SPINAL RESEARCH

fighting paralysis... and winning

6-7 September 2013

The 15th Spinal Research Network Meeting

ABSTRACTS

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

POSTER PRESENTATIONS

Poster session is scheduled from 4.30pm in Hendrix & Madonna Suite at the end of the first day, immediately after the main meeting, on Friday, 6th September. There will also be time during the coffee and lunch breaks on Friday to view the posters.

SCIENTIFIC ORGANIZING COMMITTEE

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King's College, London

Dr John Riddell BSc PhD
University of Glasgow

Professor Mark Tuszynski MD PhD
University of California, San Diego

Professor Joost Verhaagen PhD
Netherlands Institute for Neuroscience

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Session I: MOLECULAR REGULATORS OF AXONAL REGENERATION

Chair: Gennadij Raivich

Functional genomics and spinal cord injury

Vance Lemmon

Miami Project to Cure Paralysis, Department of Neurological Surgery, University of Miami, Miami, FL, USA

vlemmon@miami.edu

Injury to the central nervous system (CNS) results in irreversible loss of function, partly due to a lack of robust regeneration by CNS axons following severing or other damage. Regeneration failure results both from neuron-intrinsic limitations on regeneration and from inhibitory factors in the injury environment. Unfortunately, effective treatments to improve CNS regeneration are still lacking. Such treatments will likely require small molecules that can target more than one source of regeneration failure.

Axon growth is regulated by protein kinases (PKs) and phosphatases. Although there are more than 300 PKs, only 50-60 have been investigated for their roles in axon growth signaling. A comprehensive picture of PK signaling in axon growth is being developed using kinase inhibitor (KI) compounds as tools. A large number (about 1500) KIs are being screened for their ability to promote or inhibit axon growth, using phenotypic assays with primary neurons. Chemotyping (chemical structure/activity analysis) and informatic analysis, especially integration with kinase inhibitor profiling data, will allow us to identify the PKs and their networks targeted by the active KIs. Model network predictions are being tested using gain- and loss-of-function phenotypic analyses in primary neurons. Preliminary data from this project correctly identifies a number of classic PKs, such as ROCK, previously implicated in axon growth, but also suggests that ROCK inhibitors have off-target effects that contribute to their efficacy in growth stimulation. Our data also implicate PKs not previously understood to be important in axon growth pathways.

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Combinatorial expression of regeneration-associated transcription factors to promote axonal regeneration after spinal cord injury

Matthew Mason, Joost Verhaagen

Laboratory for Neuroregeneration, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

m.mason@nin.knaw.nl, j.verhaagen@nin.knaw.nl

Many neurons of the central nervous system respond only weakly to axotomy, in contrast to peripheral neurons which robustly upregulate many hundreds of regeneration associated genes (RAGs). Stimulating a strong regenerative response in axotomised neurons after spinal cord injury may promote regeneration in the spinal cord. Many transcription factors have been identified which are upregulated after axotomy and so may contribute to regulation of the RAG response. It is known that transcription factors (TFs) act together, and it is likely that gene regulation that governs a specific biological process is made specific by the combinations of TFs available to activate promoters. We are attempting to activate RAG expression in CNS neurons by identifying *combinations of transcription factors* which synergistically act on RAG promoters and expressing these in axotomised neurons after spinal cord or dorsal root injury[1].

We used AAV5 vectors with a compact dual promoter to deliver multiple transcription factor genes to DRG neurons[2,3]. We over-expressed either the combination of c-Jun, ATF3, STAT3, SMAD1 and GFP, ATF3 with GFP, or GFP only. This combination of TFs was chosen because each of these factors has been linked to successful axonal regrowth and are known to have functional or physical interactions. We examined regeneration after dorsal root and dorsal column injury. ATF3 alone caused an increase in regeneration in the dorsal root injury model. The combination of 4 TFs also increased regeneration but surprisingly the effect was not greater than that of ATF3 alone.

To identify other TFs that may represent the key TFs in RAG regulation we have developed and employed an algorithm to identify over-represented TF binding sites in RAG promoters. This led us to identify a number of transcription factors that may be important for regulating the RAG response. We performed a combinatorial screen of these factors (see also poster of Callan Attwell et al.) and identify several combinations that show powerful synergistic effects on neurite growth in F11 cells (a DRG - neuron like cell line). We are currently testing the efficacy of some of these combinations *in vivo* in spinal cord injury models.

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Structure and functions of extracellular matrix microenvironments for neural plasticity

Andreas Faissner

Department of Cell Morphology and Molecular Neurobiology, Ruhr-University, Bochum, Germany

andreas.faissner@rub.de

The past decades have shown that radial glia and astrocytes play important roles in the regulation of development, plasticity and regeneration of the CNS. For example, early astrocytes construct complex temporary scaffolds that guide migrating neurons and growth cones. On the other hand, glial cordons segregate neuronal assemblies and interfere with axonal regeneration in glial scars. The laboratory has established a research line that characterizes glia-derived extracellular matrix (ECM) molecules with regard to their roles in axon growth and guidance, and in the realm of inhibition of regeneration and the glial scar. This work resulted in the identification of repulsive and stimulatory domains in the ECM glycoprotein tenascin-C, and in the definition of the neurite outgrowth promoting DSD-1-carbohydrate epitope. Currently, the laboratory focuses on complementary peptide domains in ECM components and their receptors. Recently, the team has developed an interest in perineuronal nets (PNNs) that represent specialized and condensed ECM superstructures on the surface of neurons. Current research aims at elucidating the roles of PNNs for synaptic function and plasticity.

Synapses represent specialized cell-cell contact sites between nerve cells. These structures mediate the rapid and efficient transmission of signals between neurons and are surrounded by glial cells. Former investigations have shown that astrocytes and astrocyte-derived extracellular matrix (ECM) components are important for formation, maintenance and function of synapses in the CNS. In order to study the effects of glial-derived ECM components on synaptogenesis we established an *in vitro* transwell co-culture system, where E18 hippocampal neurons and primary astrocytes were cultured together without direct contact in long term periods. We detected the existence of perineural nets and the expression of several ECM proteins in the transwell co-culture system. Furthermore, we were able to demonstrate that the digestion of the chondroitin-sulfate proteoglycans (CSPGs) with the enzyme chondroitinase ABC leads to increased co-localization of the perisynaptic protein Bassoon and the postsynaptic protein proSAP1 and enhanced synapse formation within the first two weeks in the transwell co-culture system. Using a genetic mouse line that misses the ECM glycoproteins tenascin-C and tenascin-R as well as the CSPGs Neurocan and Brevican, we could establish that the neural ECM is important for synapse function and stability. In order to explore the synaptic deficit in the mutant on the the network level, our co-culture system to monitor synapse formation has been adapted to the use with multi-electrode arrays (MEAs).

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Functional testing of candidate therapeutic genes in the injured corticospinal tract

Murray G. Blackmore

Department of Biomedical Sciences, Marquette University, Milwaukee, WI, USA

murray.blackmore@marquette.edu

Recovery from spinal cord injury is limited in part by neuron-intrinsic constraints on axon growth that arise during early postnatal development. The intrinsic capacity for axon growth varies in different neuronal populations, but appears to be particularly low in the corticospinal tract (CST), a critical mediator of fine motor control. Comparative gene profiling has been widely used to identify genes whose expression correlates with regenerative success. More recently, our lab and others have used high content screening technology to narrow the search for regulators of axon growth by functionally testing large sets of candidate genes *in vitro*. Ultimately it is essential to directly test the ability of these candidate genes to promote axon regeneration and behavioral recovery in the *in vivo* setting. To do so we use adeno associated virus (AAV8) to manipulate gene expression in the adult murine cortex, and then challenge CST axons with cervical dorsal hemisection. We showed previously that overexpression of an active form of the KLF7 transcription factor enhances CST axon growth *in vivo*. We have now tested a number of additional manipulations in the CST *in vivo*, including overexpression of cJUN, doublecortin (DCX), DLK-1/MAP3K12, and knockdown of PTEN and PTPsigma; none of these manipulations were found to significantly increase axon growth. In contrast, forced overexpression of the transcription factor Sox11, like KLF7, promotes CST axon growth after injury. Importantly, both KLF7 and Sox11 appear to promote axon growth when administered 8 weeks after injury, raising the prospect of efficacy in the chronic injury state. Despite the improved CST axon growth, however, behavioral tests including horizontal ladder crossing, cylinder rearing, and pellet retrieval have failed to detect improvements in KLF7-treated animals, and in some cases show reduced function in Sox11-treated animals. Overall these findings illustrate the promise of genetic manipulations to enhance axon growth, while highlighting new challenges in the restoration of proper connectivity and function.

Supported by t*he* ***International Spinal Research Trust***, NIH R01 NS083983, the Craig H. Neilsen Foundation, SCIS, and the Bryon Reisch Paralysis Foundation

Session II: CORTICOSPINAL AXON REGENERATION: WHERE DO WE STAND?

Chair: Lyn Jakeman

The reactivation of somatosensory cortex and the recovery of hand use after lesions of the dorsal columns of the spinal cord

Jon H. Kaas

Department of Psychology, Vanderbilt University, Nashville, TN, USA

jon.h.kaas@vanderbilt.edu

After complete or nearly complete unilateral lesions of the dorsal columns at a high cervical level of the spinal cord in monkeys, the hand region of contralateral somatosensory cortex becomes unresponsive to tactile stimuli, hand use is impaired, and monkeys behave as if they cannot feel an object in their hand. Over a period of weeks, much of the hand region of somatosensory cortex typically recovers responsiveness to touch on the hand, objects in the hand are felt, and they are skillfully retrieved. The reactivation of cortex is more extensive when some of the dorsal column afferents have been preserved, and the somatotopy of the reactivated cortex is closer to normal. However, even with complete or nearly complete lesions, some reactivation of hand cortex occurs, and behavior improves. With larger lesions, the reactivation appears to depend on the preservation of a few dorsal column afferents which are then over represented in contralateral cortex, but second-order neurons in the spinal cord that project to the dorsal column nuclei likely contribute. The resulting cortical map of the hand after larger lesions is both incomplete, with portions of the hand missing, and disorderly, with digits represented in atypical locations. Yet, qualitative comparisons of the response properties of reactivated neurons demonstrate that they approximate those of neurons in intact monkeys. We suggest that the cortical reactivation, and the recovery of behavior, is the result of a spontaneous growth and spread of preserved dorsal column primary afferents and second-order spinal cord afferents into the dorsal column nuclei to activate larger than normal populations of neurons. Additional growth of connections to activate deprived neurons may occur in the contralateral somatosensory thalamus and cortex.

While reactivation of cortex is extensive, and apparently useful, after even nearly complete or complete dorsal column lesions, the extent of the reactivation by preserved dorsal column afferents can be increased by the treatment of the dorsal column nuclei with chondroitinase ABC at the time of the dorsal column lesion. This treatment likely promotes the sprouting and growth of preserved dorsal column primary afferents, and the projections of secondary spinal cord neurons into the deprived dorsal column nuclei to enhance the reactivation of dorsal column nuclei, thalamic, and cortical neurons.

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Using what's left: the contribution of brainstem pathways to recovery after corticospinal lesion

Stuart Baker

Institute of Neuroscience, Medical School, Newcastle University, Newcastle upon Tyne, UK

stuart.baler@ncl.ac.uk

Multiple descending pathways link the cortical motor areas with spinal circuitry, providing the route by which intention can be translated into action. In humans, the corticospinal tract is the dominant system, and is especially important for control of fine movements, such as the dexterous manipulative abilities of the hand. Whereas the corticospinal tract forms a direct link between cortex and spinal cord, other descending pathways have an additional synapse within the brainstem. The most important such pathway in humans is the reticulospinal tract; this is usually associated with control of gross movements, such as locomotion or maintenance of posture.

Our recent work has shown that the primate reticulospinal tract can make a contribution to control of hand and forearm movements, alongside the dominant influence of the corticospinal tract. Reticulospinal neurons make mono- and di-synaptic connections to spinal motoneurons; this even includes motoneurons innervating intrinsic hand muscles [1]. Intermediate zone interneurons in the cervical enlargement often receive input from both corticospinal and reticulospinal tracts; this includes interneurons involved in the control of hand movements[2].Finally, neurons in the primate reticular formation modulate their activity during isolated fine movements of the digits, suggesting that these connections are actually used in the control of hand movements[3].

This work suggest that, to some extent, the reticulospinal tract can form a parallel pathway for motor control in primates. Whilst subservient to the corticospinal tract in the healthy state, it may become especially important following corticospinal lesions such as after spinal cord injury. We tested this by making near-complete unilateral lesions of the corticospinal tract at the medulla in monkey, and then allowing the animals to recover[4]. We showed that connections from the reticulospinal tract to motoneurons strengthened compared to control animals. However, the changes in connectivity were not uniform, but were confined to flexor and intrinsic hand muscles. This may underlie the unbalanced recovery often seen in patients recovering from lesions, who frequently develop flexor hyperactivity (possibly leading to spasticity), but are left with extensor weakness.

Understanding the function of primate reticulospinal circuits, including the principles involved in plasticity of connections, may open new avenues for rehabilitation after injury. This could allow patients to use all surviving tissue surrounding a spinal lesion for maximal recovery of motor function.

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Key elements for functional recovery from partial spinal cord injury; spinal cord, cortex and beyond

Tadashi Isa

National Institute for Physiological Sciences, Myodaiji, Okazaki, Japan

tisa@nips.ac.jp

After brain or spinal cord injuries, patients experience severe paralysis but some recovery can occur through rehabilitative training, however, the underlying neuronal mechanism is still not well understood. We have been studying the neuronal mechanism of recovery after partial spinal cord injury using non-human primate models by combining multidisciplinary approaches.

It is generally accepted that direct connection from the motor cortex to spinal motoneurons is first established in higher primates and the direct pathway has been supposed to be the basis of dexterous hand movements in these species. However, in addition to the direct pathways, there exists an indirect pathway mediated by propriospinal neurons (Alstermark and Isa, 2012). Recently, we clarified that after lesion of the direct pathway, such indirect pathway can compensate for the dexterous hand movements, first by classical lesion experiments, and more recently by a newly developed genetic tool that enabled pathway-selective and reversible transmission blockade with double viral vectors in macaque monkeys (Kinoshita et al. Nature, 2012). Moreover, we showed that various cortical areas including ipsilateral M1 and ventral premotor cortex are causally involved in the functional recovery (Nishimura et al. Science, 2007). In addition, we found that ventral striatum (VSt) including the nucleus accumbens increases the activation during the recovery from the spinal cord injury in association with the motor cortex (Nishimura et al. PLos One 2011), and causally contributes to the recovery by local inactivation technique. This may underlie the mechanism of how the motivation facilitates the functional recovery. Such knowledge about the systems underlying the recovery will contribute to development of novel therapeutic strategies against the neuronal damage.

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Session III: STEM CELLS IN CNS REPAIR

Chair: Robin Franklin

Stem cell therapy for SCI

Mark H. Tuszynski, Ken Kadoya, Ephron Rosenzweig, John Brock, Lawrence Goldstein, Paul Lu

University of California – San Diego, Veterans Administration Medical Center, La Jolla, CA, USA

mtuszynski@ucsd.edu

Grafts of neural stem cells to sites of severe spinal cord injury exhibit an ability to survive, integrate into host tissue, and extend axons over remarkably long distances. Reciprocally, host axons innervate the implanted neural stem cell grafts, resulting in the formation of neural relays across the lesion site that support functional improvement. These effects are consistent across stem cell sources and species, both with regard to donor cell source and species into which cells are grafted. Moreover, axons derived from these neural stem cell implants readily extend through the inhibitory milieu of adult CNS white matter. A defined extracellular grafting matrix and inclusion of growth factors at the time of grafting is essential to optimize cell distribution and survival in the lesion site. The potent biological properties of early stage neural precursors suggest an intriguing possibility for clinical translation.

Cell replacement therapy for acute and chronic spinal traumatic injury

Martin Marsala, Sebastiaan van Gorp, Karl Johe, David Weingarten, Joseph D. Ciacci

Department of Anesthesiology, University of California, San Diego, La Jolla, CA, USA

mmarsala@ucsd.edu

Intraspinal grafting of neural stem cells represents a promising approach to promote recovery of function in a variety of spinal neurodegenerative disorders including spinal trauma. It is believed that such a treatment may serve to**: i)** provide trophic support to improve survival of host neurons, **ii)** improve structural integrity of spinal parenchyma by reducing syringomyelia and scarring in trauma-injured regions, and **iii)** provide neuronal populations to form relays with host axons, segmental interneurons, and/or α-motoneurons.

Recently we have characterized: 1) Study 1: the effect of intraspinal grafting of clinical grade human fetal spinal cord-derived neural stem cells (HSSC) on recovery of neurological function in immunosuppressed SD rats with lumbar (L3) compression injury (efficacy study). Cells were grafted at 3 days post-injury and animals survived for 2 months after cell grafting. 2) Study 2: HSSC survival and differentiation after spinal grafting in immunodeficient rats after Th10 contusion injury (safety study). Cells were grafted at 7 days post-injury and animals survived for 10 months after cell grafting. 3) Study 3: HSSC survival and differentiation after spinal grafting in immunosuppressed minipigs with Th12 contusion injury (feasibility study). Cells were grafted at 24 hrs post-injury and animals survived for 4 weeks after cell grafting.

