stepwise condition, while weaker contralateral dorsal and ventral activity was more prominent in the block design. Stepwise results also revealed consistent activity in regions of the rostral medulla known to be involved in sensory processing and in a region in close proximity to the periaqueductal grey region in the midbrain.

Results from this study lay the foundation for ongoing studies to assess the changes that occur at each of these relay points as a result of trauma and may elucidate neurological changes which affect functional outcomes.

Monitoring evolution of a spinal motor bridge with indwelling spinal electrodes

Samit Chakrabarty[1], O.Uemura[2], T. Drew[3], C. J. Winfree[4], J H. Martin[5]

[1] School of Biomedical Sciences, University of Leeds, UK
[2] National Hospital Organization Murayama Medical Center, Japan
[3] Dept of Physiol., Univ. of Montreal, Montreal, QC, Canada
[4] Dept of Neuro-Surgery, Columbia Univ., New York, NY, USA
[5] Dept of Physiol., City College of New York, NY, USA

S.Chakrabarty@leeds.ac.uk

Previously we showed that motor control could be restored after a spinal cord injury by reconnecting the brain with spinal motor circuits below the lesion in a rat (Campos et al. J Comp Neurol 2008, Uemura et al. SFN 2008). A re-innervated motor nerve bridge around the injury to target local motor circuits directly is an important alternative to promoting axon regeneration through the injury. A thoracic nerve from above the injury was detached from the muscle it innervates and the cut end inserted caudally into the lumbar gray matter caudal to the injury, where thoracic motor bridge axons regenerate and form functional synaptic connections with spinal cord neurons, including lumbar motoneurons.

Here we demonstrate similar approach in the adult cat. As in the rat, a thoracic nerve from above a lateral column lesion was isolated, cut from its muscle target, and the cut end inserted into the lumbar spinal cord caudal to the injury. To determine outgrowth, in the first set of animals we injected an anterograde tracer into the bridge nerve close to the insertion, between 7 and 24 hours before euthanizing the animal. This revealed the presence of labelled axons in the spinal cord. In a subset of these animals we electrically stimulated the bridge nerve during the terminal tracer injection experiment and this evoked hind leg muscle contraction. In another set of animals, we identified outgrowths electrophysiologically using indwelling spinal microwire electrodes recording the bridge nerve responses evoked by simulation through an indwelling bridge nerve cuff electrode. Animals were followed over several months. A progressive shortening of the latency of the evoked response and an increase in the amplitude of the response was continuously monitored. Response amplitude scaled with stimulus amplitude. In selected experiment, both in the awake and anesthetized animal, bridge nerve stimulation evoked, in short sequence (<10 ms), a lumbar local field response followed by hind leg muscle contraction. In one of these animals we were able to demonstrate the role of this new bridge in control of hindlimb locomotor activity.

It is evident that there is sufficient outgrowth making effective connections with motoneurons, evoking a hind leg muscle contraction. Using indwelling stimulation and recording techniques we monitored progressive plastic changes in the synaptic connections that the bridge nerve made with spinal cord neurons. Our findings suggest that this approach might, one day, be tried in humans.

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

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

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

dc501@cam.ac.uk

A debilitating consequence of spinal cord injury (SCI) is loss of bladder fullness perception and micturition. The development of a hyper-reflexive bladder provides some relief. However, the dyssynergia between detrusor and sphincter results in incomplete expression and potential infection. An effective way to restore self-directed micturition in humans is artificially, through implantation of a Sacral Anterior Root Stimulator (SARS) (Brindley 1974, Brindley *et al.,* 1986, Brindley 1994). The critical limitation of this device is the inability to detect bladder fullness, and therefore, when expression is required. The project aims to use afferent information during bladder filling, to drive SARS output. This is to be accomplished through the design and implantation of recording electrodes contained within microchannels, onto the dorsal roots of rats. When optimised, these prostheses will provide indication for timely bladder expression in dogs suffering SCI, with the ultimate goal of translation to humans. To date, in collaboration with Finetech Medical Ltd, a newly designed SARS book electrode for the bilateral S2 ventral roots has been implanted successfully, with maintained efficacy, in nine dogs with SCI. Current work has focussed on identifying bladder afferent activity at acute and chronic implantation stages, as well as optimising device design in rat. We have electrophysiologically identified bladder afferents during artificial bladder infusion using a variety of microchannel devices. Bladder afferent action potentials are small in amplitude compared to cutaneous and muscle spindle, so the root must be 'teased' into fascicules and insulated within 100 µm microchannels to improve the signal output. Implants containing electrodes are fabricated from polydimethylsiloxane (PDMS), and the dorsal root is teased apart using fine glass capillaries maintaining continuity with the spinal cord. At 1, 4, and 12 weeks after L6/S1 dorsal root implantation, axons show survival and functionality, without detrimental consequences to the bladder. In response to this positive pre-clinical data, three dogs have been implanted acutely or chronically with dorsal root recording devices in conjunction with the SARS implant.

Ex-vivo diffusion tensor imaging (DTI) in a mouse model of contusion spinal cord injury

G. Buonincontri[1,4], **Matteo Donegà[2-4]**, C. Alfaro-Cervello[2-4], G.E. Tyzack[2,4], A.O. Ward[1.4], S.J. Sawiak[1,4], T.A. Carpenter[1,4], S. Pluchino[2-4]

[1]Wolfson Brain Imaging Centre, [2]Centre for Brain Repair; [3]Stem Cell Institute; [4]Department of Clinical Neurosciences, University of Cambridge, UK

md574@cam.ac.uk; spp24@cam.ac.uk

Spinal Cord Injury (SCI) is damage or trauma to the spinal cord that results in partial or complete loss of function below the site of damage, causing reduced mobility or feeling. The current management of SCIs in the clinical practice addresses the initial acute event and includes both medical and surgical stabilization followed by an acute and long-term rehabilitative period. Unfortunately, there is no available effective therapy for chronic SCI patients other than rehabilitation. Several pre-clinical *in vivo* SCI models are available to investigate its pathophysiology as well as to test experimental therapeutics. The development of non-invasive imaging techniques, dynamically addressing the changes in the anatomy and function in SCI compared to standard histopathology are highly desirable. Here we have subjected 14 C57Bl/6 male mice to severe (70 kdynes) T12 contusion SCI and assessed their locomotor recovery by Basso mouse scale (BMS) score for a total of 10 weeks after contusion. Ex-vivo 4.7 T magnetic resonance imaging (MRI) of the whole spine was performed on SCI mice at both 4 and 10 weeks after contusion (ex-vivo), corresponding to the chronic disease stage. The spinal cords were scanned using spin-echo diffusion tensor imaging (SE-DTI) over the site of injury, at the level of the perilesional area as well as in normal-appearing healthy tissue imaged at 7 mm rostral to the lesion.

We discriminated between damaged tissue, perilesional tissue and healthy tissue, with scan times compatible with in-vivo examination. We also identified the anisotropy index (AI) as potential biomarker of tissue damage that, if combined with systematic behavioral analyses and validated by histopathology, will provide a valuable tool in the evaluation of the differences in the lesioned tissue and their relation to the clinical outcome. Further implementation with in-vivo imaging will allow longitudinal monitoring of the evolution of the naturally occurring cord damage. The combination of advanced behavioral tests, histopathology and MRI may dramatically improve sensitivity to track changes in both CNS structures and functions after injury and the effect of interventional treatments.

Supported by Wings for Life (grant XBAG/163 to SP), the European Research Council (ERC) under the ERC-2010-StG Grant agreement n° 260511-SEM_SEM and the European Community (EC) 7th Framework Program (FP7/2007-2013) under Grant Agreement n° 280772-iONE.

