“Spinal Cord Research on the Way to Translation”

Meeting in Ittingen, Switzerland, 26th–28th August 2010

CHRISTOPHER & DANA REEVE FOUNDATION
TODAY'S CARE. TOMORROW'S CURE.

SPINAL RESEARCH
fighting paralysis... and winning

26th-28th August 2010

The 2nd joint Spinal Cord Meeting of the Christopher and Dana Reeve Foundation (CDRF/NACTN), Internationales Forschungsinstitut fuer Paraplegiologie (IFP/EMSCI) and the International Spinal Research Trust (ISRT)

ABSTRACTS

ABSTRACTS

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

POSTER PRESENTATIONS

Poster session is scheduled from 19.45 in Untere Aula at the end of the first day, immediately after the main meeting on Thursday, 26th August.

SCIENTIFIC ORGANIZING COMMITTEE

Volker Dietz MD FRCP
University Hospital Balgrist, Zürich

John Priestley PhD MA DPhil
Queen Mary University of London

Martin Schwab PhD hon. MD
University of Zürich

Thursday, 26th August

12:30-13:30	*Lunch*	
	Welcome and Introduction to the Symposium	
13:30-13:50	Martin Schwab and Volker Dietz	
	Optimizing training approaches	Chair: Albert Aguayo
13:50-14:20	Volker Dietz: Neuronal dysfunction in chronic spinal cord injury	
14:20-14:50	Grégoire Courtine: Turning the balance of plasticity to your advantage	
14:50-15:30	*Coffee Break and Check-In*	
	Assessment tools	Chair: Robert Grossman
15:30-16:00	Susan Harkema: Neuromuscular recovery with activity dependent plasticity after neurologic injury	
16:00-16:30	Armin Curt: Advancing the appreciation of segmental changes in SCI	
16:30-16:50	Huub van Hedel: Does "no pain, no gain" apply to sensory-motor recovery after spinal cord injury?	
16:50-17:10	Rüdiger Rupp: From diagnostics to therapy - The possibilities of realtime gait analysis in the rehabilitation of incomplete spinal cord injured subjects	
17:10-17:40	**Panel Discussion: Readouts for Clinical Trials**	Chair: Roger Lemon
	Check-In, Poster Set-up	
18:15	*Dinner*	
19:45	*Poster Session*	

Friday, 27th August

	Regeneration and Plasticity I	Chair: Lorne Mendell
8:30-9:00	James Fawcett: Increasing the intrinsic regenerative ability of spinal cord axons	
9:00-9:30	Martin Schwab: Spontaneous, training- and anti-Nogo-A antibody induced recovery after CNS injury	
9:30-10:00	Zhigang He: PTEN deletion enhances the regenerative ability of adult corticospinal neurons	
10:00-10:30	*Coffee Break*	
	Regeneration and Plasticity II	Chair: Sue Barnett
10:30-11:00	Joost Verhaagen: Molecular target discovery for neural repair in the functional genomics era	
11:00-11:30	Mark Tuszynski: Combinatorial Approaches to SCI	
11:30-12:00	Heike Vallery: Therabotics 2030	
12:00-13:15	*Lunch*	
	Stem Cell Treatments	Chair: Jens Zimmer
13:15-13:45	Fred Gage: Modeling human spinal cord injury *in vitro*	
13:45-14:15	Sam Pfaff: Preparation of clinical grade human astrocyte precursors from stem cells	
14:15-14:50	**Panel Discussion: Treatment Combinations and New Treatments in Development**	Chair: James Fawcett
14:50-15:30	*Coffee Break*	
	Clinical Trials	Chair: Armin Curt
15:30-16:00	Michael Fehlings: Repair and regeneration of the injured spinal cord: from molecule to man	
16:00-16:30	Jane Lebkowski, Geron: Development of human embryonic stem cells for therapeutic applications	
16:30-17:00	Klaus Kucher, Novartis: Therapeutic anti-Nogo-A antibodies in acute spinal cord injury - Latest safety and pharmacokinetic data from ongoing first-in-human trial	
17:30-18:30	*Guided Cloister Tour*	
18:45	*Dinner*	
20:00	**Panel Discussion: Problems of Clinical Trials in SCI**	Chair: Naomi Kleitman