Results:

1) Study 1: Intraspinal grafting of HSSC led to a progressive and significant improvement in lower extremity paw placement, amelioration of spasticity, and normalization in thermal and tactile pain/escape thresholds at 8 weeks post-grafting. No significant differences were detected in other CatWalk parameters, motor evoked potentials, open field locomotor (BBB) score or ladder climbing test. MRI volume reconstruction and immunofluorescence analysis of grafted cell survival showed near complete injury-cavity-filling by grafted cells and development of putative GABA-ergic synapses between grafted and host neurons.

2) Study 2: Analysis of grafted cells at 10 months in Th10-contused immunodeficient rats showed continuing injury-cavity-filling effect by grafted cells and extensive rostro-caudal migration of grafted hNUMA+ cells. No deterioration of motor or sensory function was seen in any animal.

3) Study 3: Consistent engraftment and neuronal differentiation of grafted cells was seen in Th12 contused minipigs at 4 weeks after cell grafting. No significant differences in the recovery of neurological function were identified in this time point.

Summary:

Intraspinal grafting of HSSC represents a safe and effective therapy to modulate some aspects of spinal dysfunction and pathology associated with traumatic spinal cord injury. A Phase I clinical trial in chronic spinally injured patients (ASIA A) was recently approved by the FDA.

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The regenerative activity of Mesenchymal stem cells

W. Eustace Johnson

Life & Health Sciences, Aston University, Aston Triangle, Birmingham, UK

w.e.johnson@aston.ac.uk

Mesenchymal stem or stromal cells (MSCs) were initially investigated for cell replacement therapies due their ability to differentiation into mature cells that form connective tissues, e.g. bone or cartilage. However, recent studies have shown that the regenerative activity of MSCs extends well beyond their own ability to form differentiated functioning end cells and includes a wide variety of paracrine effects on endogenous cells already present in diseased or damaged tissues. Transplantation of MSCs into models of spinal cord injury (SCI) has been associated with decreased inflammation, immunomodulation, angiogenesis, neural sparing, guided axonal regeneration and increased sensory and motor function. Such paracrine activity in MSCs has been seen also in other disease or injury situations, including multiple sclerosis, chronic ulcers and myocardial infarction, which may contribute to MSCs being one of the most increasingly investigated adult stem cells in clinical trial. This talk will consider how MSCs are thought to contribute to wound healing and tissue regeneration, including in vitro data on the effects of MSC secreted products on neural and other cell types1-3 as well as work with the University of Fukui4-5 on the effects of MSC transplants in SCI. These and other studies have contributed to a growing consensus that MSCs may enable the repair or regeneration of diseased and damaged neural tissue by acting as a "drugstore"6-7, which can both provide a beneficial effect in its own right as well as promoting the effects of other interventional strategies. These newly identified functions of MSCs provide an opportunity to exploit their regenerative activity more widely.

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Session IV: NATHALIE ROSE BARR PHD STUDENTSHIP PRESENTATIONS

Chair: Stephen McMahon

Promoting spinal cord regeneration and functional recovery using viral GMCSF

Carolina Acosta-Saltos[1,3], Roman Gonitel[2], Lucas Topham[1], Elisabeth Thubron[1], Harmony Ubhi[1], Seetal Chavda[1], Alejandro Acosta-Saltos[1], Li Ying[3], Geoffrey Raisman[3], Adrian Thrasher[4], Patrick Anderson[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 Neurology, University College London, UK
[4]Institute of Child Health, University College London, UK
[5]Cell and Developmental Biology, University College London, UK

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

Background: Microglial activation around the cell bodies of injured PNS neurons is associated with a profound cell body response, vigorous axonal regeneration and functional recovery. However, corticospinal neurons display little response to spinal cord injury and no perineuronal microglial activation. Most research aiming to enhance regeneration of corticospinal axons has focused on therapies treating the lesion site. The aim of my PhD research has been to determine whether chronic perineuronal inflammation induced by the microglial mitogen GMCSF enhances the regenerative responses of corticospinal neurons and results in improved functional recovery following spinal cord tract injury.

Methods and Results: A range of doses of a non-integrating lentiviral vector encoding GMCSF and the tracing protein eGFP were stereotactically injected into the motor cortex of Sprague Dawley rats with a right dorsal C4 corticospinal tract injury. Delivery of medium to high doses of GMCSF resulted in a widespread inflammatory response and expression of the growth-associated genes c-jun and ATF3 in the motorcortex. In the spinal cord, this pro-regenerative response was associated with reduced axonal retraction and increased axonal sprouting. To evaluate whether the sprouting promoted by GMCSF expression resulted in enhanced functional recovery, a battery of post-injury motor function assessments, including a directed forepaw reaching test, rearing test and grid walking assessment, have been carried out on GMCSF-treated and control rats.

Conclusion: Prolonged perineuronal inflammation induced by lentiviral delivery of GMCSF results in an upregulation of growth-associated genes in the motorcortex and a pro-regenerative effort by corticospinal tract axons. Analysis of behavioural assessments are currently being performed to determine if these effects also promote improved functional recovery of rats with spinal injuries.

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Development of methods for application of fMRI in the spinal cord

Moreno Pasin[1], M.C. Yiannakas[1], A. Toosy [2], C.A.M. Wheeler-Kingshott[1]

[1]NMR Research Unit, Department of Neuroinflammation, [2]Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, University College London, London, UK

moreno.pasin.10@ucl.ac.uk

PURPOSE: The purpose of this study was to present a protocol to assess stimulus-related activation in the spine using SE-ZOOM-EPI sequence, and a post-processing analysis that aims to limit the physiological confounds that usually reduce the sensitivity and the specificity of fMRI studies.

MATERIALS and METHODS: Acquisition - Ten healthy volunteers were scanned with a paradigm that involved localized sensory stimulation of the C6 dermatome over the palmar surface of the thenar eminence, using an MR-compatible electric rotating brush. The surface area of stimulation was 0.8x0.8cm^2. For each subject, left and right hands were stimulated in separate, consecutive fMRI sessions. All volunteers were right-handed, 3 women and 7 men (mean age = 35). A 3T MRI scanner (Philips Achieva TX, Best, Netherlands) with a 16 channel neurovascular coil was used for this study. All scans were performed with the Spin Echo ZOnally-magnified Oblique Multislice EPI (SE-ZOOM-EPI) sequence *[1]*, using a reduced field of view for targeted areas of fMRI activations. The imaging parameters were TR=3600ms, TE=30ms, voxel size=1.19x1.19x4mm^3 with 1mm gap between slices (reconstructed to 1.19x1.19x4mm^3), acquisition matrix=64x40, 9 slices. The block design comprised 10 epochs of rest alternated with 10 epochs of activation, each one lasting 36 seconds. During each session 200 volumes were acquired in about 12 minutes. The slices were always centred transverse to the cord and the central slice was always placed in the middle of the C6 spinal segment (Fig.1). A oxymeter was used to record the cardiac trace and a respiratory belt was applied on the diaphragm of volunteers to record the respiratory trace.

Data analysis - The DRIFTER toolbox *[2]*, implemented in SPM8 software, was applied to clean up the time-series from physiological noise due to cardiac pulsations and respiration. Each dataset was 2D-spatially realigned using FSL software. Matlab codes were created in order to obtain slice-wise movement regressors from rototranslation matrixes and CSF regressors from CSF masks to be included in the GLM implemented in SPM8. Only activations localized in the slices covering the C6 segment, plus one slice above it (in the C5 segment) and one slice under it (in the C7 segment), were taken into account. It resulted in 6 slices and 19 regressors analysis for each scan. For the activated voxels: average signal change was computed, time-series were checked and qualitatively compared with the predicted neural activity and the ipsi- or contra-lateral location was assessed. TSNR measurements were performed as well in a resting state scan. Results have been reported as single subjects study and not as group study.

RESULTS: In all volunteers activations were found inside the spinal cord and, generally, both ipsi- and contralaterally (Fig.2). Random and spurious activations were found outside the spinal cord for all volunteers. Significant activity was reported at $p<0.01$ (uncorrected) and mean T-score was reported for voxels above the $p<0.01$ threshold (Fig.3). Average signal change was 2.52 +/- 0.77 (mean+/-std). Time-series of activated voxels qualitatively correlated with the stimulus. Measured TSNR in the grey matter after the post-processing was 55.

DISCUSSION: Activations during a sensorial stimulation can be found ipsi- and contralaterally as reported by previous studies *[3]. The extra-spine* activations, found in different slices, were random and never falling in the same area in different acquisitions and in different subjects. The average signal change is in agreement with the results obtained in previous studies *[4].* The value of calculated TSNR implies a good sensitivity to signal changes detection. The ZOOM-EPI used is a Spin-Echo sequence, less sensitive than Gradient-Echo sequences to venous contribution and draining veins effects to the signal changes, which are known to contribute to fMRI signal in the spinal cord *[5]*. The high pixel bandwidth and reduced echo train length of the ZOOM-EPI sequence helped to reduce susceptibility artefacts *[6]*. This is an ongoing research study about detecting spinal fMRI and further investigations comprising group analysis statistics will follow.

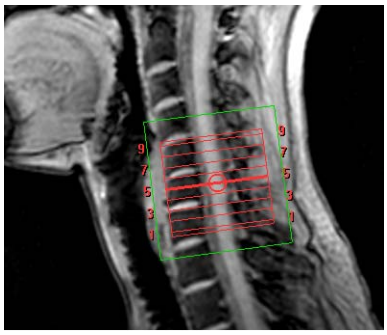

Fig.1: Screenshot showing the positioning of the 9 slices of the SE-ZOOM-EPI sequence

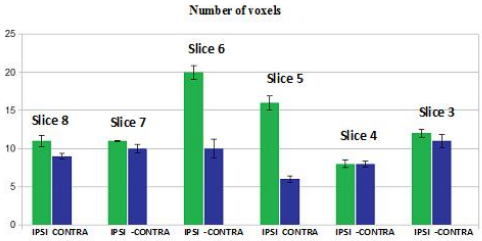

Fig.2: Number of activated voxels in the ipsilateral (in green) and contralateral (in blue) grey matter .

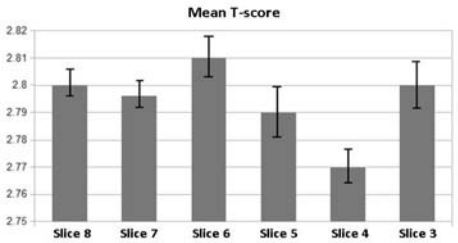

Fig.3: Mean T-score for voxels above the p=0.01 threshold .

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

Danial Kahani, Conway B.A., Allan D.B.

University of Strathclyde and Queen Elizabeth National Spinal Injuries Unit, Glasgow, Scotland, UK

Danial.kahani@strath.ac.uk

Robot-assisted technology platforms incorporating body weight support can be used to provide intensive treadmill based gait rehabilitation that can enhance walking outcomes for patients with incomplete spinal cord injury. Using powered orthoses to provide limb guidance and progressively modifying body weight support these systems create movement related sensory feedback that many believe is a critical stimulus for engaging the adaptive mechanisms that can have positive effects on recovery of over ground walking capability. Commercial devices such as the Lokomat (Hocoma AG, Switzerland), can guide the hip and knee joint motion in a way that simulates walking and allows the therapy team to vary both the level of robotic guidance and degree of body weight support in response to a patient's performance capability and progression.

In this project we have been developing instrumentation that can be incorporated into rehabilitation sessions with devices such as the Lokomat or other systems that make use of body weight support with the aim to further enhance the phase related sensory feedback that occurs during gait training. Specifically we have been interested in developing ways to provide patterned sensory stimulation that mimics the spatial and temporal activations of plantar foot afferent activation during the stance phase of walking. The rational for this is that when compared to normal walking the sensory feedback from plantar afferents (which signal ground contact and ground reaction forces during stance) are significantly diminished when high levels of body weight support are provided. We are therefore are interested to know whether stimulation of plantar foot afferents, in a way that would mimic the events of stance can be incorporated into training programmes and whether this augmented sensory feedback aids in the rehabilitation of patients undergoing gait rehabilitation.

The work completed has focused on (1) developing a system for vibratory stimulation of the foot sole that can act as a surrogate stimulus for ground contact and (2) an examination of the physiological efficacy of the stimulus in static conditions and during treadmill walking with body weight support in normal subjects. Technically, we have developed an insole device that incorporates miniature vibrators that locate below the heel and metatarsal heads. The vibrators can be controlled via pressure sensors that are also built into the insole (thereby providing closed loop control over the activity of the vibrating motors) or by external triggers that can be used to provide the on/off timing signals linked to a robot driven step cycle if needed. In developing the insole we have evaluated the effectiveness of the vibratory signals in producing physiological activations of ascending pathways by studying event related sensory evoked potentials and modulating transmission in spinal reflex pathways under static conditions and during treadmill gait with partial body weight support. We used the soleus H-reflex as a measure of motor neuron excitability and of transmission within spinal circuits. In agreement with previous studies investigating the effect of applied pressure of the foot sole on H-reflex excitability (Knikou & Conway 2001) we have demonstrated a similar depression occurs in response to vibratory stimulation during standing and during treadmill walking. During body weight assisted treadmill walking, foot sole vibration generates a larger depression of H-reflex excitability than when compared to unsupported treadmill walking illustrating its effectiveness as a stimulus that can modulate reflex pathways during procedures analogous to those encountered in gait rehabilitation.

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Session V: NEW TECHNIQUES / NEW TOOLS FOR SCI

Chair: Joost Verhaagen

Biomaterial bridges and gene delivery in spinal cord regeneration

Lonnie D. Shea[1], Aileen J. Anderson[2], Brian J. Cummings[2], Stephanie Seidlits[1], Aline Thomas[1], Kiran Powar[2], Daniel Margul[1], Ryan Boehler[1]

[1]Northwestern University, [2]University of California Irvine, USA

l-shea@northwestern.edu

The failure to regenerate after injury is caused by a multitude of factors, such as inflammation, formation of the glial scar, release of myelin associated inhibitory factors, and an insufficient supply of growth promoting factors. CNS neurons, however, are able to re-grow when presented with a permissive environment. We have been investigating the design of biomaterial scaffolds, termed bridges, to provide a permissive environment that promotes spinal cord regeneration. Porous, multiple channel bridges implanted into the injury provide stability to limit secondary damage and support cell infiltration that limits cavity formation. The channels provide a path that physically directs axon growth across the injury. Axons grew into and through the channels, and the density increased over time, resulting in the greatest axon density at 6 months post implantation, despite complete degradation of the bridge by that time point. Axons within the channels had both motor and sensory origins, and extensive myelination was observed throughout the bridge at 6 months. Furthermore, using a transgenic model with GFP labeling of the corticospinal tract, axons were observed to enter, cross the bridge, and re-enter the host tissue caudal to the injury. These studies demonstrate that the bridge structure can support substantial, long-term axon growth and myelination with limited scar formation.

Bridges have also been developed as a vehicle for the localized delivery of gene therapy vectors in order to induce the persistent expression of tissue inductive factors. Implantation of lentivirus loaded multiple channel bridges produced transgene expression that persisted for at least 8 weeks, with maximal expression at the implant and decreased rostral and caudal to the bridge. Cells transduced include macrophages, Schwann cells, fibroblasts, and astrocytes within the bridge and adjacent tissue. The delivery of lentivirus encoding the neurotrophic factors NT3 or BDNF significantly increased the extent of axonal growth into the bridge relative to empty scaffolds. In addition to promoting axon growth, the induced expression of neurotrophic factors led to myelination of axons within the channels of the bridge, where the number of myelinated axons was significantly enhanced relative to control. Gene delivery has also been employed to limit scar formation, and to modulate immune responses locally. Finally, gene delivery has been employed to enhance recruitment of glial-restricted progenitor cells toward an oligodendrocyte lineage, leading to enhanced myelination by oligodendrocytes. Taken together, gene delivery provides a versatile tool to investigate the impact of one or more factors on spinal cordregeneration.