Plasticity of sphincter function induced by repetitive transcranial magnetic stimulation after incomplete spinal cord injury

N. Vasquez[1], **Peter H. Ellaway[3],** A. Gall1, S. Knight[1], J. Susser[1], M.D. Craggs[1,2]

[1]The London Spinal Cord Injuries Centre, Royal National Orthopaedic Hospital NHS Trust, Stanmore, UK
[2]University College London, UK
[3]Imperial College London, UK

p.ellaway@imperial.ac.uk

In stable incomplete male spinal cord injury (iSCI) subjects with severely compromised voluntary control of the sphincters the aberrant "guarding reflexes" can be monitored by recording the pudendo-anal reflex (PAR). We find this reflex to be facilitated in control subjects by single pulses of transcranial magnetic stimulation (TMS) delivered optimally 30 ms prior to reflex stimulation. The facilitation appears weaker in most iSCI subjects. We are currently investigating whether known neuro-plasticity inducing protocols of repetitive TMS elicit long term potentiation of sphincter responses.

Male control and chronic (stable) iSCI subjects have been assessed using dorsal penile nerve (DPN) stimulation to elicit a PAR, single pulse TMS to elicit an anal sphincter motor evoked potential (MEP) and single pulse TMS to condition the PAR. The effect on these responses of two different regimes of excitatory repetitive TMS has been assessed: (1) 5Hz repetitive TMS of the motor cortex at a strength just below resting motor threshold for the anal sphincter MEP, and (2) paired associative stimulation (PAS), employing DPN coupled with TMS, both just above motor threshold, and with an interval of 40 ms at 0.25Hz.

5Hz continuous repetitive TMS (up to 900 stimuli) has enhanced the facilitation of the PAR by conditioning TMS in control subjects. However, the higher cortical thresholds in iSCI and technical limitations have precluded application of 5 Hz rTMS in iSCI subjects. PAS at lower frequencies is acceptable and technically feasible in iSCI. Interpretation of results from application of both regimes has had to allow for significant adaptation of the PAR to repeated DPN stimuli. Preliminary results in control subjects have shown PAS (120 paired stimuli) to facilitate the sphincter MEP and increase the TMS facilitation of the PAR. Our preliminary results in iSCI show considerable variability which might be explained by the likely unique character of the lesions of each of the subjects. As a result, we have included an additional PAS protocol consisting of DPN paired with sham TMS in an attempt to identify cause with effect. These preliminary results will be presented.

PAS is proving to be a more practical protocol than 5Hz repetitive TMS to examine plasticity of sphincter function in iSCI.

Supported by INSPIRE & The Royal National Orthopaedic Hospital Charity

Co-cultures of olfactory ensheathing cells and postnatal spinal cord organotypic slices to investigate neuron outgrowth

Karen Gladwin, Stuart Law, Daqing Li, David Choi

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

K.gladwin@ucl.ac.uk

Olfactory ensheathing cells (OECs) are unique glial cells with potential therapeutic properties for the treatment of spinal cord injuries such as spinal root avulsions. When transplanted into spinal cord lesions, OECs have been shown to enhance axon regeneration by mechanisms that are currently unclear. Present hypotheses suggest that OECs provide trophic support and a permissive pathway for regenerating axons through the inhibitory environment of the CNS. After ventral root avulsion injury, OEC therapy may enhance the survival of ventral horn neurons and improve axon regeneration through CNS white matter to reach the re-implanted ventral root.

The aim of this study was to investigate the interaction of OECs with spinal cord tissue and to quantify the effects of OECs on the survival and outgrowth of spinal neurons in an *in vitro* spinal cord slice co-culture. Cervical spinal cord slices from P8 Sprauge Dawley (SD) rats were cultured on collagen coated membrane inserts. OECs were harvested from adult SD rats, cultured for a period of 14 days and transfected with GFP prior to co-culture with spinal cord slices in a collagen gel. Our results indicate that OECs have a positive effect on neurite outgrowth and morphology.

This work was **supported by** the European Research Council and the International Spinal Research Trust

Viral delivery of interleukin-4 reprograms the macrophage response to traumatic spinal cord injury

Jodie C.E. Hall[1], Kristina A. Kigerl [1], Kevin D. Foust [1], Alexander D. Roszman[1], Brian K. Kaspar[1,2], Phillip G. Popovich[1]

[1] Center for Brain and Spinal Cord Repair, Wexner Medical Center, The Ohio State University, Columbus, Ohio, USA
[2] Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA

Jodie.Hall@osumc.edu

Macrophages are a pathological hallmark of spinal cord injury (SCI) and most adopt an M1 phenotype. M1 macrophages are extrinsic barriers to CNS regeneration and cause axonal "die-back". Emerging data indicate that it is possible to reduce M1 macrophage burden at sites of inflammation and promote the formation of M2 macrophages, cells that we have shown previously can support axonal growth without concurrent toxicity *in vitro*. Here we test the hypothesis that systemic (intravenous; i.v.) post-injury injection of a novel adeno-associated virus (AAV9) engineered to produce interleukin-4 (IL4) is capable of reprogramming the phenotype of the endogenous macrophage response after SCI. Specifically, significantly more M2 (arginase 1+) macrophages (25%) occupy the injury site of AAV9-IL4 injected mice compared to control mice injected with AAV9-GFP (0.6 %). In the spinal cord, mostly astrocytes but also a few neurons and oligodendrocytes (but not microglia), were transduced with the AAV9 vector. Transduced cells were evident over ~6 mm of spinal cord. In the periphery, widespread systemic transduction also occurred prompting us to consider the long-term consequences of systemic IL4 delivery and the breadth of cellular responses. Western blot analyses of IL4 receptor α (IL4Rα) and pSTAT6 activation (signaling intermediate downstream of IL4Rα) revealed IL4Rα in several organs including the spleen, thymus, lung and heart. In the spleen of SCI mice, pSTAT6 was activated only in mice injected i.v. with AAV9-IL4, indicating that systemic effects of IL4 are likely. To maximize the potential for reprogramming intraspinal macrophages that will support axon regeneration and limit die-back of injured axons while circumventing potential problems associated with systemic IL4Rα activation, we have developed a 2nd generation AAV9 vector. This vector, which is currently being characterized for use *in vivo*, will restrict IL4 expression to the spinal cord.

Laser microdissection of *in vivo* regenerating spinal neurons identifies genes including ptpn2 that promote neurite outgrowth

Thomas H. Hutson[1,*], Lawrence D.F. Moon[1,2,*], Ronald E. van Kesteren[3], Kerstin Iffland[3], Jorge Torres-Muñoz[4], Carol K. Petito[4], Christopher P. Bowen[2], James B. Aimone[5], William J. Buchser[2], Vance P. Lemmon[2], John L. Bixby[2], August B. Smit[3], Fred H. Gage[5], Mary Bartlett Bunge[2]

*** Joint first authors**

1 Wolfson Centre for Age-Related Diseases, King's College London, London, UK
2 The Miami Project to Cure Paralysis, University of Miami, Miami, FL, USA
3 Center for Neurogenomics and Cognitive Research, VU University, Amsterdam, The Netherlands
4 Department of Pathology, University of Miami, Miami, FL, USA
5 The Salk Institute for Biological Studies, La Jolla, CA, USA

thomas.hutson@kcl.ac.uk

Following injury to the central nervous system (CNS), neurons show a very limited axonal regenerative response due to a reduced intrinsic growth state and the presence of growth inhibitory molecules that form a molecular and physical barrier to regeneration. In this study we aim to enhance the intrinsic growth state of CNS neurons, enabling them to overcome a growth-inhibitory substrate *in vitro* and to increase regeneration *in vivo*. To identify novel targets for spinal cord repair, a novel strategy was used to identify genes that promote CNS axon regeneration. We laser microdissected spinal neurons that regenerated axons into a Schwann cell bridge implanted following complete transection of the adult rat cord. Microarray comparison of mRNAs from regenerating *vs.* nonregenerating neurons identified 552 known and novel regeneration-associated genes (RAGs). For example, the expression level of *Protein tyrosine phosphatase non-receptor type 2* (*Ptpn2*) was increased twofold after axotomy and this level was sustained in regenerating neurons. Functional screening of >500 genes using a medium-throughput electroporation assay showed *inter alia* that over-expression of *Ptpn2* increased the neurite outgrowth of CNS neurons on two different growth-inhibitory substrates (cells expressing myelin-associated glycoprotein and on chondroitin sulfate proteoglycans) and on a growth-permissive substrate (poly-L-lysine). Regarding the mechanism by which *Ptpn2* increases neurite outgrowth we demonstrate that the DNA binding domain and not the phosphatase domain of *Ptpn2* is required to enhance neurite outgrowth. Furthermore we have now developed bicistronic AAV vectors that over-express either *Ptpn2*-2A-*eGFP* or *mCherry*-2A-*eGFP* and are currently testing these in a rat model of spinal cord injury. *Ptpn2* is a novel potential target for promoting axon regeneration after spinal cord injury.