Saturday, 28th August

	Physiology of the Injured Human Spinal Cord I	Chair: Peter Ellaway
8:30-9:00	Steve McMahon: Cortical overexpression of neuronal calcium sensor 1 induces functional plasticity in spinal cord following unilateral pyramidal tract injury in rat	
9:00-9:20	Phil Waite: Studies on pain after dorsal root injury	
9:20-9:40	John Riddell: Electrophysiological assessment of function in animal models of spinal cord injury	
9:40-10:10	*Coffee Break*	
	Imaging and Characterization of the Injured Human Spinal Cord	Chair: Charles Tator
10:10-10:40	Patrick Stroman: Mapping of function in the injured human spinal cord by means of functional MRI	
10:40-11:10	Spyros Kollias: Advanced techniques for imaging the spinal cord	
	Physiology of the Injured Human Spinal Cord II	Chair: Geoffrey Raisman
11:10-11:40	Karim Fouad: The challence with the balance: wanted versus unwanted treatment effects	
11:40-12:10	Lynn Jakeman: Glial bridges and endogenous cellular repair strategies in spinal cord injury	
12:10	*End of the symposium*	

Introduction Joint Meeting CRDF/ISRT/IFP 2010

"Spinal Cord Research on the Way to Translation"

We are very happy to welcome you at this joint meeting of the Christopher and Dana Reeve Foundation (CRDF), the International Spinal Research Trust (ISRT) and the International Institute for Research in Paraplegia (IRP Zurich/ Geneva).

Three years ago the idea was born to consolidate forces of these foundations by sharing recent progress made in basic, applied and clinical research at a joint meeting. While the first joint meeting in 2007 carried the title "State of the Art in Spinal Cord Injury Research and Clinical Application" the focus of this year's meeting will be more on the translational aspects in spinal cord research.
The program will be rather dense allowing for senior scientists, clinicians and industrial representatives to talk about recent achievements in talk rounds and more junior researchers to present their data in shorter talks and on posters. Translational aspects will also be discussed in three panels representing also industry and public authorities.

The meeting venue is a former monastery of the middle ages in the countryside of northern Switzerland. We hope that this remote historical locality will foster new ideas, collaborations, and interactions in clinical and basic science and give rise to new friendships.

We thank you for attending and contributing to this hopefully inspiring meeting!

John Priestley, Volker Dietz and Martin Schwab

Christopher and Dana Reeve Foundation

The Christopher & Dana Reeve Foundation is dedicated to developing treatments and cures for spinal cord injury by funding innovative research, and improving the quality of life for people living with paralysis through grants, information and advocacy.

The Reeve Foundation's dynamic research continuum spans bench to bedside. The Individual Research Grants Program casts a wide net, nurturing new ideas and both new and established investigators. Grants cover the spectrum of injury: neuroprotection, regeneration, growth inhibition, remyelination, axon guidance, rehabilitation and concomitant function. The Reeve International Research Consortium is a multi-disciplinary, collaborative approach to spinal cord research focused on optimizing the intrinsic capacity of the nervous system to repair and remodel itself and eliciting robust regeneration after injury. The NeuroRecovery Network (NRN) is a cooperative network of rehabilitation centers that is developing and delivering activity-based therapies to promote functional recovery and improved health and quality of life for people living with paralysis. Funded through a Cooperative Agreement with the Centers for Disease Control, the NRN translates the latest scientific advances into effective activity-based rehabilitation treatments. The North American Clinical Trials Network (NACTN) was organized to move promising SCI therapies from the laboratory to the clinic in a manner that will insure patient safety and meaningful, interpretable data. NACTN also gathers and documents patient medical information in a data registry to better understand the body's natural course of recovery after injury; uses standardized patient assessment protocols and is leading an international team of scientists and clinicians to develop new, more reliable and sensitive outcome measures. It began its first clinical trial in April, a Phase I safety study of the neuroprotective drug Riluzole.
The Christopher & Dana Reeve Paralysis Resource Center provides information, education and referral services for people throughout the world living with paralysis. Its Quality of Life program addresses the challenges and needs of those individuals through Actively Achieving, Bridging Barriers and Caring and Coping grants.

www.christopherreeve.org

International Institute for Research in Paraplegia (IRP) Zurich and Geneva

The International Institute for Research in Paraplegia Zurich and Geneva (IRP) was founded on private initiatives in 1991 and 1995. The purpose of the IRP is to promote and support basic science and clinical research in the field of spinal cord injury, regeneration and rehabilitation. Projects are selected on the basis of an open call published in Nature annually. Until today IRP has supported 77 research projects world-wide. The institute also sponsors assistant professorships, as well as scientific symposia with the specific aim to foster collaboration between fundamental science and applied, clinical research.
To recognize outstanding scientific work on key questions of spinal cord injury, the IRP Foundation awards each year the Schellenberg Prize For Research of 100'000 francs.