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Nanotechology for the repair of the injured spinal cord

John A. Kessler, Samuel I. Stupp, LiuLiu Pan, Hilary North, Vicki Tysseling, Vibhu Sahni, Eric Berns, Shantanu Sur

Northwestern University's Feinberg School of Medicine, Chicago, Illinois, USA

jakessler@northwestern.edu

Axonal outgrowth and guidance are complex biological processes that require diverse sets of molecular cues present in the extracellular matrix (ECM). One of the scientific roadblocks for the repair of spinal cord injuries (SCI) is the development of molecularly designed neuro-active materials that can create an extracellular environment in the injured spinal cord that is conducive to axon regeneration. We have been examining the use of peptide amphiphile (PA) molecules that self-assemble *in vivo* into supramolecular nanofibers as a therapy for experimental SCI. Because self-assembly of these molecules is triggered by the ionic strength of the *in vivo* environment, nanoscale structures can be created within the extracellular spaces of the spinal cord by simply injecting a liquid. The molecules are designed to form cylindrical nanofibers that display to cells in the spinal cord various epitopes including the laminin epitope IKVAV, the integrin-interacting epitope RGDS, or a tenascin C mimetic. Injection of PAs containing each of these epitopes inhibits glial scar formation after SCI, promotes sensory and motor axon elongation, and enhances functional recovery. The inhibitory effects of the IKVAV and RGDS PAs on astrogliosis are mediated by β1-integrin signaling through integrin-linked kinase (ILK), thus identifying ILK as an important target molecule for treatment of SCI. Although the mechanical properties (e.g. "stiffness") of ECMs are known to alter many biological processes including astrocyte lineage commitment, the effects of the PAs are not dependent upon their mechanical properties. Efficient regeneration of axons ultimately will require directional cues to neurons that can promote and guide neurite outgrowth. We have therefore developed PAs in which the nanofibers are aligned. Presentation of IKVAV or RGDS epitopes enhanced the growth of neurites from neurons encapsulated in the scaffold, while the alignment guided these neurites along the direction of the nanofibers. Scaffolds encapsulating neural progenitor cells were formed *in situ* within the spinal cord and resulted in the growth of oriented processes *in vivo* both from the encapsulated cells and from ingrowth of both descending and ascending axons. To efficiently repair a damaged spinal cord, we envision a biomaterial with the following capabilities: 1) has the capacity to display multiple signals, 2) is highly aligned at nano-, micro- and macroscales to guide axons, 3) biodegrades over time, 4) minimizes inflammatory responses and immune rejection,

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Session VI: MEASURES OF PAIN AND AUTONOMIC DYSFUNCTION AFTER SCI
Chair: Peter Ellaway

Modelling and assessing post-spinal cord injury neuropathic pain

Ahemed Emraja, Andrew Toft, **John Riddell**

Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, Scotland, UK

John.riddell@glasgow.ac.uk

Approximately 70% of spinal cord injured patients suffer from pain and in around 40-50% of these, the pain is of central neuropathic origin (Siddall et al. 2003). Understanding of the underlying mechanisms is poor and dependent on appropriate animal models and behavioural assessments that reflect pain. These should ideally provide for the investigation of pain of different modalities, pain perceived to originate at, above or below the level of injury and include both evoked and spontaneous pain. The talk will describe studies in which we have evaluated whether contusion injuries at different segmental levels allow investigation of these different aspects of post-spinal cord injury pain and have attempted to optimise techniques for their assessment.

After low thoracic (T9) contusion injuries of 200 kdyn (infinite horizons impactor), robust signs of tactile allodynia, thermal hyperalgesia and cold allodynia developed in the forepaws. In addition, observations on the forepaws suggested evidence of spontaneous pain which has not previously been described in spinal cord injury models. Testing over the back at locations confirmed electrophysiologically to involve sensory processing at/above or below the injury level indicated increased sensitivity at/above, but not below level. Although the hindpaws also showed responses that would normally be interpreted as mechanical allodynia and thermal hyperalgesia, supraspinally mediated behaviours (licking following heat stimuli) were absent. Operant testing (Baastrup et al, 2010) indicated that cortical processing leading to the sensation of pain occurred for stimuli at/above, but not below, the injury level. Tract tracing suggested that the absence of signs of pain below the injury level may be due to interruption of ascending nociceptive pathways. In animals with less severe injuries (150 kdyn) forelimb tests showed similar results to 200 kdyn animals except that all signs were less pronounced. Furthermore, testing over the back indicated increased sensitivity below the injury level, not evident in the 200 kdyn model.

The effect of moving the site of injury to a segmental level closer to that where sensory input from the forepaws is processed was also investigated. Animals with injuries at the T3/T4 level showed the same behavioural signs of pain as seen in the 200 kdyn low thoracic injury animals, but all of the signs were more pronounced, especially signs of spontaneous pain. This model may therefore be optimum for the assessment of at/above level pain. Injuries at the C6 level also lead to behavioural signs of pain but tests on the back were more useful than the forelimbs and indicators of spontaneous pain were not evident following these injuries.

These studies show that different aspects of neuropathic pain following spinal cord injury can be assessed in rodent models of spinal cord injury. However, the choice of injury model and accompanying assessments is important and will determine those aspect of pain (origin above or below, evoked versus spontaneous and different modalities) that can be reliably assessed. The results also reinforce the idea that observations that rely on hindpaw assessments should be cautiously interpreted.

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Maladaptive induction by spinal cord injury of an adaptive nociceptor state drives chronic pain

Edgar T. Walters, Qing Yang, Zizhen Wu, Robyn J. Crook

Department of Integrative Biology and Pharmacology, University of Texas Medical School, Houston, Texas, USA

edgar.t.walters@uth.tmc.edu

Neuropathic pain develops in approximately half of all people after SCI and for many this chronic pain is debilitating and intractable. Numerous alterations within central pain pathways have been described after SCI, and central pathology is widely assumed to be the continuing cause of ongoing pain long after the spine is injured. Another possibility is that SCI generates multiple signals (neural, inflammatory, humoral) that are detected by widely distributed primary nociceptors and interpreted as signs of severe peripheral injury. Primary nociceptors are the neurons specialized for detecting injury and inflammation, and for driving adaptive responses to bodily injury, including pain. Adaptive hyperexcitability and spontaneous electrical activity arising in the cell bodies of primary nociceptors are hypothesized to be part of a natural hyperfunctional state in sensory neurons that compensates for loss of innervation to peripheral regions that are severely injured, serving to maintain painful awareness of disabled, vulnerable body parts (Walters, 2012). If neuropathic pain after SCI is driven by chronic activity in primary nociceptors (Bedi et al., 2010) that inadvertently enter this hyperfunctional state, then interventions that selectively reduce nociceptor activity should ameliorate signs of SCI pain. TRPV1 channels are expressed most abundantly in primary nociceptors. We found in rats that TRPV1 is upregulated in L4 and L5 dorsal root ganglia (DRG) after spinal contusion, that dissociated small DRG neurons become more sensitive to even very low concentrations of the TRPV1 activator, capsaicin, and that reflex hypersensitivity to mechanical and heat stimuli is reversed after SCI by either antisense knockdown of TRPV1 or injection of a specific TRPV1 antagonist (Wu et al., 2013). In the nervous system, Na^+ channel Nav1.8 is expressed only in primary sensory neurons, and primarily in nociceptors. We found that Nav1.8 is important for spontaneous activity in dissociated nociceptors after SCI, that SCI upregulates Nav1.8 in DRG, and that antisense knockdown of Nav1.8 reverses behavioral hypersensitivity (Yang et al., 2012). Preliminary data indicate that both TRPV1 function and Nav1.8 function are also important for spontaneous pain (as distinct from hyperreflexia and evoked pain) after SCI. These observations suggest that drugs selectively targeting hyperexcitable nociceptors may eventually provide a new approach for treating chronic pain after SCI.

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Changes in the function of organs innervated by the autonomic nervous system following spinal cord injury

James A. Brock

Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Australia

j.brock@unimelb.edu.au

The autonomic nervous system provides the neural control of most tissues in the periphery and plays a key role in the maintenance of homeostasis via its regulatory actions on smooth and cardiac muscle, exocrine and endocrine glands, tissue metabolism, immune cells, etc. The preganglionic neurons of sympathetic and sacral parasympathetic neural pathways reside in the intermediolateral cell columns of the spinal cord and receive descending excitatory and inhibitory synaptic inputs from supraspinal levels as well as local inputs from spinal interneurons. Spinal cord injury (SCI) severs supraspinal inputs to autonomic preganglionic neurons below the lesion, resulting in loss of supraspinal control while leaving the neural circuits mediating spinal reflexes largely intact and unopposed by descending inhibitory control. As a consequence cardiovascular, bladder, bowel and sexual dysfunctions are common after SCI and have a major impact on the wellbeing and quality of life of SCI individuals. However, the consequences of SCI for the function of sympathetic and sacral parasympathetic pathways and their effectors have generally been ignored.

Our studies have focused on the effects of SCI on blood vessel, bladder and bowel function in SCI rats. We have investigated plasticity at the sympathetic neurovascular junction that follows disruption of sympathetic pathways. We demonstrated that SCI markedly enhances constriction to nerve activity in arterial vessels to which ongoing sympathetic activity has been reduced following SCI (1, 2). Data from humans (3) suggest that these findings in experimental animals are likely to apply after SCI and contribute to unusual episodes of high blood pressure (autonomic dysreflexia) evoked by sensory stimulation below the level of the lesion.

In bladder, SCI acutely produces a hemorrhagic cystitis that is accompanied by breakdown in the barrier function of the urothelium that increases permeability to urine and urine-borne substances and greatly increases the risk of urinary tract infection. Evidence suggests this cystitis is triggered by autonomic nerve activity as it is delayed by ganglionic blockade (4). While the urothelium is rapidly repaired following SCI, there are substantial and persistent changes in the bladder wall that are likely to contribute to continued bladder dysfunction. Whether similar changes occur acutely following SCI in humans remains to be established.

Neurogenic bowel dysfunction is common following SCI leading to constipation and fecal incontinence. We have investigated whether capromorelin, a compound that causes defecation by stimulating ghrelin receptors within the lumbosacral defecation centers, is effective after SCI. Capromorelin caused robust propulsive activity in the colorectum soon after its application (5). The compound was similarly effective in sham-operated and SCI rats. On the basis of these findings we have initiated an open label clinical trial to assess whether capromorelin is a compound that could be used to relieve constipation by triggering defecation SCI subjects.

Together these findings demonstrate that SCI produces substantial changes in autonomic effectors that contribute to their dysfunction. In addition, they demonstrate that it is possible to chemically stimulate the remaining neural circuits within the spinal cord to elicit coordinated activity of effectors. It remains to be established if the observed changes in autonomic effectors can be prevented or reversed.

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Session VII: COMBINATORIAL TREATMENTS

Chair: Geoffrey Raisman

Use of an *in vitro* model of SCI to assess the effect of combined treatments on repair

Susan C. Barnett, Michael McGrath, Miles D. Housley[1], Stephanie D. Boomkamp

Institute of Infection, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, UK
[1] Institute of Pharmaceutical Sciences, King's College London, London, UK

Susan.Barnett@glasgow.ac.uk

Spinal cord injury (SCI) is a devastating condition which usually leads to paralysis. From many years of research it is apparent that a combination of therapies would be the best course of action for treatment of SCI. However this approach greatly increases animal cohorts and time to establish the most effective course of treatment. Using our previously described myelinating cultures we have developed an *in vitro* model of SCI by cutting across the cultures using a scalpel blade. A persistently neurite-free area is created which is accompanied by many features of SCI, including demyelination and reduced neurite density adjacent to the lesion and the presence of reactive astrocytes within the lesion. These changes can be quantified by immuocytochemistry, PCR and Western blotting and the model validated with reagents known to induce repair *in vivo* models of SCI. Thus, the cultures can be used as a moderate throughput screen to analyse combined therapies and allow a more detailed analysis at the mechanistic level.

We have focused on cyclic adenosine monophosphate (cAMP), a promising therapeutic target for the treatment of SCI in our *in vitro* SCI model and studied the mechanisms by which it promotes neurite outgrowth and myelination, to identify more specific therapeutic targets. Using enantiomers of the cAMP degrading phosphodiesterase-4 (PDE4)-specific inhibitor, rolipram the HARBS, rather than the LARBS, PDE4 conformer, promotes neurite outgrowth and myelination, demonstrating its pivotal role in SCI repair. Rather than protein kinase A, the 'traditional' effector of cAMP action being involved, we show that exchange protein activated by cAMP (Epac) activation by cAMP directs myelination and neurite outgrowth. Moreover, astrocytic expression of the chemokine CXCL10, a factor known to inhibit myelination, was markedly elevated upon Rho inhibition and that such an effect was ablated by inhibition of not only ROCK, but also PDE4. Thus, PDE4 inhibitors targeted at the HARBS conformer as well as Epac agonists may provide promising novel targets for the treatment of SCI. These data show how combination of factors can be analysed in an *in vitro* model of SCI prior to confirmatory studies using animal models.

Combining treatments that target axons, matrix, myelin and rehabilitation

James Fawcett

John van Geest Centre for Brain Repair, University of Cambridge, Cambridge, UK

jf108@cam.ac.uk

Recovery from SCI needs plasticity and axon regeneration. Current treatments have their main effect through stimulating plasticity. They enhance sprouting and alter perineuronal nets. If the refinement of exuberant projections that occurs during development is a model for post-injury events, then one would expect that appropriate rehabilitation would stabilise useful new circuits at the expense of inappropriate connections. There is evidence that combining rehabilitation with measures that affect plasticity or excitability is beneficial. There is also evidence for combinatorial effects of regenerative treatments.

Effective axon regeneration after SCI is still an unsolved problem. We know what effects can be obtained by blocking inhibitory molecules singly or in combination, and this is insufficient. It will also be necessary to increase the intrinsic regenerative ability of the axons. We are starting to understand why axons lose the ability to regenerate with development. As axons mature transport becomes increasingly selective, and growth-related molecules such as integrins and ribosomal proteins are excluded at the axon initial segment, leaving axons crippled from the perspective of growth. This selective transport block involves a change in the directionality of transport of some transport endosomes, controlled by the state of activation of Arf6. In addition, expression of some growth-related molecules such as integrins is greatly decreased during development. We need to understand the developmental events that trigger these changes.

PDGFRα-positive progenitor cells from myelinating oligodendrocytes and Schwann cells following spinal cord contusion injury

Peggy Assinck*[1], Gregory J. Duncan*[1], Nathan J. Michaels[1], Douglas R. Brown[1,2], Jason R. Plemel[1], Micheal J. Lee[1], Jie Liu[1], **Wolfram Tetzlaff**[1,2]

*Authors contributed equally to this work
[1]ICORD and Dept. of Zoology[2], University of British Columbia, Vancouver, BC, Canada

tetzlaff@icord.org

Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons which impairs signal transduction and may leave axons vulnerable to degeneration. Both oligodendrocytes (OL)s and Schwann cells remyelinate denuded axons in the subsequent weeks and months following SCI. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which may serve as a source of new OLs following SCI. PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic spinal cord injury. In the uninjured spinal cord we found that YFP was expressed in NG2+ OPs at very high efficiency, as well as αSMA+ pericytes and fibronectin+ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express the PDGFRa+, Olig2 and NG2, indicative they have remained as OPs, but substantial differentiation into new mature oligodendrocytes (CC1+) was observed, particularly in the spared ventral and lateral white matter. Near the lesion site, YFP+ cells expressed fibronectin while others contributed to scarring with some limited differentiation at the lesion into GFAP+ astrocytes. Strikingly, the majority of P0+ Schwann cells expressed YFP, suggesting they originated from central nervous system PDGFRα+ OPs, and not the spinal roots as previously thought. However, further work is required to characterize if other YFP+ populations like αSMA+ pericytes or the peripheral fibrocytic-like cells can contribute to the formation of myelinating Schwann cells or OL's in the injured CNS. Overall, this work reveals a large phenotypic plasticity of PDGFRα precursors following spinal cord injury as a source of the new remyelinating Schwann cells and oligodendrocytes in the injured spinal cord.

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Session VIII: CLINICAL SESSION

Chair: James Guest

Effects of spinal epidural stimulation and training following a motor complete injury

Susan Harkema, Enrico Rejc, Claudia Angeli

Department of Neurological Surgery, University of Louisville, Kentucky Spinal Cord Injury Research Center, Frazier Rehab Institute, Louisville, KY, USA

susanharkema@kentuckyonehealth.org

Previously, we reported that one individual who had a motor complete, but sensory incomplete spinal cord injury regained voluntary movement after 7 months of epidural stimulation and stand training. We presumed that the residual sensory pathways were critical in this recovery. However, we now report in three more individuals voluntary movement occurred with epidural stimulation immediately after implant even in two who were diagnosed with a motor and sensory complete lesion. We demonstrate that neuromodulating the spinal circuitry with epidural stimulation, allows completely paralyzed individuals to process conceptual, auditory, and visual input to regain relatively fine voluntary control of paralyzed muscles. We show that neuromodulation of the sub-threshold motor state of excitability of the lumbosacral spinal networks was the key to recovery of intentional movement in four out of four individuals diagnosed as having complete paralysis of the legs. We have uncovered a fundamentally new intervention strategy that can dramatically affect recovery of voluntary movement in individuals with complete paralysis even years after injury. In other experiments we studied how the spinal sensory-motor pathways associated with posture can be modulated when different stimulation parameters are applied. We also assessed how the spinally evoked responses to different stimulation parameters are modulated in the presence of different patterns of cutaneous and proprioceptive input to the spinal cord circuitry that is functionally isolated from the brain. These results have important implications with respect to: 1) how postural spinal networks can be selectively modulated by varying the stimulation frequency, 2) the impact of proprioceptive input and how and which pathways can be modulated with epidural stimulation, and 3) identifying strategies that are likely to be most efficacious in enabling improved motor function for standing after complete paralysis.