This work was **supported by** the Christopher and Dana Reeve Foundation International Research Consortium (M.B.B.), the Buoniconti Foundation (M.B.B.), the Miami Project to Cure Paralysis (M.B.B.), the National Institutes of Health (S10 RR15694-01, CKP), the Center for Medical Systems Biology in the framework of the Netherlands Genomics Initiative (R.v.K., A.B.S), the Henry Smith Charity (L.D.F.M., T.H.) a Friends of Guy's Hospital Research Grant (L.D.F.M), a Biotechnology and Biological Sciences Research Council's Doctoral Training Grant (T.H.) and the UK Medical Research Council (L.D.F.M.)

Gene delivery of chondroitinase ABC promotes functional repair following contusion injury at thoracic or cervical level

Nicholas D. James, Jessie R. Shea, Karen D. Bosch, Katalin Bartus, John H. Rogers, Stephen B. McMahon, Bernard L. Schneider, Elizabeth M. Muir, Elizabeth J. Bradbury

Regeneration Group, Wolfson Centre for Age-Related Diseases, King's College London, London SE1 1UL, UK

nicholas.n.james@kcl.ac.uk

Spinal cord extracellular matrix is densely packed with growth inhibitory chondroitin sulphate proteoglycans (CSPGs), which become more abundant after injury. Thus, matrix modification has become a leading experimental strategy for promoting repair following spinal cord injury. Despite the beneficial effects that have been achieved by digesting CSPGs with the bacterial enzyme chondroitinase ABC (ChABC), the potential for achieving long term efficacy in traumatic injuries that mimic a human spinal cord injury has not yet been realised. Gene therapy offers a route to achieving stable continuous delivery of ChABC and therefore, here we deliver genetically modified ChABC via a lentiviral vector (LV-ChABC) to the adult rat spinal cord and assess the efficacy of chronic gene delivery using a spinal contusion injury model. Contusion injury represents the most common form of spinal cord injury in humans and, therefore, provides a clinically relevant tool for assessing the efficacy of potential therapeutic interventions. Adult rats received a moderate severity thoracic (T10) contusion injury and LV-ChABC or a control LV-GFP was immediately injected rostral and caudal to the injury site. We demonstrate prolonged and widespread CSPG degradation with LV-ChABC and, using both behavioural and electrophysiological outcome measures, we show improved function in animals treated with LV-ChABC. We saw a dramatic increase in spinal conduction through the injury site as well as a significant improvement in performance on the horizontal ladder test. Using an additional electrophysiological technique we also saw evidence of plastic changes in the form of reorganisation of spinal circuitry below the level of the injury. In order to enhance the potential clinical applications of this study we have now also assessed the effects of LV-ChABC in a moderate severity cervical (C5) contusion injury. Approximately 50% of all human spinal cord injuries occur at the cervical level making this injury model of particular clinical relevance as well as allowing us to assess a number of additional functional outcomes such as forelimb grip-strength, sensory and motor function during sticky-tape removal, and proprioception using the inclined plane. We again saw significant improvements in the horizontal ladder task as well as a striking increase in spinal conduction; in addition there were also modest improvements in forelimb grip-strength. Thus, we demonstrate the potential advantages of gene delivery of ChABC for achieving sustained and widespread CSPG degradation and that this is associated with functional improvements following contusion injury.

Supported by: The U.K. Medical Research Council, the International Spinal Research Trust, the Darwin Trust of Edinburgh and the International Federation for Research in Paraplegia

Human adipose-derived mesenchymal stem cells cultured on medical grade silicon secrete LL-37 peptide and have antibacterial activity: implications for the treatment of chronic skin ulcers

D. Al-dhahri, N. Kohli, T. Worthington, A.C. Hilton, *S. MacNeil, L. Jeys, M. Snow, **W. Eustace B. Johnson**

Life & Health Sciences, Aston University, Birmingham, B4 7ET, UK
*Kroto Institute, University of Sheffield, UK

w.e.johnson@aston.ac.uk

Patients with neurological deficits following spinal cord injury are susceptible to pressure ulcers, which are often compounded by bacterial infection. Helping bacterial clearance whilst also enhancing wound healing seems a reasonable therapeutic approach. Recent studies have shown that bone marrow-derived mesenchymal stem cells (BM-MSCs) have skin wound healing properties [1] and antibacterial activity [2]. Therefore, this study has investigated the anti-bacterial activity of adipose-derived MSCs (AD-MSCs), as these cells are more readily generated in large numbers than BM-MSCs and thus potentially advantageous for the treatment of acute and chronic wounds. Herein, we show that human AD-MSCs have antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* through inhibition of bacterial survival/growth as well as reduced biofilm formation. In order to exploit these findings in clinic, we tested the compliance of AD-MSC with a medical grade silicon-coated membrane that has been used as a keratinocyte delivery system for the treatment of acute burns and diabetic non-healing skin wounds [3, 4]. This demonstrated that the AD-MSCs adhered and remained viable on the silicon cell membranes and maintained their anti-bacterial activity. Moreover, it was shown that the anti-bacterial activity was related to the time-dependent secretion of soluble factors into AD-MSC culture conditioned medium (MSC CM) following bacterial challenge, which was associated with an up-regulation of the antimicrobial peptide LL-37 for *S. aureas* and that acted on both types of bacteria, independently of the initial type of bacterial challenge. Therefore, this study has demonstrated that the delivery of AD MSC has therapeutic potential in the treatment of infected skin wounds.

References

1. Walter MN, Wright KT, Fuller HR, MacNeil S, Johnson WE. Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an *in vitro* study of fibroblast and keratinocyte scratch assays. Exp Cell Res. 2010 Apr 15;316(7):1271-81.
2. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells. 2010 Dec;28(12):2229-38.
3. Hernon CA, Dawson RA, Freedlander E, Short R, Haddow DB, Brotherston M, MacNeil S. Clinical experience using cultured epithelial autografts leads to an alternative methodology for transferring skin cells from the laboratory to the patient. Regen Med. 2006 Nov;1(6):809-21
4. Moustafa M, Bullock AJ, Creagh FM, Heller S, Jeffcoate W, Game F, Amery C, Tesfaye S, Ince Z, Haddow DB, MacNeil S. Randomized, controlled, single-blind study on use of autologous keratinocytes on a transfer dressing to treat nonhealing diabetic ulcers. Regen Med. 2007 Nov;2(6):887-902.

Supported by the Foreign and Commonwealth Office, Aston University and the Royal Orthopaedic Hospital, Birmingham

The S120G mutant form of Nm23H1 stimulates neurite outgrowth and inhibits non-neuronal cell migration from embryonic chicken dorsal root ganglia

*****A.J. Lilly, L. Toyne, **K.T. Wright, *C.M. Bunce, **W. Eustace B. Johnson**

Life & Health Sciences, Aston University, Birmingham, B4 7ET, UK
*Biosciences, University of Birmingham, UK
** Institute for Science & Technology in Medicine, Keele University, UK

w.e.johnson@aston.ac.uk

The nucleoside diphosphate (NDP) kinase, Nm23H1, is expressed at high levels in the developing CNS; furthermore, transfection of neuronal cell model systems has been associated with increased differentiation and neurite growth. Previously, we reported that extracellular recombinant Nm3H1 stimulates chick and rat DRG neurite outgrowth and formed a preferred culture substrate compared with collagen type I [1]. These neurostimulatory activities were not dependent on NDP kinase activity, but otherwise the mechanism of action remained unknown. Here, we have shown that an S120G mutant form of recombinant Nm23H1, which forms a dimer rather than the predominant hexamer seen in the wild type protein, has an enhanced neurostimulatory activity on chicken DRG explants. Neurite outgrowth was denser with increased branching on culture substrates coated with S120G Nm23H1 compared to collagen type I and other forms of the recombinant protein, including wild type, P96S or the H118F kinase deficient form. The S120G form of Nm23H1 also markedly inhibited the migration of non-neuronal cells from the DRG explants compared to collagen type I. These studies suggest that the activity of extracellular Nm23H1 on neuronal systems may depend on whether the protein is present in dimerised versus hexamer form.