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Spontaneous and therapy-induced plasticity of CNS circuits

Martin E. Schwab

Brain Research Institute, University of Zurich and ETH Zurich, Zurich, Switzerland

schwab@hifo.uzh.ch

Large spinal cord or brain injuries lead to life-long major functional impairments. In contrast, small lesions of the CNS often have a good prognosis with extensive functional recovery; the underlying mechanisms are not well understood, however. Major changes in the neuronal wiring including formation of new circuits and maps were found after spinal cord lesions in adult rats. A spinal cord injury transecting the hindlimb corticospinal tract (CST) induced spontaneous sprouting of the lesioned fibers in the upper spinal cord leading to new connections of former hindlimb CST fibers to the forelimb. Forelimb sensory connections also expanded into the former hindlimb motor cortex. A similar re-wiring and map shift was observed after focal cortical strokes: destruction of the forelimb cortex led to sprouting of hindlimb fibers into the cervical spinal cord. These anatomical changes may form the basis for the functional recovery observed behaviourally. However, in all these cases extent and length of fiber growth was limited to about 0.2 - 2 mm.

20 years ago, the presence of specific neurite growth inhibitory factors in myelin of the CNS was discovered. The membrane protein Nogo-A is currently the most potent known neurite growth inhibitor. Function blocking antibodies against Nogo-A have been applied to rats and macaque monkeys with spinal cord injuries as well as animals with strokes in the sensory-motor cortex. Biochemical readouts showed an up-regulation of the neuronal growth machinery in lesioned and in intact animals. On the anatomical level, injured fibers showed enhanced regenerative sprouting as well as long-distance regeneration with formation of large terminal arbors. Spared fiber tracts showed enhanced compensatory sprouting, often covering relatively long distances. In animals with cortical strokes, fibers from the intact corticobulbar or corticospinal system crossed the midline, supplying innervation to the denervated brain stem or spinal cord under the influence of anti- Nogo-A antibodies. Behavioral tests for locomotion, grid and beam walk, swimming, as well as skilled forelimb reaching showed marked improvements of functional recovery in the Nogo-A antibody treated injured animals. Antibodies against human Nogo-A are currently used in clinical trials for spinal cord injury, MS and ALS.

Revisiting magnesium – a new clinical development program for acute neuroprotection in SCI

Andrew R. Blight

Acorda Therapeutics, Inc., Ardsley, NY, USA

ablight@acorda.com

Disruption of the ionic microenvironment is one of the most rapidly occurring effects of trauma to the central nervous system (CNS). This disruption includes a dramatic and relatively long lasting loss of divalent cations from the extracellular space, as calcium ions pass into the intracellular space and magnesium ions are lost from the tissue. Divalent cations are normally important for the maintenance of cellular membrane properties, and magnesium is specifically involved in the control of permeability at the NMDA channel as well as in the maintenance of effective mitochondrial function. Hence, there has been considerable interest over many years in the potential for restoration of magnesium ion levels to limit secondary pathological processes in CNS injury.

The major difficulty in translating beneficial effects seen in animal models of injury has been that CNS magnesium levels are not readily changed by elevation of magnesium concentrations in the blood, and the degree to which plasma magnesium concentrations can be elevated is limited by adverse systemic effects of higher concentrations of magnesium in the clinical setting. AC105 is a proprietary magnesium formulation in polyethylene glycol, which appears to increase accessibility of magnesium ions to the CNS environment. AC105 has demonstrated neuroprotective properties, leading to improved functional recovery and tissue preservation in a number of animal models of SCI and traumatic brain injury, when treatment was initiated within several hours of injury. AC105 has completed a Phase 1 study in healthy volunteers and Acorda is now in the process of initiating a human Phase 2 clinical trial in acute SCI at several trauma centers in North America. This is a randomized, placebo controlled study that will examine the safety and feasibility of early treatment in human injury. It will also provide an initial exploration of the potential effects of this treatment on long term outcome. Meanwhile, additional laboratory studies are under way to further explore the underlying mechanisms of this approach.

The key issues involved in taking such a program from translational studies into clinical development will be discussed, and the implications for development of other potential clinical approaches to acute and chronic SCI will be considered.

Session IX: DISCUSSION FORUM / DEBATE

Chair: James Fawcett

Translational issues in spinal cord repair: how do we improve preclinical study design?

Panel members: Adam Ferguson, Stephen McMahon, Hans-Werner Mueller, Oswald Steward, Wolfram Tetzlaff
Chaired by James Fawcett

Adam.Ferguson@ucsf.edu, stephen.mcmahon@kcl.ac.uk, hanswerner.mueller@uni-duesseldorf.de, osteward@uci.edu, tetzlaff@icord.org

The session will discuss two topics:
1. Design of preclinical treatment trials
2. Use of large scale data analysis

In June, 2012, the National Institute of Neurological Disorders and Stroke (NINDS) held a workshop entitled: "Optimizing the Predictive Value of Preclinical Research" in response to growing concern that promising preclinical findings in a number of different disorders could not be replicated. The results of explicit replications of promising studies in spinal cord injury have led to some understanding of the factors that may contribute to the failure to replicate (Steward et al., 2012, Exp. Neurol., 233, 597-605). Major conclusions of the workshop were that there is need for increased rigor in experimental design and execution, and full transparency in reporting of methods and results (Landis et al., 2012, Nature 490, 187-191). Of particular relevance for preclinical studies in SCI are issues arising from pooling of data from experiments done over time, lack of explicit randomization, under-powered experiments, use of multiple endpoint measures, testing to a foregone conclusion, lack of self-replication, and publication bias for positive results. O. Steward will summarize the recommendations for changes in review criteria for grants that NIH is beginning to implement and standards for reporting that some journals are beginning to adopt.

"How much evidence is enough in order to justify a trial?". What animal models (species, type of injury, spinal levels, severities), intervention timelines and effect sizes should we see? We will review the efforts of Dr. Brian Kwon and myself gathering opinions. The stroke field has been leading with the CAMARADES (Collaborative Approach to Meta-analysis and Review of Animal Data in Experimental Studies) initiative and similar analyses of publication bias and quality criteria have been proposed (including a 9 point score). We will discuss whether a similar approach might work for SCI.

Other points will be: Distinguishing between exploratory and hypothesis-testing research, Shared evaluation of data, Facility to publish experiments with negative outcomes, Long term (rather than short term) assessment/citation measures.

Both replication (across laboratories) and translation (across species) fundamentally deal with the question of the experimental generality: How do research findings generalize across different laboratories or across different species? Hans Werner Müller and Adam Ferguson will discuss large-scale, big-data projects that seek to determine the generality of research findings from computational perspective. A. Ferguson will discuss efforts to build a large-scale database integrating raw research data from 13 SCI research laboratories, and provide examples of the novel insights that can be provided through multivariate statistical integration of these data (Ferguson et al., 2011, Transl Stroke Res, 2, 438-454; Ferguson et al., 2013, PLoS ONE 8, e59712; Rosenszweig et al., 2010, Nat Neurosci 13, 1505-1510). H. Werner Müller will discuss efforts to curate SCI knowledge from the published literature on CNS injury through the development of a novel database of preclinical research findings hosted by the Center for Neural Regeneration in Düsseldorf (CNR (www.cnr.de) and complementary efforts by other groups (e.g., Kwon et al., J Neurotrauma 28, 1525-43). The fundamental premise of these knowledge-curation projects is that integration of big-data can deliver powerful decision-support tools for judging translational potential through large-scale data-mining, automated pattern-detection and large-scale data visualization.

Table of Contents – Poster Abstracts

Distribution of perineuronal net molecules in the adult rat spinal cord and in cultured embryonic hippocampal neurons

Louise Adams, Yi Zhang, Adina T. Michael-Titus, John V. Priestley, Xuenong Bo

Centre for Neuroscience & Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK

L.Adams@qmul.ac.uk

As part of an on-going project to promote plasticity by manipulating the density of the perineuronal net (PNN) *in vivo*, we aimed to characterise the distribution of these structures throughout the adult rat spinal cord. Formed of a network of extracellular matrix proteins including chondroitin sulphate proteoglycans, tenascin-R, link protein and hyaluronan, the PNN is believed to have a role in the limitation of neuroplasticity in the adult nervous system. For example, PNNs may contribute to the poor functional recovery observed after injury to the brain or spinal cord.

We noted strong PNN labelling for two component molecules, link protein and neurocan, around neurons in both the intermediate grey matter and ventral horns of the rat spinal cord. However, only a fraction of these cells were also labelled by lectin from Wisteria Floribunda Agglutinin (WFA), a widely used marker of the PNN. Particularly strong PNN labelling for this lectin was seen in a subpopulation of non-cholinergic cells in lamina X, that receive weak serotonergic, but strong inhibitory input. Interestingly, WFA labelling in the ventral horn shows minimal overlap with link protein and neurocan. WFA labelling is strong in the medial ventral horn, in contrast to neurocan and link protein, which are abundant in PNNs around large motor neurons located in the lateral ventral horns. Although there are regional variations in the distribution of link protein, neurocan and WFA in the spinal cord, high power confocal imaging revealed a similar 'honeycomb' substructure of the PNN with all three markers.

To investigate the mechanisms regulating the development of the PNN in the nervous system, we used neuronal cultures from the embryonic rat hippocampus (E18). A small proportion of these cells produce PNN components during the first week in culture, and form a mature PNN that is found associated with the plasma membrane during the second week. Similarly to that observed in the spinal cord, WFA only labels a subpopulation of cultured neurons and does not always co-localise with link protein or neurocan. In future studies this culture system will be used to examine the effect of polysialic acid removal on the morphology of the PNN.

This work was **supported by** a Nathalie Rose Barr Studentship from the ***International Spinal Research Trust***

Increased expression of chondroitin sulphate proteoglycans in the lumbar spinal cord of bipedally step trained spinal rats

Yazi D. Al'joboori[1], B. C. Marsh[1], H. Zhong[2], R. R. Roy[2], J. W. Fawcett[3], V. R. Edgerton[2], R. M. Ichiyama[1]

[1]Sch. of Biomed. sciences, Univ. of Leeds, Leeds, UK
[2]Physiological Sci. and Neurobio. and Brain Res. Institute, Univ. of California, Los Angeles CA, USA
[3]Dept. of Clin. Neurosci., Univ. of Cambridge, Cambridge Ctr. for Brain Repair, Cambridge, UK

bsydaj@leeds.ac.uk

Failure of axons to regenerate after spinal cord injury (SCI) is in part due to the presence of numerous inhibitory molecules. Chondroitin sulphate proteoglycans (CSPGs) are extracellular matrix molecules that form perineuronal nets (PNNs) around some neurones. Evidence suggests that these molecules restrict neuronal plasticity after SCI as the combination of motor training and the addition of an enzyme which degrades CSPGs, chondroitinase ABC, allows for further functional recovery in reaching and grasping tasks. After a complete spinal cord transection, we have shown that epidural stimulation (ES; L2) produces co-ordinated, weight-bearing bipedal stepping that is further improved with a combination of serotonergic and monoaminergic agonists and daily locomotor training. In this study we determined how the expression of CSPGs and PNNs in the spinal cord caudal to the lesion site change after injury and with rehabilitation.

Adult Sprague-Dawley rats received a complete spinal cord transection (T9/T10). CSPG expression was examined 7, 21, and 67 days-post-injury (dpi) in locomotor trained and non-trained groups. Trained rats received ES (L2; 40 Hz) with quipazine (0.3 mg/kg; 5-HT agonist) and bipedal step training (6-21 cm/s) 5 days/week (30 min/day). Immunohistochemistry was used to observe expression levels of CSPG components using antibodies against aggrecan, cartilage link protein-1 (Crtl-1) and the lectin Wisteria floribunda agglutinin (WFA). PNN thickness and intensity was measured using WFA staining of alpha motor neurones (α-MNs) and the expression of CSPG components was measured by mean intensity of staining in the ventral horns (L5).

After SCI CSPGs and PNN expression levels were upregulated caudal to the lesion. This upregulation increased with time after injury: the 67 dpi group expressed the highest levels of CSPGs. Surprisingly, locomotor training under ES and quipazine produced a further increase in expression of CSPGs and PNNs. Trained rats (exhibited higher expression of CSPGs when compared to their non-trained counterparts. PNN thickness and intensity of staining in alpha motor neurons were significantly increased in trained than non-trained. Moreover, no PNNs were detected surrounding gamma motor neurones (γ-MNs).

The absence of PNNs around γ-MNs supports the hypothesis that PNNs are important in the regulation of synaptic plasticity. We have recently shown that after spinal cord transection γ-MNs are more susceptible to synaptic inputs and losses than α-MNs. These findings also suggest that application of ChABC in the lumbar cord may result in further recovery of locomotor function after SCI and rehabilitation.

Support provided by NIH, Christopher and Dana Reeve Foundation, ***International Spinal Research Trust***

Combinatorial expression of regeneration associated transcription factors in a high throughput F11 neurite outgrowth assay

Callan L. Attwell[1], Nitish Fagoe[1], August B. Smit[2], Ronald E. van Kesteren[2], Joost Verhaagen[1], Matthew R.J. Mason[1]

[1]Neuroregeneration Group, Netherlands Institute for Neuroscience, The Netherlands
[2]Center for Neurogenomics and Cognitive Research, VU University Amsterdam, The Netherlands

c.attwell@nin.knaw.nl

The central nervous system (CNS) has a limited intrinsic ability to regenerate after damage, in contrast to the peripheral nervous system (PNS) which regenerates efficiently after injury. The characteristics of dorsal root ganglia (DRG) neurons are ideal for research into neuron-intrinsic mechanisms controlling regeneration. DRG neurons possess a central and a peripheral axon projection. Injury to the central projection (into the CNS) alone does not initiate a robust regenerative response, but after a conditioning lesion to the peripheral projection the central projection will regenerate more effectively. Based on microarray analysis of axotomized neurons many regeneration associated genes (RAGs) have been previously identified and investigated. Promoter analysis performed on genes from RAG datasets indicated a number of transcription factors (TFs) which are thought to be involved in the response to injury and coordination of the intrinsic regenerative capabilities seen in the PNS. Some of these TFs are already described in relation to regeneration (e.g. c-Jun, ATF3, KLF7, STAT3, Sox11) and others have not previously been linked to regeneration (MEF2, HOX factors, SRF).

We hypothesized that the gene expression program in PNS neurons which results in regeneration following injury is coordinated by a relatively small number of TFs acting synergistically. We therefore sought to determine the effects of expressing combinations of regeneration associated transcription factors in a neurite outgrowth assay which utilizes the F11 cell line (a rat DRG/ mouse neuroblastoma hybrid cell line), automated plate imaging and neurite length analysis with the expectation that some combinations would have a greater effect on neurite outgrowth than the sum of their individual effects.

Starting with 9 regeneration associated TFs, chosen because they were strongly indicated by RAG promoter analysis, we performed a systematic screen to identify the most potent combination of TFs for promoting neurite outgrowth. We quantified neurite outgrowth induced by all single TFs, all possible pairs of TFs and the triple combinations of those TFs which in a pair caused significantly higher neurite outgrowth than the best single TF.

Of the selected TFs only KLF7 and MEF2 caused significantly more neurite outgrowth than the control (GFP-only) when expressed alone. When expressed in pairs, two combinations of TFs, c-Jun/MEF2 and KLF7/MEF2 caused significantly higher neurite outgrowth than the best single TF alone (MEF2). Of the triple TF combinations selected, two of these, ATF3/KLF7/MEF2 and STAT3/KLF7/MEF2 caused significantly higher neurite outgrowth than the best double TF combination (both inducing 2.85 times the outgrowth of GFP alone). We conclude that these TFs show strong synergistic effects on neurite outgrowth and that expression of this combination in injured spinal neurons that do not normally regenerate may be a powerful way to induce axonal regeneration in the injured spinal cord.

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Neuregulin-1 plays a role in Schwann cell-mediated axonal remyelination following spinal cord injury

Katalin Bartus[1], J. Galino Barres[4], F.R. Fricker[4], J.M. Dawes[4], N.D. James[1], N. Zhu[4], A.N. Garratt[2,3], C. Birchmeier[2,3], D.L.H. Bennett[4], and E.J. Bradbury[1]

[1]King's College London, Regeneration Group, Wolfson Centre for Age-Related Diseases, London, UK
[2]Max Delbrueck Center for Molecular Medicine, Berlin, Germany
[3]Charité Universitätsmedizin Berlin, Charitéplatz 1, Berlin, Germany
[4]Nuffield Department of Clinical Neurosciences, West Wing John Radcliffe Hospital, Oxford, UK

katalin.bartus@kcl.ac.uk

One of the responses following traumatic spinal cord injury is demyelination of spinal axons. However, whether demyelination persists in chronic spinal cord injuries and the impact this may have on function remains controversial. We have recently found that, although there is extensive remyelination of axons following a spinal contusion injury, a significant proportion of axons remain chronically demyelinated. We also demonstrated that remyelination is largely mediated by Schwann cells. Invasion and/or proliferation of Schwann cells into the CNS (Schwannosis) is commonly observed in the chronically injured human spinal cord, where axons become associated with peripheral myelin. These Schwann cells may originate in the peripheral spinal roots and migrate to the spinal cord as a result of injury-induced compromise of the central glial barrier, or they may derive from CNS-resident oligodendrocyte precursors. However, little is known about mechanisms governing Schwannosis following spinal cord injury. It is well established that the growth factor neuregulin-1 (NRG1) plays a crucial role in communication between Schwann cells and axons during peripheral myelination in development. However, whether it plays a role in adulthood following spinal cord injury remains unclear. Here we examined the role of NRG1 in remyelination following spinal contusion injury using an inducible NRG1-null mouse, where NRG1 expression is suppressed in adulthood in order to avoid the confounds of its developmental roles. Adult NRG1 null mice and wildtype littermate controls received a moderate severity spinal contusion injury. Eight weeks following injury in wildtype animals a proportion of dorsal column axons at the lesion epicentre were remyelinated by Schwann cells, revealed by their distinctive morphology and expression of the peripheral myelin marker P_0. Strikingly, however, this Schwann cell-associated re-myelination was absent in NRG1-null injured spinal cords. We have also assessed the impact of the lack of NRG1 on functional outcome following spinal contusion injury and demonstrate a significantly poorer degree of spontaneous recovery of NRG1-null mice. These data indicate that NRG1 plays a key role in Schwann cell-mediated remyelination following spinal cord injury, which also impacts on the degree of spontaneous recovery following spinal cord contusion injury.