References

1. Wright KT, Seabright R, Logan A, Lilly AJ, Khanim F, Bunce CM, Johnson WE. Extracellular Nm23H1 stimulates neurite outgrowth from dorsal root ganglia neurons *in vitro* independently of nerve growth factor supplementation or its nucleoside diphosphate kinase activity. Biochem Biophys Res Commun. 2010 Jul 16;398(1):79-85.

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

Danial Kahani, Bernard Conway

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

Danial.Kahani@strath.ac.uk

Spinal cord injury results in severe physical disability and a range of progressive medical complications. Robotics-assisted technology platforms can be used to enhance walking rehabilitation outcomes based on protocols incorporating intensive repetitive sensori-motor stimulation. By moving limbs and progressively modifying trunk support, the patterned sensory information arising from the robotic guidance of movement is considered to increase the potential for the recovery by mechanisms that lead to adaptive change. For patients with incomplete spinal cord injury treadmill training incorporating robotic devices has been demonstrated to have positive effects on over ground walking. Commercial devices such as the Lokomat can guide the hip and knee joint motion in a way that simulates walking. The level of guidance can be adjusted in relation to the patient's ability and rate of recovery.

Deficits in walking in spinal cord injured patients are often revealed as deficits in ankle control and it is recognised that the loss of control over ankle dorsiflexion is associated with abnormal corticospinal function. Accordingly, it is believed that successful recovery of stepping requires a degree of sparing in the corticospinal pathways that subserve ankle joint control. We therefore have begun experiments to examine if additional phasic activation of sensory afferents implicated in control of walking when introduced to a body weight support rehabilitation program can facilitate improved voluntary stepping in patients. The work has focused on developing a system for vibratory stimulation of the foot sole that can act as a surrogate stimulus for ground contact and we are currently examining the effects of the stimulus in static conditions in normal subjects prior to tests with spinal cord injured patients.

Previously we demonstrated the ability of this form of sensory stimulation to evoke activity in cortical sensori-motor areas associated with ankle function. To assess the spinal effects of localized vibration of the foot sole we have now used the soleus H-reflex as a measure of motor neuron excitability and of pathways associated with ankle control. The soleus H-reflex is strongly depressed during loading of muscle such as occurs in stance and we were keen to learn if localize vibration of the plantar surface produced a similar effect before commencing testing with patients. Vibration was applied to the foot over the metatarsal heads or the heel. The strength of vibration was set as 1.5 times perceptual threshold, at 100Hz and the duration of vibration was varied between 50ms and 3s. Vibration of the metatarsal area of the foot sole generates a larger depression of the H-reflex compare to heel stimulation. the peak depression is seen between 200ms and 400ms. When these tests are conducted with the subjects standing the pattern of depression remains the same but the magnitude of the depression is increased. These results demonstrate the effectiveness of the cutaneous vibratory stimuli as a method to influence pathways associated with the control of ankle musculature as does the dependency on the size of effect associated with the area of skin stimulated. The results observed serve to provide a set of control data and proof of principle prior to tests on patients with spinal cord injury.

Supported by the International Spinal Research Trust

Peripheral delivery of recombinant human Neurotrophin-3 for Spinal Cord Injury

Claudia Kathe, Thomas Hutson, Stephen McMahon, Lawrence Moon

Neurorestoration Department, Wolfson CARD, King's College London, London, SE1 1UL, UK

claudia.kathe@kcl.ac.uk

Spinal Cord Injury is a debilitating pathology and can result in some form of paralysis due to immediate axotomy and neuronal loss. Our goal is to increase sprouting of axons and functional recovery, which occurs to a limited degree spontaneously after SCI. Neurotrophin-3 has been shown to facilitate corticospinal tract sprouting, plastic changes in the sensorimotor cortex and functional recovery after central nervous injuries in animal models such as stroke.
Our first aim is to test an intramuscular treatment of neurotrophin-3 after spinal cord injury in rodent models. We hypothesise that neurotrophin-3 leads to sprouting of intact corticospinal tract fibers across the midline in the spinal cord after unilateral injuries, and that will facilitate functional recovery. In our study, rats received a unilateral pyramidotomy severing one of the motor tracts. 24 hours after the injury, intramuscular neurotrophin-3 protein or saline infusions were started, continuing for 4 weeks, into the triceps. We performed different behavioural tests (that assessed sensory and motor function recovery) and electrophysiology (that tests for strengthened output to muscles after stimulation of spared descending tracts). Our second aim is to establish mechanisms of neurotrophin-3 action. We want to address multiple questions about neurotrophin-3 neuronal uptake, transport and release in the spinal cord. We developed two AAV vectors expressing genetically tagged neurotrophin-3, which we will assess in various CNS injury paradigms.

Future Directions: Systemic delivery of high doses of neurotrophin-3 has been shown to be safe and effective in human clinical trials for a neuropathy and constipation. If intramuscular neurotrophin-3 treatment improves functional recovery after acute spinal cord injury it will be the first step towards a clinical application of neurotrophin-3 for this pathology.

Supported by the International Spinal Research Trust, Nathalie Rose Barr Studentship

Forelimb neuroplasticity following incomplete cervical spinal cord injury in adult rat

Gonzalez-Rothi E.J., Rombola A., Fernandez L., Alappattu M., Daly A., Rousseau C.A., Federico R.A., O'Steen B.E., Vandenborne K.V., Reier P.J., Fuller D.D., **Michael Lane**

University of Florida, Gainesville, USA

malane@ufl.edu

Injury in the cervical spinal cord (cSCI) significantly impairs function in the upper extremitie. Although some spontaneous functional recovery has been demonstrated, the extent of recovery is limited and the underlying mechanisms are poorly defined. Using a well-established rodent model of high cSCI, lateral C2 spinal cord hemisection (C2Hx), the present works examines the muscular and neuroanatomical substrates underlying upper extremity dysfunction and recovery following incomplete cSCI. Gross forelimb motor function was assessed prior to, and at 1- and 8-weeks post-injury using the limb-use asymmetry (cylinder) test. Immunohistochemical techniques were used to assess forelimb muscle fiber cross sectional area (CSA). The neuroanatomical circuitry of the forelimb was assessed using retrograde transneuronal tracing techniques. Initial results indicate dramatic reductions in ipsilateral forelimb use ($p<0.01$) and muscle fiber CSA ($p<0.05$) at 1-week post-C2Hx. By 8-weeks post-injury, improvements in ipsilateral forelimb use ($p<0.01$) and increased muscle fiber CSA ($p<0.05$) were observed. Taken together, these initial results indicate substantial muscular remodeling and modest recovery of forelimb function following cSCI. Preliminary results from tracing studies reveal the location and distribution of forelimb motoneurons and interneurons in the cervical spinal cord and associated neurons within the brainstem and motor cortex of uninjured animals. Ongoing studies are exploring the neuroanatomical circuitry associated with forelimb dysfunction and recovery after injury. These experiments are the first to examine the combined muscular and neuroanatomical mechanisms underlying neuroplasticity and recovery of upper extremity function following cSCI and may aid in identifying potential therapeutic targets for rehabilitation interventions.