Intraneural ATP injection strongly stimulates regeneration of primary sensory axons in the spinal cord

Dongsheng Wu, Sena Lee, Juan Luo, Storm Lonsdale, Haijian Xia, Peter M. Richardson, 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, London, UK

x.bo@qmul.ac.uk

Weak intrinsic regenerative capacity of injured neurons is a major obstacle for neural repair. Injury to the peripheral axons of sensory neurons strongly enhances the regeneration of their central axons in the spinal cord. Better knowledge of the molecular events underlying this phenomenon could lead to practical methods to boost their intrinsic regenerative capacity. Since ATP is released extracellularly by nerve and other tissue injury, we hypothesized that injection of ATP into a peripheral nerve might mimic the stimulatory effect of nerve injury on the regenerative state of the primary sensory neurons. We found that a single ATP injection quadrupled the number of axons growing into a lesion cavity in spinal cord after a concomitant dorsal column transection. A second ATP injection one week after the first one markedly reinforced the stimulatory effect of a single injection. Single ATP injection increased expression of pSTAT3 and GAP43, two markers of regenerative activity, in sensory neurons. Double ATP injections sustained the activation of pSTAT3 and GAP43, which may account for the marked axonal growth across the lesion. Levels of interleukin-6 in both injected sciatic nerve and dorsal root ganglion were increased by ATP injection. ATP injection caused little Wallerian degeneration judged by histochemical examination and behavioural tests showed no significant long-term adverse effects on sciatic nerve functions. The results in this study reveal possible mechanisms underlying the stimulation of regenerative programmes and suggest a practical strategy for stimulating axonal regeneration following spinal cord injury.

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A novel knowledge database for information management of pre-clinical published data of spinal cord injury

Nicole Brazda[1,2], Fabian Kruse[1], Michael Kruse[1], Tarek Kirchhoffer[1], Roman Klinger[3], Philipp Cimiano[3], Hans Werner Müller[1,2]

[1]Center for Neuronal Regeneration, Life Science Center Düsseldorf, Germany
[2]Molecular Neurobiology Laboratory, Heinrich Heine University Düsseldorf, Germany
[3]Cognitive Interaction Technology Excellence Cluster (CITEC), University Bielefeld, Germany

Nicole.brazda@hhu.de

World-wide therapy development for spinal cord injury is done predominantly in experimental animal models. The amount of data already published on spinal cord and brain injury exceeds 150,000 peer-reviewed publications (PubMed result on July 26th:152,335 publications). It is obvious that conservative manual data management, even if supported by reference management systems is not suited to provide a profound overview of existing knowledge in this field. We present a pilot beta version of a free, open access database for published pre-clinical data in the field of spinal cord injury (SCI) which is hosted by the non-profit CNR organization (Center for Neuronal Regeneration, www.cnr.de). The major purpose of the CNR is to support and speed up clinical translation of pre-clinical approaches to SCI-therapy by offering a tool for structured query, analysis and evaluation of already published knowledge on SCI treatments to basic and clinical scientists, as well as funding organizations and pharmaceutical companies working in this field.

In the current pilot version of the CNR SCI database basic functionalities are presented and can be tested on-site. Data collected include basic information on the experimental setup of a published pre-clinical treatment trial in an animal model, e.g. animal species, age, gender, injury model, severity and height of lesion, and also treatment paradigm, compound, concentration etc. The most demanding type of data comprises the outcomes of publications which can be classified as functional (behavioral, electrophysiological, etc.) and non-functional (histological, biochemical, etc.) outcomes. For each outcome of a published experiment, the type of outcome, experimental group size, methods used and authors judgement of the result, concerning benefit for the injured individual, is modeled in the CNR SCI database. These data can be computed by the user via filter and grouping tools, but importantly, visualization of the data allows an easy overview and direct comparison of outcomes generated by different treatment paradigms.

The second major function of the CNR SCI database is the implementation of a grading system as described by Kwon et al. (2011, J. Neurotrauma 28: 1525-43) to evaluate the level of evidence for a treatment strategy. The grading system was developed for neuroprotective non-invasive SCI therapies and was previously applied manually for a set of 117 publications by the authors. Briefly, scores are assigned to treatment strategies and depend on animal species, injury type, therapeutic time window and clinically meaningful efficacy. Moreover, independent replication of positive outcomes or description of negative results influence the final score of a treatment. Importantly, the scoring system does not judge upon a single publication or laboratory but describes the level of evidence for a therapy which could change with each new publication about this treatment. In the CNR SCI database, the rules for score assignment as developed by Kwon et al are implemented, but the points for each field can be adjusted by the user individually, allowing a personalized view upon the data, depending on the users own experience and expertise.

The pilot version of the CNR SCI database comprises 143 publications with over 800 outcomes in total and was filled manually with the presented pre-clinical data. Parallel to database development, an automated information extraction system is currently being developed. The ontology-based analysis system will aid data extraction from full text articles and thus allow collection of big datasets into the CNR SCI database. As it is widely accepted that lack of published negative results biases objective judgement upon therapeutic approaches, the CNR would like to collect unpublished replication data or negative data of SCI treatments and render them citable for the scientist. Furthermore, future implementation of published clinical data on SCI and (pre-)clinical data on traumatic brain injury is envisaged and feasible.

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Developing a tool to measure synaptogenesis and investigate functional plasticity following regenerative therapies to treat spinal cord injury

Emily R. Burnside, Karen D. Bosch, Nicholas D. James, Stephen B. McMahon, Elizabeth J. Bradbury

Neurorestoration Group, Wolfson Centre for Age-Related Diseases, King's College London, London, UK

emily.burnside@kcl.ac.uk

Conventional tracing techniques reveal projection patterns of particular neuronal populations but do not themselves provide evidence of synaptic connectivity. The corticospinal tract (CST) is an important decending motor pathway involved in locomotion, posture and voluntary skilled movements. Therefore regeneration and anatomical reorganisation of this projection is often examined in experimental SCI studies. Techniques such as BDA tracing have been combined with immunolabelling for synaptic proteins or electron microscopy to elucidate connectivity; however whether active synaptogenesis occurs following SCI and potential therapeutic manipulations has not been studied. Genetically encoded reporters of presynaptic function represent novel tools to assess synaptogenesis and gain insight into the anatomical and functional status of new connections. Via stereotaxic intra-cortical injection, adeno-associated viral (AAV) vectors were used to label the corticospinal tracts of naïve rats with one of two fluorescently tagged presynaptic proteins, AAV-SynaptopHluorin (AAV-SpH; AAV-VAMP-superecliptic GFP) or AAV-Synaptophysin-GFP. Both constructs were found to transduce neurons in the sensorimotor cortex and label axons in the CST and putative presynaptic terminals in grey matter, though with different patterns of expression. AAV-SpH intensely labeled CST axon bundles at the level of the pyramidal decussation and in the dorsal columns of the spinal cord as well as putative presynaptic boutons, whereas AAV-synaptophysin localised more distally to presynaptic terminals. These results suggest usefulness for studying anatomical plasticity and synaptogenesis in a specific spinal pathway following SCI. We therefore went on to use the vectors in a clinically relevant contusion model. AAV-SpH was utilised to label the CST in rats which had undergone moderate severity thoracic contusion injury. We are currently analysing the projection patterns and optimising a method of unbiased stereological quantification of putative synaptic boutons. Future studies will investigate synaptogenesis following experimental therapeutics that have previously been shown to promote plasticity following SCI. An additional property of AAV-SpH is pH-dependent fluorescence, a feature which we eventually aim to utilise to study active synaptogenesis in an *ex vivo* spinal cord slice preparation, imaging real time with two-photon microscopy.

Muscle strength changes recorded using dynamometry during robot assisted gait training

Galen S.[1], Clarke C.[2], Allan D.[3], **Bernard A. Conway**

Biomedical Engineering, University of Strathclyde, Scotland, UK
[1]Physical Therapy Program, Wayne State University, Detroit, USA
[2] Rehabilitation Sciences, University of East Anglia, UK
[3]Queen Elizabeth National Spinal Injuries Unit, Scotland, UK

b.a.conway@strath.ac.uk

Background: The Lokomat (Hocoma) is a commercially available robot assisted BWSTT. Although previous studies have shown functional improvements in gait following Lokomat training in ISCI, the strength changes in lower limb muscles following BWSTT has received little attention. The aim of this study was to record changes in muscle strength in key lower limb muscle groups in acute and chronic ISCI patients undergoing daily Lokomat training.

Methods: Eighteen patients (acute = 13, chronic= 5) with ISCI participated in this study (Age range: 26-63 years, mean = 49.33 + 11.04 years). Each patient underwent a six week Lokomat training program consisting of a daily target of 1 hour of BWSTT. In this study we used the Lokomat's ability to measure isometric hip and knee joint moment in order to chart weekly changes in the maximal voluntary strength changes in the flexor and extensor muscles groups of those joints. Isometric measurements were made prior to the start of Lokomat training and during the middle of each training week. Patients were positioned in an upright standing posture within the Lokomat and instructed to make maximal efforts of flexor and extensor muscle groups of the hip and knee against a fixed orthosis. The best of three attempts of each exertion was taken as an estimate of the maximal voluntary strength. Rest periods between each exertion were given in order to avoid fatigue.

Results: For all muscle groups tested there was a significant increase in the maximal strength recorded in the acute patient group ($p<0.05$). The time course of the changes reveals that in responding patients who demonstrate functional improvements in gait that there is clear evidence of strength increases in the hip and knee musculature within 3 weeks of training onset. This increase in strength continues to rise throughout the training program but not in a simple linear fashion. In the chronic patient group strength changes were also seen but were of a lesser magnitude than those seen in the acute group.

Discussion/Conclusions: This study objectively measured the changes in muscle strength following robot assisted BWSTT. For patients receiving Lokomat rehabilitation the observation that increased hip and knee moments in responding patients can be seen by week 3 is indicative of treatment efficacy. However, in patients lacking a positive increase in strength by week 3 may denote that the patient is receiving no physiological benefit from the robot assisted BWSTT. Incorporating, regular dynamometry may therefore assist in revising treatment planning for patients who are considered for Lokomat or other forms of BWSTT.

Taking a new look at corticospinal tract involvement in spinal cord injury in macaque monkeys

Corinna Darian-Smith, Alayna Lilak, Karen-Amanda Irvine, Joseph Garner

Dept. of Comparative Medicine, Stanford University School of Medicine, Stanford CA, USA

cdarian@stanford.edu

The primate corticospinal tract comprises nine or more functional subdivisions and is the major descending pathway mediating voluntary hand movements. We recently showed that following a cervical dorsal rhizotomy (DRL) (Darian-Smith et al., 2013), the corticospinal tract (CST) projections originating from primary somatosensory motor cortex responded quite differently to the injury. Terminal projections from the somatosensory cortex shrank to only 60% of the contralateral side. Conversely, motor CST projections did not shrink, and sprouting was observed in the dorsal horn ipsilateral to the lesion. Thus the CST subdivisions were shown to play a different functional role in post-injury recovery, with motor cortex (area 4) having a much greater role in the recovery process than primary somatosensory cortex (S1).

In the work described here, we asked what happens when a central injury is added to the equation. Monkeys received either a unilateral DRL, or a combined DRL/ cuneate fasciculus lesion. Three months later, electrophysiological recordings were made to identify the reorganized region of D1-D3 representation, and anterograde tracers injected bilaterally to assess spinal terminal labeling. Remarkably, terminal projections from the reorganized S1 cortex extended bilaterally into the intermediate and ventral horns well beyond terminal territories observed in normal animals or following a DRL. Rostrocaudally, S1 CST input projected from C1 through T4, or at least 3-4 segments beyond what is observed in normal animals. Terminal labeling from the reorganized motor cortex similarly extended bilaterally beyond that seen following a DRL, though the rostrocaudal extent of labeling from C1 through C5 was no more than 1 segment beyond that observed in normal animals (C1-T4). These data were highly significant and indicate a very different response from sensorimotor cortex following a combined DRL/central dorsal column lesion versus a DRL alone. The extensive sprouting from the S1 CST has not been observed previously, and these data raise important questions about S1 CST involvement in recovery following SCI.

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Bioinformatics analysis of high throughput data reveals novel pathways and mechanisms underlying tissue remodelling following spinal cord injury

Athanasios Didangelos, Katalin Bartus, Michaela Iberl, Nicholas James, Elizabeth Bradbury

Wolfson CARD, King's College London, UK

athanasios.didangelos@kcl.ac.uk

Spinal cord injury research has generally focused on the investigation of few, preselected candidate molecules and linear pathways and has largely ignored the complex, systems-wide interactions of biological networks. Spinal cord injury is characterized by aggressive tissue remodeling, dynamic changes in the extracellular matrix and a dysregulated inflammatory response. In this study, we used a systems-wide approach to investigate novel mechanisms of tissue remodelling following spinal cord injury. Advanced network analysis of microarray data from a rat spinal contusion study revealed a distinct immune response, highly interconnected with an unusual extracellular matrix signature. Modulation of the inflammatory response following spinal cord injury could facilitate tissue repair and promote neuronal plasticity. More specifically, polarization of the local and infiltrating population of macrophages to either an aggressive, cytotoxic M1 phenotype or the alternative, immunoregulatory M2 phenotype has received particular attention, influenced by paradigms from other pathologies. However, macrophage polarization following spinal cord injury is poorly understood in the rat, an animal model, which closely mimics human pathology. Therefore, in adult rat contused spinal cords we initially measured the expression of known and novel markers of macrophage polarization, as well as novel effectors of matrix remodelling, identified in the bioinformatics analysis. TaqMan qPCR and western blotting of injured spinal cords revealed a mixed polarization profile two weeks post injury. To further characterize macrophage polarization in the injured spinal cord, rat spinal contusion microarray data was analysed and the network of differentially regulated genes (1012 genes) was aligned and compared with expression networks generated from a microarray study of *in vitro* human macrophage polarization. Gene co-expression revealed that the injured rat spinal cord has a mixed polarization profile, with a shift towards M2. Given that polarization of human macrophages is extensively studied, we then stimulated human unpolarized (M0) macrophages with conditioned medium taken from injured rat spinal cord explants. The rat conditioned medium had a clear activating effect on the human M0 cells. Validation of the 42 most regulated M1 and M2 human markers indicated, once again, a mixed polarization profile. Interestingly, treatment of injured spinal cords with chondroitinase increased the expression of the M2 markers, IL10 and FN1 and decreased the expression of two M1 markers, IL12β and DDX58. Notably, IL10 and IL12β are prototypical M2 and M1 polarizing cytokines, respectively. In depth investigation of the underlying mechanisms is currently underway and future studies will include the comparison of genomics data with proteomics signatures, to characterize the accumulation and proteolytic processing of matrix proteins in the injured cord.

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Significant axon regeneration and functional outcome in chronic severe spinal cord injury following treatment with PEG

Veronica Estrada[1], Nicole Brazda[1], Christine Schmitz[1], Heinrich Blazyca[2], Rudolf Martini[2], Hans Werner Müller[1]

[1]Molecular Neurobiology Laboratory, Department of Neurology, Heinrich-Heine-University Medical Center Duesseldorf, Germany
[2]Developmental Neurbiology, Department of Neurology, University Medical Center Wuerzburg, Germany

veronica.estrada@hhu.de

Spinal cord injury (SCI) generally results in life-long severe impairments for the patients. Present research in the field of SCI largely targets acute SCI. However, the majority of SCI patients are those with chronic lesions who may benefit insufficiently from therapeutic treatments designed for acute application. Compared to treatments of acute experimental SCI the efficacy of therapies promoting axonal regeneration seems impaired in chronic models [1]. After the time period of five weeks the scar is fully developed at the injury site while the biosynthesis of most scar-associated CSPGs has subsided [2] and spontaneous behavioural recovery has reached its plateau [3].

Careful surgical resection of the scar and filling-in of scaffolding matrices into the resulting cavity could be a possible regeneration-supporting therapy for chronic SCI. We identified a suitable biomatrix that remarkably improved axon regeneration and functional outcome after partial and complete chronic spinal cord injury in rat. Five weeks after dorsal thoracic hemisection-injury the lesion scar was resected via aspiration and the resulting cavity was filled with different biopolymers such as Matrigel^{TM} (MG), alginate-hydrogel (ALG) and polyethylene glycol 600 (PEG) all of which have not previously been used as sole graft-materials in chronic SCI. Immunohistological staining revealed marked differences between these compounds regarding axon regeneration, invasion of astrocytes, fibroblasts, endothelial and Schwann cells, revascularization, and collagen deposition. According to axon regeneration-supporting effects the biopolymers could be ranked in the order: PEG >> ALG > MG. Even after complete chronic transection, the PEG-bridge allowed long-distance axon regeneration through the grafted area and for, at least, 1 cm beyond the lesion. As revealed by electron microscopy, bundles of regenerating axons within the matrix area received myelin ensheathment from Schwann cells. The beneficial effects of PEG-implantation into the resection-cavity were accompanied by long-lasting significant locomotor improvement over a period of 8 months. Following complete spinal re-transection at the rostral border of the PEG-graft the locomotor recovery was aborted, suggesting a functional role of regenerated axons in the initial locomotor improvement. In conclusion, scar resection and subsequent implantation of PEG into the generated cavity leads to remarkable tissue recovery, axon regeneration, myelination and functional improvement that have not been achieved before in severe chronic SCI.

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The K^+-Cl^- cotransporter KCC2: a good target to restore endogenous inhibition and to treat both spasticity and neuropathic pain after spinal cord injury

Gackière Florian, Bos R., Brocard C., Liabeuf S., Viallat-Lieutaud A., Boulenguez P., Vinay L.

Institut de Neurosciences de la Timone (INT) - CNRS - UMR 7289. Aix-Marseille Université. Campus Santé Timone. Marseille, France

florian.gackiere@univ-amu.fr

In healthy mature spinal motoneurons (MNs), γ-aminobutyric acid (GABA) and glycine are inhibitory neurotransmitters given the low intracellular chloride concentration maintained by the potassium-chloride outward cotransporters KCC2. Previous work of our team demonstrated that spinal cord injury (SCI) induces a down-regulation of KCC2 expression, which in turn reduces the strength of neuronal inhibition. This has been associated with the emergence of both spasticity and neuropathic pain. The goal of this study was: 1/ to determine to what extent a change in KCC2 function affects the strength of inhibition and 2/ to investigate whether a pharmacologically-induced increase of KCC2 function after SCI is able to restore endogenous inhibition and to reduce both spasticity and neuropathic pain.

Reciprocal inhibition through Ia-interneurons prevents the co-contraction of antagonist muscles. We studied the involvement of KCC2 cotransporters in reciprocal inhibition during the first postnatal week in rodents. Pharmacological blockade of KCC2 function, down-regulation of KCC2 expression following SCI as well as genetic reduction of KCC2 expression all resulted in a reduction of inhibition. This result demonstrates the involvement of KCC2 and intracellular chloride homeostasis in flexor-extensor coordination. Reduction of KCC2 expression could therefore account for the occurrence of synchronous contraction of antagonistic muscles following SCI.

KCC2 therefore appears as an attractive target to restore endogenous inhibition and thereby reduce spasticity and neuropathic pain after SCI. We looked for compounds that are able to up-regulate KCC2 function. We demonstrated that the specific activation of $5\text{-}HT_{2A}$ receptors by TCB-2 increases the expression of KCC2 in the plasma membrane of MNs, restores inhibition through a reduction of intracellular concentration of chloride ions, and reduces signs of spasticity and neuropathic pain *in vivo* in adult rats after SCI. More recently, we showed that zinc chloride also up-regulates KCC2 functionality and partially restores the strength of reciprocal inhibition *in vitro* in neonate rats that had the spinal cord transected at birth. Altogether, these results open new avenues for the treatment of both spasticity and neuropathic pain after SCI.

Differential functions of infiltrating macrophages and resident microglia after spinal cord injury

Andrew D. Greenhalgh, Samuel David

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

andrew.greenhalgh@mail.mcgill.ca

Macrophages in the injured spinal cord arise from resident microglia and infiltrating, peripherally derived monocytes. It is still not clear if macrophages derived from these two populations differ in their roles after spinal cord injury (SCI). The aim of this study was to investigate the contribution to the phagocytic response and the clearance of damaged axons by macrophages derived from resident microglia in comparison to macrophages of a peripheral, blood-borne origin. The LysM-eGFPki transgenic mouse tags haematogenous macrophages, but not microglia, and allows the study of these two previously indistinguishable cell populations without the need for chimeric experiments. We used a combination of immunofluorescence, flow cytometric and neuronal tracing techniques (using fluorescently labelled dextran to trace axons in the dorsal column) we show that microglia contact damaged axons early (24 h) after SCI and are the predominant type of macrophage to contain phagocytic material at 3 days. Thereafter, infiltrating macrophages become the predominant cell in contact with degenerating axons and contain more phagocytic material. Furthermore, after phagocytosing myelin *in vitro*, bone marrow derived macrophages are much more susceptible to apoptotic and necrotic cell death than CNS microglia. These data show that peripherally derived macrophages are the main cell type to phagocytose degenerating axons after CNS injury despite the presence of microglia, but may not be as well equipped to process the CNS material. Overall, these data highlight the differential roles played by different types of macrophages, depending on their origin, and provide further information for cell specific targeting of inflammatory response after SCI.

Tailored computer tools to deal with the complexity after CNS regeneration events

Cohen K.[1], Hunter L.[1], Baumgartner W.A. Jr.[1], Ries A.[2], Pape D.[3], **Grimpe Barbara**[3]

[1] Computational Bioscience Program, University of Colorado, Denver/Aurora CO, USA
[2] Max Planck Institute of Biochemistry, Martinsried, Germany
[3] Applied Neurobiology, Department of Neurology, Medical Center of the Heinrich Heine University, Düsseldorf, Germany

Kevin.Cohen@gmail.com, Larry.Hunter@ucdenver.edu, William.Baumgartner@ucdenver.edu, ries@biochem.mpg.de, Pape.Daniel@med.uni-duesseldorf.de, **Barbara.Grimpe@med.uni-duesseldorf.de**

Paralysis caused by spinal cord injury remains incurable despite persistent research for over a century. In due time a multitude of single factors were discovered which act somehow as promoters and inhibitors of regeneration, yet a clear scheme for the regeneration process and its failure has not emerged due to the bewildering number of possible relevant interactors and interactions. We develop two software tools adapted to this problem to deal with this complexity.

Our first tool aims to discover how the proteins and genes with relevance to regeneration interact and what they do. It consists of a computer model of protein interactions extended from a seed list of proteins with a reported role for regeneration. Then, it associates molecular and biological function extracted from natural language sources and various databases available on-line. Our prototype was able to predict influence on regeneration processes for hitherto neglected protein-protein-interactions and single proteins (Ries et al., 2007). In the end we want to obtain a comprehensive protein and gene function map with respect to nerve regeneration.

Our second tool will be an improved and refined version of the Hanalyzer (Leach et al., 2009). This program convolves empirical data with a knowledge representation in the regeneration neurobiology domain in order to predict assertions with high relevance to the empirical data. Since the whole process runs automatically it allows scanning a vast amount of possible assertions without human bias or neglect. Input empirical data will be obtained from gene microarray and high-through screenings of animal models of spinal cord injury (SCI) over time. The large regeneration-specific knowledge representation is built from several ontologies, biochemistry databases and extensive natural language text mining of publications significant to CNS paralysis.

To test hypotheses derived from promising assertions we measure the effect on neurite outgrowth and regeneration in cell culture experiments as well as in an established rat SCI contusion model in combination with a suitable mRNA-knockdown technique, called deoxyribozyme. We were able to indentify new or less investigated protein interactions and proteins with this approach. Furthermore, latest establishments in my laboratory such as training rats in treadmill exercise, provide the foundation to use interventions in animals, which has been shown to increase the quality of life of SCI patients and positively influences pain management, depression, psychological well-being and self-concept in this patient group

In sum, our international and interdisciplinary approach shows the power of systems biology in comprehending complex biological processes and might bring about new ways of treating SCI.

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AAV9-IL4 enhances reparative M2 macrophages in spinal cord but exacerbates a pathogenic systemic autoimmune response that impairs functional recovery after contusive SCI

Jodie C.E. Hall[1, 2], Kristina A. Kigerl[1, 2], Kevin D. Foust[1, 2], Alexander D. Roszman[1], Brian K. Kaspar[2,3], Phillip G. Popovich[1, 2]

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

Jodie.Hall@osumc.edu

After spinal cord injury (SCI), macrophages cause axon retraction and influence formation of the glial scar[1, 2]. The composition of activation stimuli at sites of inflammation determines the phenotype and function of macrophages. Classically activated 'M1' macrophages predominate after SCI and have neurotoxic effector functions. Conversely, alternatively activated 'M2' macrophages are not toxic and augment regenerative growth in adult sensory axons, but these cells occupy the lesion site only transiently[3]. Interleukin-4 (IL4) is a cytokine that causes newly activated macrophages to differentiate into M2 cells *in vitro*[3]. Whether IL4 also promotes M2 differentiation *in vivo* after SCI is unknown.

Injectable viral vectors engineered to produce IL4 are potential non-invasive tools for reprogramming macrophages at the injury site. Here, using two models of SCI, we test the hypothesis that gene therapy will enhance M2 macrophages at/nearby the site of SCI creating a lesion environment that is permissive for axon regeneration. Increasing M2 macrophages also is expected to reduce tissue damage and improve functional recovery.

Two hours after a mid-thoracic contusion or dorsal hemisection (dHx) SCI, female C57BL/6 mice were injected (i.v.) with an adeno-associated viral (AAV9) vector engineered to produce IL4 (AAV9-IL4; n=30) or GFP (AAV-GFP; n=30) under the control of a cytomegalovirus (CMV) promoter ($1 \times 10^{9\text{-}11}$ vg/mouse). Control groups received SCI only (n=8), laminectomy and AAV9-IL4 (n=4) or AAV-GFP (n=4) injection (1×10^{11} vg/mouse) or AAV9-IL4 (n=4), or AAV-GFP (n=4) injection only (1×10^{11} vg/mouse). Open field locomotor function was assessed regularly (weekly) for eight weeks then mice were perfused and tissues processed for western blot or histology.

Data show that AAV9-IL4 significantly increased the density of M2 macrophages in the epicenter; however, intraspinal pathology was exacerbated and functional recovery was impaired in these mice compared to those injected with control vector or SCI only. Similar results were obtained after mild (60kDyn) or moderate (75kDyn) contusive SCI. Conversely, AAV9-IL4 did not have adverse effects on uninjured mice nor did this vector exacerbate intraspinal pathology in mice receiving spinal dHx lesions. Previously, we found that contusive SCI activates pathogenic autoreactive B cells[4]. Since IL4 is a B cell growth factor, AAV9-IL4 could exacerbate a systemic autoimmune response elicited by SCI. Indeed, in contused mice injected with AAV9-IL4, splenomegaly was consistent with more intraspinal B cells and anti-CNS antibodies found in these mice. Thus, post-injury intravenous injection of AAV9-IL4 may create a more growth-permissive inflammatory environment (M2 macrophage dominant) but it also enhances pathogenic autoantibody synthesis and exacerbates functional recovery. Future work will explore intraspinal IL4 injection, use of a second-generation AAV9 vector in which IL4 production is controlled by a GFAP promoter (limit synthesis to CNS) and evaluate axon/glial interactions within the M2 dominant lesion foci of AAV9-IL4 injected mice.

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Histone modifying enzymes play a role as regulators of regenerative gene expression in DRG neurons

Arnau Hervera, Radhika Puttagunta, Luming Zhou, Simone Di Giovanni

Department of Medicine, Burlington Danes Building, Imperial College London, London, UK

hervera@gmail.com

Lack of a regenerative glial environment and of a neuronal intrinsic regenerative gene expression program compromise axonal regeneration after injury in the central nervous system. However, regenerative gene expression, mainly characterized by the induction of regeneration-associated genes (RAGs), is activated after regenerative PNS injury, including in dorsal root ganglia (DRG) neurons. Pseudounipolar sensory DRG neurons, have a central and a peripheral axonal branch belonging to the same cell body, with opposite regenerative capacity. Moreover, lack of regeneration of the injured DRG central axon in the spinal cord can be reversed by an injury to the corresponding peripheral branch (conditioning lesion) of the those DRG neurons. Interestingly, this conditioning lesion induces the expression of RAGs[1,2,3] and some level of axonal regeneration in DRG central axons, which can be achieved by overexpressing selected RAGs in those DRG neurons after spinal cord injury only[2, 3].

In search for molecular mechanisms that may rule this shift in injury induced-expression programs, we hypothesized that epigenetic modifications may play a role as master regulators. In fact, epigenetic marks including histone post-translational modifications, transduce extracellular signaling into intracellular control of gene expression. Epigenetic changes could therefore activate gene expression of RAGs only in the presence of pro-regenerative signaling following peripheral but not central damage. Consequently, identification and modulation of key epigenetic players may regulate and induce the regenerative program in non-regenerative conditions.

To this end, we employed a systematic expression analysis and screening of epigenetic regulators, with emphasis on histone modifications and histone modifying enzymes (HMEs), in DRG neurons during neurite outgrowth both in culture and *ex vivo*. By using pharmacological and genetic inhibitors we modulated the activity of key HMEs and monitored regenerative changes in DRG neurons.

Accordingly, we found that the specific patterns of histone modifications occur with regenerative gene expression after peripheral axonal lesion only. Mechanistically, inhibition of the histone acetyltransferases p300-CBP/PCAF, hinders the conditioning effect by decreasing both injury-induced outgrowth and RAG expression. Similarly, inhibition of HDAC1/2 leads to a decrease in the outgrowth of cultured DRG neurons both *in vitro* and *ex vivo*.. Moreover, we also analyzed the effect on the inhibition of other HMEs that seem to be involved in the inhibition of the regenerative program, such as SUV39-H1 and LSD1.

In summary, our results show a clear link between conditioning induced regenerative signaling pathways and epigenetic transcriptional regulation of RAGs, whose modulation may lead to axonal regeneration in spinal cord injuries.

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

Thomas H. Hutson[1,*], Lawrence D.F. Moon1[,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], and Mary Bartlett Bunge[2]

[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. Our aim is to enhance the intrinsic growth state of CNS neurons by over-expressing genes that enhance regeneration, enabling the neurons to overcome the growth-inhibitory environment and increase axon regeneration. 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.* non-regenerating 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 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 *Ptpn2*-2A-*eGFP* or *mCherry*-2A-*eGFP* and are currently testing these in models of CNS injury. *Ptpn2* is a novel potential target for promoting axon regeneration after spinal cord injury.

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Gene delivery of chondroitinase ABC promotes functional repair following spinal contusion injuries

Nicholas D. James, Katalin Bartus, Athanasios Didangelos, Karen D. Bosch, Jessie Shea, Merrick Strotton, 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, 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 number of spinal contusion injury models. Contusion injury represents the most common form of spinal cord injury in patients, this model therefore provides a clinically relevant tool for assessing the efficacy of potential therapeutic interventions. Adult rats received either a moderate cervical (225 kdyne at C5), moderate thoracic (150 kdyne at T10) or more severe thoracic (225 kdyne at T10) contusion injury and then 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 long distance sensory fibre conduction through the injury site, as well as a significant improvement in performance on the horizontal ladder test and modest improvements in forelimb grip-strength. In addition a further electrophysiological technique highlighted a trend towards improved corticospinal tract function following delivery of LV-ChABC. Functional recovery was associated with significantly reduced pathology, modulation of the inflammatory response and enhanced plasticity. Thus, here we demonstrate the potential advantages of gene delivery of ChABC for achieving sustained and widespread CSPG degradation and that this is associated with both anatomical and functional improvements following contusion injury.

Peripheral delivery of recombinant human Neurotrophin-3 for spinal cord injury

Claudia Kathe, Thomas Hutson, Stephen McMahon, Lawrence Moon

Neurorestoration Department, Wolfson CARD, Guy's Campus, King's College London, London, UK

claudia.kathe@kcl.ac.uk

Background: Spinal Cord Injury is a debilitating condition that can result in varying degrees of paralysis due to immediate axotomy and neuronal loss. Neurotrophin-3 (NT-3) has been shown to facilitate neuronal sprouting, plastic changes and functional recovery in animal models of CNS injuries. Our goal is to use NT-3 to increase the amount of axonal sprouting and functional recovery, which occurs to a limited degree spontaneously after SCI.

Aims: Our first aim is to test the intramuscular delivery of NT-3 after SCI in rodent models. We hypothesise that NT-3 leads to sprouting of intact corticospinal tract fibers across the midline of the spinal cord after unilateral injuries, which will facilitate functional recovery. Our second aim is to establish mechanisms of NT-3 action. We want to address multiple questions about the neuronal uptake, transport and downstream activation of NT-3.