Injection of ATP to sciatic nerve induces sensory axonal regeneration after spinal cord injury

Sena Lee, Dongsheng Wu*, John Yeh, Yi Zhang, Xuenong Bo

Centre for Neuroscience and Trauma, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London E1 2AT, UK

s.lee@qmul.ac.uk

It has been known for several decades that lesion of a peripheral nerve can significantly enhance the regenerative capacity of the corresponding dorsal root ganglion (DRG) neurons, a phenomenon called conditioning lesion. As conditioning lesion is not a clinically feasbile approach to enhance the regeneration of injured sensory axons, an alternative treatment is needed to mimic the condition lesion to enhance the axon regeneration. Since a large amount of ATP is released in injured nerves and extracellular ATP can induce the release of various bioactive factors such as cytokines, we postulate that ATP may be involved in the mechanism of conditioning lesion. In this study, we showed that injection of both high (10 mM) and low concentrations (150 μM) ATP into adult rat sciatic nerves enhanced neurite outgrowth of dorsal root ganglion neurons. Intraneural injection of ATP also activated the axon growth related transcription factor, signal transducer and activator of transcription 3 (STAT3), and significantly increased the expression of growth associated protein 43 (GAP43) in dorsal root ganglion neurons. Results from ELISA showed that after ATP injection neuropoietic cytokine interleukin-6 level was increased in dorsal root ganglia and at the ATP injection site, indicating that activation of STAT3 may be partially due to increased synthesis and release of interleukin-6. In a rat spinal cord dorsal column transection model, significantly more cholera toxin B subunit-labelled axons were counted at the caudal border of the lesion site and inside the lesion cavity in the nerve crush group and ATP injection groups than in the sham and saline injection groups. The results from this study demonstrate that ATP injection into rat sciatic nerve can mimic the conditioning effects of peripheral nerve injury, leading to enhanced regeneration state of the DRG neurons.

* DW carried out most of the experiments in this study

Supported by the Wellcome Trust. SL was supported by a scholarship from Korea

AAV vector allows widespread and long-term secretion of chondroitinase ABC in rat CNS

João Nuno Alves [a,b], Melissa R. Andrews [a], Anneliese Ward [b], Nicholas Michelmore [b], Joost Verhaagen [c], Elizabeth Moloney [c], Roger J. Keynes [b], James W. Fawcett [a], John H. Rogers [b], **Elizabeth M. Muir [b]**

[a] Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge CB2 0PY, UK
[b] Department of Physiology Development and Neuroscience, University of Cambridge, Downing St, Cambridge, CB2 3EG, UK
[c] Laboratory for Neuroregeneration, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105BA Amsterdam, Netherlands

emm1@mole.bio.cam.ac.uk

The bacterial enzyme chondroitinase ABC has been shown to promote axon regeneration following spinal cord injury in many models and in three species. It is therefore a promising strategy for the treatment of SCI. However, repeated injections, which would likely be required for human treatment, would increase the risk of causing further trauma and infection, therefore gene therapy is a desirable alternative route of administration. We have modified the bacterial chondroitinase gene to achieve efficient secretion of active chondroitinase from mammalian cells, as previously demonstrated with lentiviral vectors both *in vitro* and *in vivo*, assessed by staining for the products of digestion (stub antibody 2B6). We then inserted the gene into AAV vectors, since these vectors are currently considered the safest for the treatment of patients. AAV-GFP and/or AAV-chondroitinase vectors were injected into adult rat cortex and expression profiles were examined 4 weeks later. Both AAV5 and AAV8 gave efficient transduction around the injection site. GFP was expressed by many neurons and their axons, including the corticospinal tract. Chondroitinase was expressed strongly and widely as indicated by 2B6 staining. Injections into vibrissal-motor cortex led to chondroitinase secretion in the midbrain, including the substantia nigra. Injections that spread into adjacent areas of cortex and hippocampus led to additional chondroitinase secretion which in some areas extended beyond the visible GFP-positive fibres, probably because it is efficiently secreted from very thin, terminal arborisations from some neuronal types. At 12 weeks post-injection, chondroitinase expression was still widespread.

Test re-test reproducibility for 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

Functional MRI (fMRI) is a non-invasive technique that allows the inference of neuronal activation from MRI signal changes.

There is an increasing interest in performing fMRI in the spine but a lot of research must still be done in order to make it an established MRI technique, as it is in the brain. We performed 3 fMRI scans on the same healthy volunteer but on different days and then we compared the results. The aim of this work was to study the reproducibility and feasibility of this fMRI protocol. The stimulus was always delivered in the same way and it consisted of poking the palm of the left hand using a pointed object. The speed of the poking was 2Hz and the strengh was such not to hurt the volunteer's hand. We performed the scans using a 3T MRI scanner (Philips Achieva TX, Best, Netherlands) with a 16 channel neurovascular coil. The block design comprised 10 alternating epochs of rest and movement, each lasting 36 seconds, for a total of 200 volumes. We performed all the scans using the ZOnally-magnified Oblique Multislice EPI (ZOOM-EPI) sequence, using a reduced field of view for targeted areas of fMRI activations. ZOOM-EPI is a Spin-Echo sequence, with minimal contribution from venous flow to fMRI signal changes. Moreover, one of the characteristics of ZOOM-EPI is that it is less sensitive to susceptibility artefacts, given the higher pixel bandwidth and reduced echo train length. The imaging parameters were: TR=3600ms, TE=30ms, voxel size 1.2x1.2x4mm3 (reconstructed to 1.2x1.2x1mm3 with 1mm gap among slices, FOV= 44x64 mm2, we acquired 9 slices. The geometrical analysis was performed using SPM8 software and the images were analysed using the GLM after the geometrical analysis comprising of slice-timing, realignment and smoothing by two times the voxel in plane dimensions. The results showed ipsilateral and contralateral activations at the level of C6-C7 vertebrae and the activated areas were consistent among the different scan sessions.

References

P.Stroman, Magnetic Resonance Imaging of Neuronal Function in the Spinal Cord: Spinal fMRI. Clinical Medicine&Research (2005); Vol.3, Num.3 : 146-156

CAM Wheeler Kingshott et al. Investigating Cervical Spinal Cord Structure Using Axial Diffusion Tensor Imaging. NeuroImage 16, 93–102 (2002)

Supported by the International Spinal Research Trust

Signalling and down-stream targets of Jun-dependent regeneration programme

Smriti Patodia[1], Carolina Acosta-Saltos[1], Xavier Fontana[2], Mariya Hristova[1], Chloe Santos[1], Joerg Hoeck[2], Reinhard Faessler[3], Gennadij Raivich[1]

[1]Centre for Perinatal Brain Protection and Repair, University College London, UK
[2]Mammalian Genetics Laboratory, Cancer Research UK, UK
[3]Department of Molecular Medicine, Max Planck Institute of Biochemistry, Germany

s.patodia@ucl.ac.uk

Unlike the central nervous system, peripheral nerve regeneration is generally successful even if not very precise. Recent studies have shown a key role for the transcription factor c-Jun in allowing regeneration to occur (Patodia and Raivich, 2012). In the facial nerve axotomy model, deletion of c-Jun throughout brain neuroepithelium-derived cells (Raivich et al, 2004) or just in neurons (Ruff et al, 2012) abolishes most of the cell body response after axotomy including the upregulation of the regeneration-associated neuronal alpha7 beta 1 integrin. It also abolishes post-traumatic neuronal cell death, causes most of the motoneurons to atrophy, and very strongly reduces the ability of their axons to regenerate, reinnervate their peripheral targets and contribute to functional recovery. Deletion of c-Jun in peripheral nerve Schwann cells (the p0::jun mutants) produces a very different effect (Fontana et al, 2012). Neuronal cell death is increased by 2-3 fold, even though most of the cell body response is not affected. Axonal regeneration is reduced, but most of the defect in target reinnervation and functional recovery appears due to excessive neuronal cell death. Deletion of Schwann cell c-Jun interferes with local production of neurotrophins, including BDNF, LIF, GDNF and Artemin; in the same vein supplementation with exogenous GDNF and Artemin promotes neuronal survival, target reinnervation as well as functional recovery. *In vitro*, anisomycin-enhanced c-Jun phosphorylation strongly enhanced Schwann cell synthesis of GDNF and Artemin (Fontana et al, 2012).

Since both neuronal and Schwann cell c-Jun are strongly phosphorylated at its N-terminus following nerve injury, we decided to explore the effects of c-Jun phosphorylation and neuronal deletion of one of its target molecules, the beta 1 integrin. Global replacement of all 4 N-terminal c-Jun phosphorylation sites (Ser63&73, Thr91&93) with alanines (jun4A) produced a significant increase (1.8x) in neuronal cell death, an approx 40% reduction in target reinnervation and delayed functional recovery. Interestingly, neuron-specific deletion of the gene for beta 1 integrin (sBB mutants) using Cre/Lox system and synapsin-promoter driven Cre recombinase (syn::cre) produced a similar phenotype, with an approx 2.5-fold increase in neuronal cell death, a commensurate 60% reduction in target reinnervation and delayed functional recovery. Surprisingly, both mutants –jun4A and sBB - interfered primarily with the post-traumatic trophic response. The current data suggest that

(A) Jun activation in the specifically neuronal, non-peripheral part of the regeneration program is not affected by N-terminal phosphorylation, and that

(B) Neuronal beta1 integrin is a critical co-factor in trophic signalling elicited by the Jun-expressing and N-terminal phosphorylation-dependent Schwann cells.

References

Fontana X, Hristova M, Da Costa C, Patodia S, Thei L, Makwana M, Spencer-Dene B, Latouche M, Mirsky R, Jessen KR, Klein R, Raivich G, Behrens A (2012) c-Jun in Schwann cells promotes axonal regeneration and motoneuron survival via paracrine signaling. J Cell Biol 198(1):127-41.

Patodia S, Raivich G (2012) Role of transcription factors in peripheral nerve regeneration. Front Mol Neurosci 5:8.

Raivich G, Bohatschek M, Da Costa C, Iwata O, Galiano M, Hristova M, Nateri AS, Makwana M, Riera-Sans L, Wolfer DP, Lipp HP, Aguzzi A, Wagner EF, Behrens A (2004) The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. Neuron 43(1):57-67.

Ruff CA, Staak N, Patodia S, Kaswich M, Rocha-Ferreira E, Da Costa C, Brecht S, Makwana M, Fontana X, Hristova M, Rumajogee P, Galiano M, Bohatschek M, Herdegen T, Behrens A, Raivich G (2012) Neuronal c-Jun is required for successful axonal regeneration, but the effects of phosphorylation of its N-terminus are moderate. J Neurochem 121(4):607-18.

Axonal localization of importin β1 transcript is required for retrograde injury signaling

Rotem Ben-Tov Perry[1], Ida Rishal[1], Dima Yudin[1], Ella Doron-Mandel[1], Wasim Geraisy[1], Jeffery L. Twiss[2], Avraham Yaron[1], and Mike Fainzilber[1]

[1] Dept. of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel
[2] Dept. of Biology, Drexel University, Philadelphia, Pennsylvania 19104, USA

rotem.ben-tov-perry@weizmann.ac.il

Intracellular trafficking and localization of mRNA is a fundamental feature of living cells. Transport of specific transcripts into axons is usually controlled by untranslated sequences (UTR) that interact with RNA transporting proteins, however, so far, evidence is lacking for an essential role of endogenous mRNA localization in axons. Localized upregulation of importin β1 in lesioned axons coordinates a retrograde injury-signaling complex transported to the neuronal cell body. We have now identified a long 3′ untranslated region (3′ UTR) that directs axonal localization of importin β1. Conditional targeting of this 3′ UTR region in mice causes subcellular loss of importin β1 mRNA and protein in axons, without affecting cell body levels or nuclear functions in sensory neurons. Strikingly, axonal knockout of importin β1 attenuates cell body transcriptional responses to nerve injury and delays functional recovery *in vivo*.
Most recently we were able to localize the axon-targeting element in importin β1 UTR to a short distal segment that contains a novel motif. We have used this specific motif as bait for affinity proteomics to identify candidate proteins that might be involved in transport of importin β1 mRNA to axons.

WIS-neuromath enables automated neuronal morphology analyses

Ida Rishal[1*], Ofra Golani[2,3], Marek Rajman[1], Barbara Costa[1], Keren Ben-Yaakov[1], Zohar Schoenmann[1], Avraham Yaron[1], Ronen Basri[3], Mike Fainzilber[1], Meirav Galun[3*]

Departments of [1]Biological Chemistry, [2]Veterinary Resources, and [3]Computer Science and Applied Mathematics, Weizmann Institute of Science, 76100 Rehovot, Israel

ida.rishal@weizmann.ac.il

Automated analyses of neuronal morphology are important for quantifying connectivity and circuitry *in vivo*, as well as allowing high content imaging approaches on cultures of primary neurons. Here we describe a new software package called WIS-Neuromath, which provides solutions for automated quantification of neuronal morphologies in both *in vivo* and *in vitro* preparations. We demonstrate the capabilities of WIS-Neuromath in a diverse set of applications, including in dissociated and explant cultures, and histological analyses on thin and whole-mount sections. WIS-Neuromath is freely available to academic users, providing a versatile and cost-effective range of solutions for quantifying neurite growth, branching, maintenance, regeneration or degeneration under different experimental paradigms. For further information please see http://www.wisdom.weizmann.ac.il/~vision/NeuroMath/ or e-mail to wisneuromath@gmail.com.

PTEN deletion promotes regenerative sprouting in the aged rubrospinal tract

B. J. Hilton[1,2], N. Vashisht[1], **Wolfram Tetzlaff[1,2]**

[1]International Collaboration on Repair Discoveries (ICORD), [2]Department of Zoology, Univ. of British Columbia, 818 W 10th Avenue, Vancouver, BC, V5Z 1M9, Canada

tetzlaff@icord.org

The devastating paralysis that occurs following spinal cord injury (SCI) is largely the result of transected central nervous system (CNS) axons failing to regenerate. Previously Park et al. (Science 322, 963-966 [2008]) and Liu et al. (Nat Neurosci 13, 1075-1081[2010]) have shown that a major contributor to this regenerative failure is a diminished intrinsic capacity of adult CNS (retinal ganglion cellular and corticospinal) axons to grow, based largely on inactivity in the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway which is negatively regulated by phosphatase and tensin homolog deleted on chromosome ten (PTEN). The rubrospinal tract (RST), which originates in the red nucleus of the midbrain and travels contralaterally in the dorsolateral funiculus of the spinal cord, is an alternative model of CNS axon regenerative failure. In the rodent, this tract has important functions in forelimb skilled movements. Here, we assessed whether PTEN deletion would promote axon regeneration in the RST with deletion occurring in aged (7-8 month old) mice. Floxed PTEN mice were injected with adeno-associated virus serotype 2 expressing Cre and GFP (AAV2-Cre) or GFP alone for control (AAV2-GFP) into the right red nucleus. Four weeks later, mice underwent a left dorsolateral crush at cervical level C4/C5. Six weeks later, mice were injected with biotinylated dextran amine (BDA) into the right red nucleus to anterogradely trace the RST. Two weeks later, mice were sacrificed. AAV2-Cre successfully transduced rubrospinal neurons and promoted mTOR signalling. Based on independent analyses of BDA and GFP labelling, AAV2-Cre injected animals showed significantly decreased dieback and increased regenerative sprouting of rubrospinal axons through the lesion site in comparison to AAV2-GFP injected animals, for up to 100µm caudal measured from the middle of the lesion site. Our findings suggest that PI3K/Akt/mTOR activity is a significant determinant of rubrospinal regenerative potential, yet advanced age may play an important role in decreasing the ability of RST axons to regenerate long distances.