Results: We are developing new electrophysiological techniques to monitor functional recovery after SCI over multiple time-points, which involves using direct cortical stimulation of the rat's brain. Secondly, we have developed two AAV vectors expressing a tagged NT-3 variant, which we will use in different models of CNS injury. We are verifying NT-3 expression and functionality of these AAV vectors *in vitro*.

Conclusions: The techniques we have developed will allow us to design and conduct *in vivo* studies, which will allow us to answer our hypothesis that intramuscular delivery of NT-3 is a beneficial therapy after SCI and has the potential to be translated into a clinical application.

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Optical Mark Recognition – a novel method of documenting ASIA impairment score

Sreedhar Kolli, Swaroop Shanbhag

Welsh Spinal Cord Injury Rehabilitation Centre, Rookwood Hospital, Fairwater road, Llandaff , Cardiff, Wales, UK

drkollisridhar2@hotmail.com

Assessment of Spinal Injury by serial neuro-charting is essential to demonstrate progress or neurological recovery in a patient. In the rehabilitation setting, accurate documentation is important for ASIA charting to be meaningful and useful. A “traditional” paper based ASIA chart requires manual entry of the scores into an Excel or similar programme in order to allow any subsequent processing. As well as being time consuming, manual entry is prone to errors. Digital smart pen, optical character recognition or internet based tools can also be used but are not without limitations.

We have devised an Optical Mark Recognition (OMR) ASIA chart to simplify data entry of the ASIA impairment score.Optical mark recognition is the scanning of paper to detect presence or absence of a mark in a predetermined position-a process all of us are familiar as a result of our experiences with the dreaded MCQ answer sheets.

The use of an OMR-ASIA chart as an input device for data entry along with purpose-designed software simplifies Serial Neurocharting.OMR allows subsequent efficient and accurate Data extraction for patient care and research purposes. The process is simple, cost-effective, and reproducible and utilizes normal paper, an ordinary scanner and a pen or HB Pencil.

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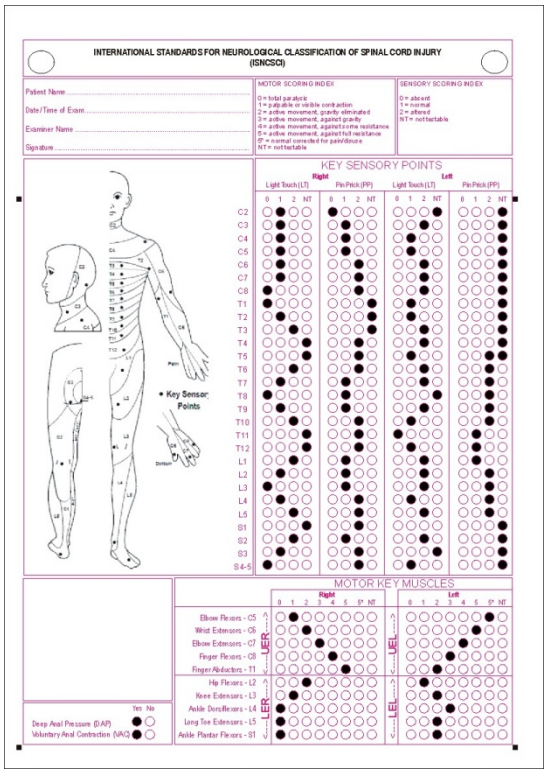

Effects of ω3-polyunsaturated fatty acids in a rodent model of cervical spinal cord injury

Zhuo-Hao Liu, Ping K. Yip, Adina T. Michael-Titus, John V. Priestley

Centre for Neuroscience and Trauma, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

z.liu@qmul.ac.uk

Recent epidemiological data indicate that 51% of spinal cord injury (SCI) patients have injuries of the cervical spine, with the most common neurological level being C5, followed by C4 and C6. Some degree of neurological recovery does occur after SCI in both humans and rodents, particularly after partial, incomplete injuries. Enhancing the plasticity responsible for this recovery is an important target of efforts to promote functional recovery. In this study, we developed a cervical SCI model comprising a hemisection lesion applied at the C4-5 level of the rat spinal cord, and tested the effects of an acute treatment with 250nmole/kg docosahexaenoic acid (DHA) delivered intravenously 30 minutes after injury. DHA has been shown to be neuroprotective in rodent thoracic SCI (Huang et al., 2007; Lim et al., 2013). One week after SCI rats received injections of biotinylated dextran amine (BDA) in the ipsilateral sensorimotor cortex.

Recovery of function was followed for 3 weeks using sensitive behavioural tests (staircase test, open field test, footprint test, and grid exploration test). In addition the cervical spinal cord was harvested for immunohistochemical analysis of the extent of the lesion and the organisation of neuronal pathways after injury (labelled generically for neurons (NeuN), synaptic terminals (synaptophysin), and serotonergic fibers (5-HT)). Corticospinal tract projections were identified based on anterograde transport of BDA. The behavioural tests showed substantial spontaneous recovery of crude forelimb function, but a long-lasting deficit in fine forelimb function. DHA treated animals showed improved functional recovery, especially in the staircase and grid exploration tests. At the lesion site, DHA treated animals had increased survival of neurons. Caudal to the injury, DHA treated animals showed increased numbers of BDA labelled axons ipsilateral to the lesion, increased staining for 5-HT and synaptophysin, and increased numbers of perisomatic contacts on motoneurons.

Our data confirms in another SCI paradigm that DHA treatment has neuroprotective effects, and indicates that it may also promote neuronal plasticity. Effects on neuronal circuitry deserve further investigation.

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Acute treatment with VEGF and PDGF preserves function after spinal cord contusion

Ben Goss[1], Fatemeh Chehrehasa[1], Mitchell Cobcroft[1], Will Young[1], **Alan Mackay-Sim**[2]

[1] Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane QLD, Australia
[2] Eskitis Institute for Drug Discovery, Griffith University, Brisbane QLD, Australia

a.mackay-sim@griffith.edu.au

Sustained inflammation of the spinal cord after traumatic spinal cord injury leads to destruction of healthy tissue. This secondary degeneration is more damaging than the initial physical damage and is a major contributor to permanent loss of functions. In our previous study we showed that combined delivery of two growth factors, vascular endothelial growth factor and platelet-derived growth factor, significantly reduced secondary degeneration after hemi-section injury of the spinal cord in the rat (Lutton et al, 2011). In those experiments, acute 1d or 7d growth factor treatment reduced the size of the lesion cavity at 30d compared to control animals. In treated animals the lesion cavity was reduced further at 90d whereas in control animals the lesion cavity was larger than at 30d. Growth factor treatment reduced astrogliosis and reduced macroglia/macrophage activation around the injury site. Treatment with individual growth factors alone had similar effects to control treatments. These observations led us to conclude that combined growth factor treatment prevented secondary degeneration by altering the inflammatory processes after spinal cord injury. The aim of the present study was to investigate whether growth factor treatment would improve locomotor behaviour after spinal contusion injury. After acute spinal cord contusion using the Infinite Horizons Impacter, the growth factors were delivered for the first 7d to the site of injury via osmotic minipump. Locomotor behaviour was monitored at 1 - 28d after injury using the BBB score and at 30d using Digigait automated gait analysis. Growth factor treatment improved locomotor behaviour to near normal levels (Control: BBB=10; Treated: BBB=18). Histological analysis confirmed that the treated animals had significantly reduced lesion cavities. We conclude that growth factor treatment preserved the spinal cord tissues after contusion injury, thereby allowing functional recovery.

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Wnt and the GSK3β inhibitor lithium differentially regulate astrogliosis *in situ*

Andrea D. Rivera, Arthur M. Butt

Institute of Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Science, University of Portsmouth, UK

Andrea.rivera@port.ac.uk

Following CNS injury, astrocytes react to form the glial scar, a physical and chemical barrier that protects the spreading of degeneration from the injury site, but which inhibits axon regeneration in the CNS. The mechanisms regulating glial scar formation are unresolved. Glycogen synthase kinase 3-β (GSK-3β) is the target of multiple receptor-mediated pathways that regulate cell proliferation, survival and differentiation. Here, we have examined the effects of GSK-3β signalling on astrogliosis in the optic nerve from transgenic mice in which GFAP drives the expression of green fluorescent protein. All procedures were in accordance with the Animal Scientific Procedures Act (1986). Optic nerves (ONs) aged postnatal day (P) 35 were isolated with the retina intact and maintained in organotypic culture for 3 days *in vitro* (DIV), in normal medium or medium containing the non-specific GSK-3β inhibitor Lithium Chloride, or Wnt3a, which acts via the canonical Wnt pathway to inhibit GSK-3β and activate β-catenin. Both agents significantly increased the number of astrocytes compared to controls ($p<0.05$, unrelated t-tests), but had differential morphogenic effects. Lithium resulted in the generation of novel astrocytes with a radial morphology, which very densely filled the nerve, whereas astrocytes developed a very simple morphology following treatment with Wnt3a. These newly generated populations of astrocytes were unlike reactive or 'scar' astrocytes in the optic nerve. To examine this further, we performed genome wide microarray to compare the genes affected by Lithium and Wnt3a with those known to be associated with reactive astroglia. Two key findings were that lithium down-regulated Bmp7, which is known to be elevated in astrogliosis and important in the cell fate specification of astrocyte like stem cells, whereas Wnt3a upregulated the expression of NG2, a potent inhibitor of axon regeneration. In addition, we identified a number of microglial genes amongst the top 10 regulated by lithium, including the novel activated microglia/macrophage WAP domain protein (AMWAP) and Csf2, which regulate proinflammatory microglia activation and are potential therapeutic targets in neurodegeneration. Pathway analysis (IOA, Ingenuity Systems) indicated Axon Guidance Signalling as one of the major pathways significantly altered by lithium, with significant effects on ephrin and semaphorin signaling, key regulators of axons growth and duidance in the optic nerve. The results provide evidence that GSK-3β and Wnt differentially regulate astrogliosis and that lithium regulates gene pathways important in CNS injury and degeneration/regeneration.

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Neural progenitor transplantation improves diaphragm function following cervical spinal cord injury

Spruance Victoria M., Sanchez D.E., Grossl G.B., Meola D.M., O'Steen B.E., Lane M.A.

Drexel University, College of Medicine, Department of Neurobiology & Anatomy, Philadelphia, PA, USA

victoria.spruance@gmail.com

Cervical spinal cord injuries (SCI) result in many devastating functional deficits, among which impaired breathing is one of the most debilitating. These injuries are not only life threatening but give rise to high costs of medical care, often exceeding $1 million in the first year alone. This is an important consideration given that the majority of human injuries occur at mid-cervical levels and there is an increased frequency of spinal injuries at higher cervical levels in recent years. Accordingly, there is an urgent need for therapeutic strategies targeted at improving respiratory function following SCI. While there is experimental and clinical evidence for spontaneous respiratory recovery following a cervical injury, the extent of lasting recovery is limited and significant deficits persist. Though the anatomical substrates that contribute to functional plasticity following SCI are not well defined, spinal interneurons have been identified as a key component of post-injury plasticity and can contribute to the formation of new anatomical pathways. The central hypothesis of this research is that spinal interneurons are a key therapeutic target for respiratory recovery following mid-cervical SCI. The goal of the present work is to harness the neuroplastic potential of these cells and test whether intraspinal transplantation of interneuronal precursors can promote the establishment of a novel circuitry capable of enhancing respiratory recovery.

Adult, female Sprague Dawley rats (n=25), received a lateral C3/C4 contusion injury using the Infinite Horizon pneumatic impactor (200KD). One week post-injury the injury site was re-exposed and dissociated fetal spinal cord (FSC; obtained from E13.5 rats) tissue was injected into the contusion cavity. After a one month recovery period, a retrograde, transneuronal tracer (pseudorabies virus, PRV) was either applied to the ipsilateral hemidiaphragm (n=8) or injected into the transplant (n=10). Animals were perfuse-fixed 72 hours post-PRV delivery. A subset of animals underwent terminal diaphragm EMG recordings to analyze function during 1) spontaneous eupneic breathing and 2) respiratory challenge, induced by exposure to hypoxia or hypercapnia. Spinal cord and brainstem tissues were immunohistochemically labeled for the presence of PRV, c-fos, tyrosine hydroxylase (TH), serotonin (5HT), as well as GABAergic and glutamatergic activity.

Terminal EMG recordings revealed enhanced diaphragm activity in animals that received FSC transplants, particularly in response to respiratory challenge. PRV immunohistochemistry revealed that transplanted neurons were synaptically integrated with the host phrenic circuitry. In addition, host spinal cord (cervical and thoracic) and brainstem neurons innervated donor tissues. Ongoing quantitative analysis will be used to expand upon these findings. Extensive c-fos staining was seen throughout transplanted tissue suggesting that many donor neurons are active. 5HT and TH labeled axons, as well as TH positive neurons, were detected within the transplant. These results suggest that transplantation of neural progenitors can facilitate improved respiratory function following cervical spinal cord injury.

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Combinatorial treatment of acute spinal cord injury with ghrelin, ibuprofen, C16 and ketogenic diet does not result in improved histologic or functional outcome

F. Streijger*, J.H.T Lee*, G.J. Duncan, M.T.L. Ng, P. Assinck, T. Bhatnagar, W.T. Plunet, **Wolfram Tetzlaff**, B. K. Kwon

International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Vancouver, BC, Canada

tetzlaff@cord.ubc.ca

Individual treatments that aim to modulate the inflammatory response, facilitate angiogenesis, and promote axonal regeneration represent appealing therapeutic approaches for spinal cord injury (SCI). However, due to the complex, multifaceted nature of SCI, it is widely believed that a combination of such approaches will be superior to individual treatments. Under this context, we employed a rat model of cervical SCI to evaluate the combination of four minimally invasive treatments that individually have been reported to be effective for acute SCI during clinically relevant therapeutic windows. These treatments included ghrelin, ibuprofen, C16 and ketogenic diet (KD); selected not only because of their previously reported efficacy in SCI models, but also for their potentially different mechanisms of action.

Administration of ghrelin, ibuprofen, C16 and KD several hours to days post-injury was based on previous observations by others that each treatment has profound effects on the pathophysiology and functional outcome following SCI. In the current study, we showed that except for a modest improvement observed in the Montoya staircase test at 8-10 weeks post-injury, combinatorial treatment of ghrelin, ibuprofen, C16 and KD did not result in any substantial improvements in rearing test, grooming test, or horizontal ladder. Histologic analysis of the spinal cords did not reveal any significant differences in tissue sparing between the treatment and control groups.

While single approaches of ghrelin, ibuprofen, C16, and KD have been reported to be beneficial after SCI; our results show that combination of the four interventions did not promote robust recovery after a cervical model of SCI. Possible differences in the injury models previously used for the efficacy of these treatmtnets as well as interactions between the treatments that may have negated their beneficial effects, might explain these negative results. This emphasizes the challenges that need to be addressed with combinatorial drug therapy for SCI.

Blockade of interleukin-6 signaling improves the survival rate of transplanted bone marrow stromal cells and increases locomotory function in mice with spinal cord injury

Kenzo Uchida[1], Hideaki Nakajima[1],Ying Tan[2], Shuji Watanabe, MD[1], Makoto Kitade[1], William E.B. Johnson[3], Hisatoshi Baba[1]

[1]Department of Orthopaedics and Rehabilitation Medicine, Faculty of Medical Sciences, University of Fukui, Fukui, Japan
[2]Department of Spine Surgery, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China
[3]Life & Health Sciences, Aston University, Birmingham, UK

kuchida@u-fukui.ac.jp

Bone marrow stromal cells (BMSCs) have the potential to improve functional recovery in spinal cord injury (SCI) [1)] ; however, they are limited by low survival rates after transplantation in the injured tissue. Our objective was to clarify the effects of a temporal blockade of IL-6/ IL-6 receptor (IL-6R) engagement, using an anti-mouse IL-6R monoclonal antibody (MR16-1) [2)] on the survival rate of BMSCs after their transplantation in mice contusion SCI. MR16-1 co-treatment improved the survival rate of transplanted BMSCs, allowing some BMSCs to differentiate into neurons and astrocytes, and improved locomotory function recovery compared to BMSC transplantation or MR16-1 treatment alone. The death of transplanted BMSCs could be mainly related to apoptosis but not necrosis. BMSC transplantation with co-treatment of MR16-1 was associated with: (i) decrease of some pro-inflammatory cytokines; (ii) increase of neurotrophic factors; (iii) decreased apoptosis rates of transplanted BMSCs; and (iv) enhanced expression of the survival factors, Akt and extracellular signal-regulated protein kinases (ERK) 1/2. We concluded that MR16-1 treatment combined with BMSC transplants helped to rescue neuronal cells and axons after contusion SCI better than BMSCs alone, by modulating both the inflammatory/immune responses and decreasing apoptosis.

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The interaction of cortico-spinal pathways and sacral sphincter reflexes in subjects with incomplete spinal cord injury (iSCI)

Natalia Vasquez[1], Balasubramaniam V[1], Kuppuswamy A[3], Knight S[1], Susser J[1], Ellaway PH[1,3], Craggs MD[1,2.]