Supported by Wings for Life

The disparity between light touch and pinprick scores in the ASIA Impairment Scale (AIS) sensory assessment of spinal cord injury

Natalia Vasquez[1], A. Gall[1], P.H. Ellaway[1,2], M.D. Craggs[1,3]

[1]London Spinal Cord Injuries Centre, RNOH, Stanmore, UK
[2]Imperial College London, UK
[3]University College London, UK

nataliavasquezp@yahoo.com

Objectives: The International Standards for Neurological Classification of Spinal Cord Injury (ISNSCI) incorporates the American Spinal Injuries Association (ASIA) Impairment Scale (AIS) that assesses cutaneous sensibility through light touch (LT) and sharp-dull discrimination, referred to as pin prick (PP). This project aimed to assess the agreement between LT and PP scores in a cohort of SCI subjects.
Methods: A retrospective analysis of LT and PP scores of 99 SCI subjects at the time of discharge (median 5 months) from acute care and rehabilitation in the London Spinal Injuries Centre was conducted. Subjects were aged 10-88 yrs (median 44 yrs; 78 male; 74 traumatic, 25 non-traumatic). There were 40 AIS A, 7 B, 18 C and 34 D subjects.
Results: A disparity ($P<0.001$) was found between LT (64.5 ±3.2, mean ±SE) and PP (54.7 ±2.9) AIS sensory scores. A similar difference in score (LT>PP) was registered both for traumatic and non-traumatic injury, but was greater for incomplete than for complete injury. Despite the difference, LT was well correlated with PP ($R = 0.87$, $P<0.001$). PP scores tended to be lower than LT scores, irrespective of the type, level or degree of spinal cord injury. PP rather than LT or motor assessments most frequently determined the neurological level of injury.
Conclusions: 1. The consistent discrepancy between LT and PP could relate to the greater complexity of the PP test or a difference in the extent of injury to the separate tracts that are generally assumed to convey LT (posterior columns) and PP (anterolateral spinothalamic) sensation. 2. The discrepancy would benefit from further study using electrophysiological quantitative measures (Hayes et al, 2002) to compare LT scores for individual dermatomes with electrical perceptual thresholds (Savic et al, 2006) or dermatomal sensory evoked potentials (Kramer et al, 2010), and PP with temperature thresholds (Nicotra and Ellaway, 2006) or contact heat evoked potentials (Kramer et al, 2012). 3. The results are relevant to the finding that PP provides the best prognostic indicator for useful motor recovery (Katoh and El Masri, 1995). With reference to future use of the AIS as an outcome measure for trials promoting recovery of function, it could be advantageous to relate the degree of recovery to pre-treatment discrepancies in LT and PP assessments.

References

Hayes et al. *Arch Phys Med Rehabil* 2002; 83: 1612-1619. Savic et al. *Spinal Cord* 2006; 44: 560-566. Nicotra, Ellaway. *Spinal Cord* 2006; 44; 617-624. Kramer et al. *Neurorehabil Neural Repair* 2010; 24: 309-317. Kramer et al. *Clin Neurophysiol* 2012; 123: 582-589. Katoh, El Masri. *Paraplegia* 1995; 33: 506-509.

Novel interactions between the c-Jun and Notch Signalling pathways regulate the Schwann cell response to peripheral nerve injury

Daniel K. Wilton[1], K. Malgapo[1], J. Shen[2], D. Meijer[3], L. Feltri[4], L. Wrabetz[4], A. Behrens[5] R. Mirsky[1], K. R. Jesssen[1]

[1]Department of Cell and Developmental Biology, University College London, UK
[2]Ctr. For Neurologic diseases, Brigham and Women's hospital, Harvard Med. Sch, Boston, MA, USA
[3]Dept of cell biology and genetics, Erasmus MC, Rotterdam, Netherlands
[4]School of Medicine and Biomedical Sciences, University of Buffalo, New York, USA
[5]Mammalian Genetics Laboratory, London Research Institute, CRUK, London WC2A 3LY, UK

daniel.k.wilton@btinternet.com

We have previously shown that in Schwann cells the transcription factor c-Jun acts as a master regulator of Wallerian degeneration and is required for successful repair following nerve injury (Wilton et al., (2009) Glia, 57 (13) S158-S158); Latouche et al., (2009) Glia, 57 (13), S158 – S158). In this study we identify a novel role for c-Jun in the activation of Notch signalling in the denervated Schwann cell. We find that c-Jun is required to activate Notch signalling, leading to upregulation of the BHLH protein Hes1. Hes1 then plays two functions in the denervated cell, promoting myelin breakdown and acting as part of a negative feedback loop to reduce c-Jun levels. As a result of this, ablating Notch signalling specifically in Schwann cells acts to increase c-Jun levels. We show that this upregulation of Schwann cell c-Jun accelerates axon outgrowth, target re-innervation and remyelination by generating a cell, which results in a more rapid functional recovery. These results identify novel functional links between the c-Jun and Notch signalling pathways. They also show that not only is Schwann cell c-Jun necessary for successful nerve regeneration, but that nerve repair can be improved by enhancing normal c-Jun signalling.

Spinal motor neurite outgrowth over glial scar associated neural inhibitors in bone marrow stromal cell co-cultures

Karina Wright[1], Kenzo Uchida[2], Sally Roberts[1], William Johnson[3], Wagih El Masri[1]

[1]ARC/TORCH Building and Midlands Centre for Spinal Injuries, RJAH Orthopaedic Hospital and Institute for Science and Technology in Medicine, Keele University, Oswestry, SY10 7AG, UK
[2]University of Fukui, Department of Orthopaedics and Rehabilitation Medicine, Fukui, Japan
[3]Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET, UK

Karina.Wright@rjah.nhs.uk

Transplantation of bone marrow cells into spinal cord lesions promotes functional recovery both in animal models and in recent clinical trials but the mechanisms responsible for these improvements are still unclear. Previously we have reported that human bone marrow stromal cells (MSC) promote the growth of extending sensory neurites from dorsal root ganglia (DRG), in the presence of some of the molecules present in the glial scar which are attributed with inhibiting axonal regeneration following spinal cord injury (SCI).

We have adapted and optimized this system replacing the DRG with a spinal cord culture to produce a central nervous system (CNS) model which is more relevant to the SCI situation. As has been reported previously with DRG, substrate-bound neurocan and Nogo-A repelled spinal neuronal body adhesion and neurite outgrowth, but these inhibitory effects were abrogated in MSC/ spinal cord co-cultures. However unlike DRG, spinal neuronal bodies and neurites showed no inhibition to substrates of myelin associated glycoprotein (MAG).

These findings provide novel insight into how MSC transplantation may promote regeneration and functional recovery in animal models of SCI and in the clinic, especially in the chronic situation where glial scars (and associated neural inhibitors) are well established. In addition, we have confirmed that this CNS model predominantly comprises of motor neurons via immunocytochemical characterisation. We hope that this model may be used in future research to test various other potential interventions for CNS repair.

Acknowledgements: This work was supported by the Institute of Orthopaedics Ltd

Delegates

First Name	Last Name	Institution	Email Address
Carolina	Acosta Saltos	UCL	francia.acosta-saltos.09@ucl.ac.uk
Louise	Adams	Queen Mary, University of London	louise_adams24@hotmail.co.uk
Zubair	Ahmed	University of Birmingham	z.ahmed.1@bham.ac.uk
Hakan	Aldskogius	Uppsala University	Hakan.Aldskogius@neuro.uu.se
Clara	Alfaro Cervello	University of Cambridge	ca386@cam.ac.uk
Robin	Ali	UCL	r.ali@ucl.ac.uk
Warren	Alilain	Case Western Reserve University	wja4@case.edu
David	Allan	National Spinal Injuries Unit for Scotland	spinalunit@ggc.scot.nhs.uk
Callan	Attwell	Netherlands Institute for Neuroscience	c.attwell@nin.knaw.nl
Mark	Bacon	ISRT	mark@spinal-research.org
Sue	Barnett	University of Glasgow	Susan.Barnett@glasgow.ac.uk
Katalin	Bartus	King's College London	katalin.bartus@kcl.ac.uk
Tracey	Baskerville	Queen Mary University of London	t.baskerville@qmul.ac.uk
Xuenong	Bo	Queen Mary University of London	x.bo@qmul.ac.uk
Marina	Boido	University of Turin	marina.boido@unito.it
Marc	Bolliger	Spinal Cord Injury Center Balgrist	mbolliger@paralab.balgrist.ch
Karen	Bosch	King's College London	karen.bosch@kcl.ac.uk
Rachael	Bosma	Queen's University	rachaelbosma@gmail.com
Nancy L	Brackett	University of Miami	nbrackett@med.miami.edu
Liz	Bradbury	King's College London	elizabeth.bradbury@kcl.ac.uk
Emily	Burnside		emily.burnside@gmail.com
Arthur	Butt	University of Portsmouth	arthur.butt@port.ac.uk
Thomas	Carlstedt	SIU, Stanmore	carlstedt.thomas@googlemail.com
Samit	Chakrabarty	University of Leeds	chakrabarty.samit@gmail.com
Daniel	Chew	University of Cambridge	dc501@cam.ac.uk
David	Choi	UCL	d.choi@ion.ucl.ac.uk
Brendan	Collins	UCL	brendan.c.collins@gmail.com
Sam	David	McGill University	sam.david@mcgill.ca
Simone	Di Giovanni	HIH, University of Tuebingen	simone.digiovanni@medizin.uni-tuebingen.de
Volker	Dietz	University Hospital Balgrist	vdietz@paralab.balgrist.ch
Matteo	Donegà	University of Cambridge	md574@cam.ac.uk
Wagih	El Masry	SIU Oswestry	wagih.elmasri@rjah.nhs.uk
Peter	Ellaway	Imperial College London	p.ellaway@imperial.ac.uk
Nitish	Fagoe	Netherlands Institute for Neuroscience	n.fagoe@nin.knaw.nl
Adam	Ferguson	University of California, San Francisco	Adam.Ferguson@ucsf.edu
Eridan	Ferreira	UCL	e.ferreira@ucl.ac.uk
Itzhak	Fischer	Drexel University College of Medicine	ifischer@drexelmed.edu
Karim	Fouad	University of Alberta	karim.fouad@ualberta.ca
Robin	Franklin	University of Cambridge	rjf1000@cam.ac.uk
Isabella	Gavazzi	King's College London	isabella.gavazzi@kcl.ac.uk
Karen	Gladwin	UCL	k.gladwin@ucl.ac.uk
James	Guest	University of Miami	jguest@med.miami.edu
Jodie	Hall	Ohio State University	jodiehall@hotmail.com
Leif	Havton	University at California at Irvine (UCI)	lhavton@uci.edu
Linda	Hsieh-Wilson	HHMI / Caltech	lhw@caltech.edu
Stephen	Hunt	University College London	hunt@ucl.ac.uk
Tom	Hutson	King's College London	thomas.hutson@kcl.ac.uk
Ronaldo	Ichiyama	University of Leeds	R.M.Ichiyama@leeds.ac.uk
Nicholas	James	Kings College, London	nicholas.n.james@kcl.ac.uk

Nick	Jeffery	Iowa State University	njeffery@iastate.edu
Eustace	Johnson	Aston University	w.e.johnson@aston.ac.uk
Danial	Kahani	University of Strathclyde	danial.kahani@strath.ac.uk
Pratipal	Kalsi	UCL	p.kalsi@ucl.ac.uk
Claudia	Kathe	King's College London	claudia.kathe@kcl.ac.uk
Peter	Kirkwood	UCL	peter.kirkwood@ucl.ac.uk
Naomi	Kleitman	NINDS	KleitmaN@ninds.nih.gov
Jeffrey	Kocsis	Yale University	jeffery.kocsis@yale.edu
Timea	Konya	ISRT	timea@spinal-research.org
Elena	Kozlova	Uppsala University	elena.kozlova@neuro.uu.se
Brian	Kwon	University of British Columbia	brian.kwon@vch.ca
Michael	Lane	University of Florida	michael.lane@mbi.ufl.edu
Stuart	Law	Spinal Repair Unit	stuart.law@ucl.ac.uk
Rosi	Lederer	Wings for Life	rosi.lederer@wingsforlife.com
Sena	Lee	Barts and The London	senadori@hotmail.com
Ying	Li	Spinal Repair Unit, UCL	ying.li@ucl.ac.uk
Daqing	Li	Spinal Repair Unit,	daqing.li@ucl.ac.uk
Zhuo-Hao	Liu	Barts and The London	z.liu@qmul.ac.uk
Luis	López de Heredia	Stoke Mandeville Hospital	luis.lopezdh@buckshealthcare.nhs.uk
Charles	Lynne	University of	clynne@miami.edu
Matthew	Mason	Netherlands Institute for Neuroscience	m.mason@nin.knaw.nl
Stephen	McMahon	King's College London	stephen.mcmahon@kcl.ac.uk
Adina	Michael-Titus	Barts and The London School	a.t.michael-titus@qmul.ac.uk
Lawrence	Moon	King's College London	lawrence.moon@kcl.ac.uk
Hans Werner	Mueller	University of Düsseldorf	hanswerner.mueller@uni-duesseldorf.de
Liz	Muir	University of Cambridge	emm1@mole.bio.cam.ac.uk
Lev	Novikov	Umeå University	lev.novikov@anatomy.umu.se
Patrick	Pallier	Queen Mary, University of London	p.pallier@qmul.ac.uk
Moreno	Pasin	UCL	moreno.pasin01@libero.it
Smriti	Patodia	UCL	smriti_17@hotmail.com
Rotem	Perry	Weizmann institute of science	Rotem.Ben-tov-perry@weizmann.ac.il
Stefano	Pluchino	University of Cambridge	SPP24@cam.ac.uk
Phil	Popovich	The Ohio State University	Phillip.Popovich@osumc.edu
John	Priestley	Barts and The London School	J.V.Priestley@qmul.ac.uk
Geoff	Raisman	UCL	g.raisman@ion.ucl.ac.uk
Gena	Raivich	UCL	G.Raivich@ucl.ac.uk
Matthew	Ramer	ICORD	ramer@icord.org
Paul	Reier	University of Florida Coll. Med.	reier@mbi.ufl.edu
John	Riddell	University of Glasgow	j.riddell@bio.gla.ac.uk
Ida	Rishal	Weizmann Institute of science	ida.rishal@weizmann.ac.il
John	Rogers	University of Cambridge	jhr11@cam.ac.uk
Anne	Rosser	Cardiff University	rosserae@cf.ac.uk
Torben	Schneider	UCL	t.schneider@ion.ucl.ac.uk
Jan	Schwab	Charité Hospital - Humboldt University	jan.schwab@charite.de
Jerry	Silver	Case Western Reserve University	jxs10@po.cwru.edu
Patrick	Stroman	Queen's University	stromanp@queensu.ca
Giorgio	Terenghi	University of Manchester	giorgio.terenghi@manchester.ac.uk
Wolfram	Tetzlaff	ICORD	tetzlaff@icord.org
Mark	Tuszynski	University of California	mtuszynski@ucsd.edu
Natalia	Vasquez	RNOH NHS Trust	nataliavasquezp@yahoo.com
Alessandro	Vercelli	University of Torino	alessandro.vercelli@unito.it
Joost	Verhaagen	Netherlands Institute for Neuroscience	J.Verhaagen@nin.knaw.nl

Laurent	Vinay	CNRS & Aix Marseille Université	laurent.vinay@univ-amu.fr
Claudia	Wheeler-Kingshott	UCL	c.wheeler-kingshott@ion.ucl.ac.uk
Stephanie	Williams	Spinal Cord Injury Network	stephanie.williams@spinalnetwork.org.au
Daniel	Wilton	UCL	daniel.wilton@ucl.ac.uk
Karina	Wright	Midlands Centre for Spinal Injuries	Karina.Wright@rjah.nhs.uk
Ping	Yip	Barts and The London School	p.yip@qmul.ac.uk