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

michael.craggs@ucl.ac.uk

Introduction: The bladder guarding reflex is a response which helps to maintain continence by increasing tone in the striated urethral sphincter as the bladder fills. It is aberrant in iSCI, with detrusor-sphincter dyssynergia[1], but the expression of these disordered reflexes cannot be predicted exactly by the nature and level of neurological level of SCI[2]. Inconsistency in presentation of disordered pelvic function in iSCI make further research into the underlying neural mechanisms desirable if suitable treatments to recover function are sought. One practical proposal for the study of the interaction between reflex and voluntary control of urinary continence is to substitute the pudendo-anal reflex (PAR) as a surrogate marker for the urethral sphincter guarding reflex[3], and this can coupled with cortical stimulation to elicit a motor evoked potential in the external anal sphincter[4]. The interaction between pudendal afferent input and corticospinal drive to the external anal sphincter has not been studied. Here we present the results from conditioning the PAR by prior transcranial magnetic stimulation (TMS) of the motor cortical representation of the anal sphincter muscle in iSCI subjects who have a neurogenic bladder.

Methods: Three neurologically normal subjects and twenty-six subjects with incomplete, supra-sacral spinal cord injuries and symptoms of a neurogenic bladder were recruited. Incontinence was assessed using the International Consultation on Incontinence Modular Questionnaire (ICIQ). Electromyographic activity of the external anal sphincter was recorded. The pudendo-anal reflex (PAR) was elicited by electrical stimulation of the dorsal penile nerve (DPN). Motor cortical excitation was achieved using transcranial magnetic stimulation (TMS).

Results: Preliminary findings in normal and iSCI subjects showed facilitation of the PAR by prior TMS with an optimal interval of 20-40ms. Of 23 iSCI subjects, 12 showed facilitation to TMS applied 30ms before DPN stimulation. Eight of the 12, and a further 5 iSCI subjects, had an anal sphincter MEP in response to TMS alone. There was a weak tendency (r^2 = 0.22, P=0.03) for those with higher ICIQ values to have larger PAR responses but no significant difference in ICIQ scores between those with (ICIQ = 4.9 ±4.0 mean ±SD) and those without (ICIQ = 7.2 ±4.7) cortical facilitation of the PAR.

Conclusion: Cortical TMS is effective in facilitating the PAR in some iSCI subjects.

Our long-term goal is to determine whether residual elements of cerebral control of pelvic function can be enhanced by neuromodulation or activity-based therapies through manipulation of the inherent neuro-plasticity of the brain and spinal cord in chronic iSCI. The aim of this preliminary study therefore was to assess the potential for application of plasticity inducing regimes based on repetitive transcranial magnetic stimulation or paired associative stimulation[5] of pudendal afferents and motor cortex in iSCI.

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Rescuing the diaphragm from paralysis: the roles of serotonin and chondroitinase ABC in promoting the activity of injured pathways following cervical spinal cord contusion

Philippa M. Warren, Basem I. Awad, Kevin C. Hoy, Davina V. Gutierrez, Warren J. Alilain

Department of Neurosciences, Metrohealth Medical Center and Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

warren.alilain@case.edu

There is a relative paucity of studies investigating cervical contusion injuries and the resulting respiratory motor deficits. The frustrating lack of data is further underscored by the fact that the majority of spinal cord (SC) injuries are at the cervical level and those afflicted usually have a higher morbidity and mortality related to respiratory insufficiency. Limiting these kinds of studies is that producing cervical contusion injuries with robust respiratory motor deficits (i.e. diaphragmatic paralysis or impairment) comes with dramatically reduced post-injury survival. Therefore, studies have resorted to hemisecting or mildly contusing the spinal cord resulting in subtle breathing deficits. However, such models do not reflect the injuries which exist within the extant human SC injury population. To counteract this, we recently devised a new cervical contusion injury model with which we can investigate potential therapeutic strategies aimed at repairing and promoting the activity of injured pathways and rescuing the diaphragm from a paralytic fate. In our new model we hemicontuse the animal at C3/4 and, at a later post-contusion time point, we perform a contralateral hemisection so that the animal depends solely on these injured pathways to breathe. We find that without intervention, the animal cannot wholly depend on contused pathways to produce diaphragmatic activity. Correlating with this profound respiratory motor deficit is a significant decrease of the neuromodulator serotonin (5-HT) and upregulation of inhibitory chondroitin sulfate proteoglycans (CSPGs) ipsilateral to the contusion and at the level of the phrenic motor nucleus. Therefore, we hypothesize that exogenous 5-HT application or treatment with chondroitinase ABC (ChABC), which degrades CSPGs and can increase endogenous 5-HT, can facilitate activity of injured respiratory motor pathways. Indeed, our preliminary experiments show that either exogenous administration of 5-HT or direct spinal injections of ChABC directly promotes activity of respiratory motor pathways injured by contusion and rescues the diaphragm from paralysis. Additionally, we find that ChABC administration increases endogenous 5-HT expression after injury. Overall, our exciting preliminary results suggest that injured bulbospinal pathways can be made more effective in the presence of 5-HT. Further to this, treatment with ChABC is able to effectively improve or remedy the contused cervical SC leading to significant functional activity of the diaphragm.

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Investigation of paracrine factors mediating mesenchymal stem cells-induced functional recovery after rat spinal cord injury

Akihito Yamamoto, Kohki Matsubara, Fumiya Kano, Minoru Ueda

Nagoya University Graduate School of Medicine, Japan

akihito@med.nagoya-u.ac.jp

Stem-cell transplantation therapy holds great promise for treating SCI. Previous studies has shown that various types of stem cells and their derivatives, when transplanted into the injured spinal cord in animal SCI models, promote substantial functional recovery through cell-replacement and tropic/paracrine mechanisms. However, most studies have reported poor differentiation and survival of the engrafted stem cells in severe acute or sub-acute SCI, suggesting that engrafted stem cells may primarily promote neuroregeneration by paracrine mechanisms.

Human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) are self-renewing mesenchymal stem cells residing within the perivascular niche of the dental pulp. They are thought to originate from the cranial neural crest, expresses early markers for both mesenchyme and neuroectodermal stem cells and are able to differentiate into the functional neurons and oligodendorocytes under appropriate conditions. Studies have reported that engrafting these pulp stem cells promote functional recovery from various types of acute and chronic CNS insult through paracrine mechanisms that activate endogenous tissue-repairing activities [1-6].

Here we show that the intrathecal administration of conditioned serum-free medium (CM) from SHED, but not bone marrow (BMSC) or fibroblast, into the severely injured adult rat SCs led to a marked recovery of hindlimb locomotor function. SHED-CM treatment inhibited SCI-induced apoptosis, preserved neural fibres and myelin sheaths, and promoted the growth of the descending 5-HT^+ axons. Importantly, we show here that these neuroregenerative activities were supported by a marked immunoregulatory function of SHED-CM, by which the pro-inflammatory M1 microglia/macrophages were directly converted to the anti-inflammatory/tissue repairing M2.

Furthermore, based on the differential secretomic analysis between SHED-CM and BMSC-CM, we identified a novel set of M2-inducer, ChemokineA and LectinB in SHED-CM, which acted synergistically with endogenous chondroitin sulphate proteoglycans to induce M2 circumstance. Administration of ChemokineA/LectinB into the acute rat SCI reduced tissue distraction, preserved 5-HT^+ axons beyond the epicenter and led to a significant functional recovery. SCI-rat receiving ChemokineA/LectinB-depleted SHED-CM failed to induce M2 and exhibited subtle recovery. LectinB physically interacted with the ChemokineA receptor in sialic acid dependent manner and activate M2 inducing program. CSPG played an essential role in the activation of ChemokineA receptor expression on native mouse microglia. Thus, taken altogether these results demonstrate that pulp stem cells secrete a previously uncovered novel set of M2 inducer that generated an anti-inflammatory/tissue-regenerative condition for functional recovery from SCI

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First Name	Last Name	Institution	Email Address
Francia Carolina	Acosta Saltos	University College London	francia.acosta-saltos.09@ucl.ac.uk
Louise	Adams	Queen Mary, University of London	louise_adams24@hotmail.co.uk
Warren	Alilain	Case Western Reserve University	wja4@case.edu
Yazi	Al'Joboori	University of Leeds	y.aljoboori@hotmail.co.uk
Patrick	Anderson	University College London	p.anderson@ucl.ac.uk
Callan	Attwell	Netherlands Institute for Neuroscience	c.attwell@nin.knaw.nl
Mark	Bacon	ISRT	mark@spinal-research.org
Stuart	Baker	Newcastle University	stuart.baker@newcastle.ac.uk
Sue	Barnett	University of Glasgow	Susan.Barnett@glasgow.ac.uk
Katalin	Bartus	King's College London	katalin.bartus@kcl.ac.uk
Etienne	Baulieu	MAPREG SAS	etienne.baulieu@inserm.fr
Catherina	Becker	University of Edinburgh	catherina.becker@ed.ac.uk
Murray	Blackmore	Marquette University	murray.blackmore@marquette.edu
Armin	Blesch	UniversitätsKlinikum Heidelberg	Armin.Blesch@med.uni-heidelberg.de
Andrew	Blight	Acorda Therapeutics Inc.	ablight@acorda.com
Xuenong	Bo	Queen Mary, University of London	x.bo@qmul.ac.uk
Liz	Bradbury	King's College London	elizabeth.bradbury@kcl.ac.uk
Nicole	Brazda	Düsseldorf University	nicole.brazda@uni-duesseldorf.de
James	Brock	University of Melbourne	j.brock@unimelb.edu.au
Emily	Burnside	King's College London	emily.burnside@kcl.ac.uk
Arthur	Butt	University of Portsmouth	arthur.butt@port.ac.uk
Thomas	Carlstedt	NSIC - Stanmore	carlstedt.thomas@googlemail.com
Darren	Carwardine	University of Bristol	darren.carwardine@gmail.com
Samit	Chakrabarty	University of Leeds	s.chakrabarty@leeds.ac.uk
Andrew	Collins	University College London	Andrew.Collins@ucl.ac.uk
Brendan	Collins	University College London	brendan.c.collins@gmail.com
Bernie	Conway	University of Strathclyde	b.a.conway@strath.ac.uk
Corinna	Darian-Smith	Stanford University School of Medicine	cdarian@stanford.edu
Athanasios	Didangelos	King's College London	athanasios.didangelos@kcl.ac.uk
Wagih	El Masry	NSIC - Oswestry	Heather.Edwards@rjah.nhs.uk
Peter	Ellaway	Imperial College London	p.ellaway@imperial.ac.uk
Veronica	Estrada	Düsseldorf University Clinics	estrada@uni-duesseldorf.de
Andreas	Faissner	Ruhr-University	andreas.faissner@ruhr-uni-bochum.de
James	Fawcett	Cambridge University Brain Repair Centre	jf108@cam.ac.uk
Adam	Ferguson	University of California, San Francisco	Adam.Ferguson@ucsf.edu
Karim	Fouad	University of Alberta	karim.fouad@ualberta.ca
Robin	Franklin	University of Cambridge	rjf1000@cam.ac.uk
Florian	Gackiere	CNRS & Aix Marseille Université	florian.gackiere@univ-amu.fr
Isabella	Gavazzi	King's College London	isabella.gavazzi@kcl.ac.uk
Nicolas	Granger	University of Bristol	nicolas.granger@bristol.ac.uk
Andy	Greenhalgh	McGill University	adgreenhalgh@gmail.com
Barbara	Grimpe	Heinrich Heine University Düsseldorf	Barbara.Grimpe@med.uni-duesseldorf.de
James	Guest	University of Miami	jguest@med.miami.edu
Jodie	Hall	Ohio State University	jodie.hall@osumc.edu
Barbara	Hanzi	King's College, London	barbara.haenzi@gmail.com

Susie	Harkema	University of Louisville	susanharkema@kentuckyonehealth.org
Arnau	Hervera Abad	Imperial College	hervera@gmail.com
John	Hick	ISRT	jwahick@waitrose.com
Thomas	Hutson	King's College London	thomas.hutson@kcl.ac.uk
Michaela	Iberl	King's College London	michaela.iberl@kcl.ac.uk
Ronaldo	Ichiyama	University of Leeds	R.M.Ichiyama@leeds.ac.uk
Tadashi	Isa	National Institute for Physiological Sciences	tisa@nips.ac.jp
Lyn	Jakeman	The Ohio State University	Jakeman.1@osu.edu
Nicholas	James	Kings College, London	nicholas.n.james@kcl.ac.uk
Jacqueline	Jeruss	Northwestern University	j-jeruss@northwestern.edu
Kristjan	Jessen	University College London	k.jessen@ucl.ac.uk
Eustace	Johnson	Aston University	w.e.johnson@aston.ac.uk
Jon	Kaas	Vanderbilt University	jon.h.kaas@Vanderbilt.Edu
Danial	Kahani	University of Strathclyde	danial.kahani@strath.ac.uk
Claudia	Kathe	King's College London	claudia.kathe@kcl.ac.uk
John	Kessler	Northwestern University	jakessler@northwestern.edu
Dr Marilyn	Kessler	Northwestern University	m-kessler@northwestern.edu
Makoto	Kitade	University of Fukui	makoto8@u-fukui.ac.jp
Isabel	Koh	University College London	isabel.koh.10@ucl.ac.uk
Sreedhar	Kolli	NSIC - Rookwood	drkollisridhar2@hotmail.com
Timea	Konya	ISRT	timea@spinal-research.org
Michael	Lane	Drexel University	mlane.neuro@gmail.com
Stuart	Law	University College London	stuart.law@ucl.ac.uk
Rosi	Lederer	Wings for Life	rosi.lederer@wingsforlife.com
Vance	Lemmon	University of Miami	vlemmon@med.miami.edu
Zhuo-Hao	Liu	Barts and The London School	z.liu@qmul.ac.uk
Ann	Logan	University of Birmingham	a.logan@bham.ac.uk
Alan	Mackay-Sim	Griffith University	a.mackay-sim@griffith.edu.au
Martin	Marsala	University of California San Diego	mmarsala@ucsd.edu
Matthew	Mason	Netherlands Institute for Neuroscience	m.mason@nin.knaw.nl
Kouki	Matsubara	Nagoya University	k.matsu@med.nagoya-u.ac.jp
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
Liz	Muir	University of Cambridge	emm1@mole.bio.cam.ac.uk
Hans Werner	Müller	University of Düsseldorf	hanswerner.mueller@uni-duesseldorf.de
Karen	Oprych	University College London	k.gladwin@ucl.ac.uk
Moreno	Pasin	University College London	moreno.pasin01@libero.it
Giles	Plant	Stanford University	gplant@stanford.edu
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	University College London	g.raisman@ion.ucl.ac.uk
Gena	Raivich	University College London	G.Raivich@ucl.ac.uk
Leanne	Ramer	King's College London	Leanne@ICORD.org
Matthew	Ramer	ICORD	ramer@icord.org
Paul	Reier	University of Florida	reier@mbi.ufl.edu

Peter	Richardson	London Clinic	p.richardson@qmul.ac.uk
John	Riddell	University of Glasgow	j.riddell@bio.gla.ac.uk
Andrea	Rivera	University of Portsmouth	andrea.rivera@port.ac.uk
John	Rogers	University of Cambridge	jhr11@cam.ac.uk
Jane	Roskams	University of British Columbia	roskams@zoology.ubc.ca
Martin	Schwab	University of Zurich	schwab@hifo.uzh.ch
Jan	Schwab	Charité Hospital - Humboldt University Berlin	jan.schwab@charite.de
Lonnie	Shea	Northwestern University	l-shea@northwestern.edu
Peter	Shortland	University of Western Sydney	p.j.shortland@qmul.ac.uk
Victoria	Spruance	Drexel University	Victoria.spruance@gmail.com
Os	Steward	University of California Irvine School of Medicine	osteward@uci.edu
Patrick	Stroman	Queen's University	stromanp@queensu.ca
Merrick	Strotton	King's College London	merrick.strotton@kcl.ac.uk
Wolfram	Tetzlaff	ICORD	tetzlaff@icord.org
Chinnaya A	Thiyagarajan	NSIC - Stoke Mandeville	catrajan@btinternet.com
Irene	Tracey	University of Oxford	irene.tracey@ndcn.ox.ac.uk
Mark	Tuszynski	University of California, San Diego	mtuszynski@ucsd.edu
Kenzo	Uchida	University of Fukui	kuchida@u-fukui.ac.jp
Natalia	Vasquez	NSIC - Stanmore	nataliavasquezp@yahoo.com
Joost	Verhaagen	Netherlands Institute for Neuroscience	J.Verhaagen@nin.knaw.nl
Isabelle	Villey	MAPREG SAS	isabelle.villey@gmail.com
Laurent	Vinay	CNRS & Aix Marseille Université	laurent.vinay@univ-amu.fr
Terry	Walters	University of Texas Medical School at Houston	Edgar.T.Walters@uth.tmc.edu
Claudia	Wheeler-Kingshott	University College London	c.wheeler-kingshott@ion.ucl.ac.uk
Liang-Fong	Wong	University of Bristol	L.Wong@bristol.ac.uk
Akihito	Yamamoto	Nagoya University Graduate School of Medicine	akihito@med.nagoya-u.ac.jp
Ping	Yip	Queen Mary University of London	p.yip@qmul.ac.uk