Further information please visit: www.ifp-zh.ch and www.irp.ch

International Spinal Research Trust

The goal of *The International Spinal Research Trust* is to create a future where spinal cord injury no longer leads to permanent paralysis and disability. Set up in the early 1980s *Spinal Research* (as it is now know) is the only medical research charity based in the UK dedicated to funding research on an international scale. To date *Spinal Research* has committed more than £18 million to research projects around the world.

The Trust has a number of funding streams that run concurrently that are closely linked. These grants support research projects in areas ranging from basic neuroscience to clinical studies as well as PhD studentships and a Translational Awards scheme to support the transition from discovery to clinical application.

Spinal Research has a long history of planning its research in a highly strategic manner. To this end, we have recently published our third strategic research review document (Adams *et al*., (2007)). This review document guides our funding and is implemented by an international Scientific Committee of experts in spinal cord injury.

Spinal Research has recently launched its ***Translational Initiative*** which aims to strengthen, now and for the future, i) the pre-clinical pipeline of treatments for SCI and ii) facilitate better interaction between basic and clinical research, and iii) prepare the UK for forthcoming clinical trials activity. The Trust will deliver benefits to the health of people with SCI through this initiative by operating in three key areas with a common purpose:

- Establishing a grant scheme to encourage academia and its partners to undertake translational project activities
- Establishing one or more centres of research excellence to act as a focal point for basic, translational and clinical research in SCI
- Establishing a patient registry and clinical trials network involving existing spinal injury centres in the UK

Further information is available on our website at www.spinal-research.org

Adams, M *et al*., (2007) International Spinal Research Trust research strategy. III: A discussion document. *Spinal Cord* 1:2-14

Additional Sponsors

Swiss Balgrist Association

Balgrist University Hospital is a private not for profit institution managed according to business principles. It has three core competencies:

1. As a highly specialised diagnostic, treatment and rehabilitation centre for patients with difficult problems of the musculoskeletal system

2. As a teaching hospital contracted to the University of Zurich for undergraduate training in the subjects of spinal cord injuries and orthopaedics as well as postgraduate training for doctors and healthcare professionals in spinal cord injuries, orthopaedics, rheumatology, anaesthesiology and radiology

3. As a research centre to improve the quality of future healthcare

Balgrist University Hospital developed from the "Balgrist Institution" founded in 1912. The objective of the original institution was to treat and care for physically disabled children, educating and integrating them into working life (if necessary free of charge).
Today, as then, "Balgrist" is funded by the **Swiss Balgrist Association** which was set up in 1909. Over time the demands on the hospital grew and became ever more complex. In 1945 it became the Orthopaedic Hospital of the University of Zurich. The Spinal Cord Injuries Centre was opened in 1990, in a spacious and purpose-built complex. The **Swiss Balgrist Association** manages also the **ParaCare Fund**, which is specifically intended to financially support projects of the Spinal Cord Injuries Centre. To meet and finance the many demands on the **Swiss Balgrist Association**, we are grateful to all our sponsors for their active and generous support.

For more information, please check our website at: www.balgrist.ch

The KGF (Kontaktgruppe für Forschungsfragen) includes the companies Ciba, Novartis, F. Hoffmann-La Roche, Merck Serono, and Syngenta and coordinates research policies and matters of common interest to its member companies.

It facilitates the interactions between its member companies and external partners, e.g., individuals or groups at Swiss research institutions, by acting as a homogeneous discussion partner or sounding board, providing harmonized opinions, recommendations, or action plans.

On behalf of its member companies the KGF can decide on joint support of scientific events or donations to research and educational institutions or organizations. Alternatively, the KGF may issue recommendations to the appropriate decision bodies within the member companies.

Hocoma – **We move you**

Hocoma – your partner for functional rehabilitation

Hocoma is the leader in robotic rehabilitation therapy for neurological movement disorders. We are a globally active medical technology company based near Zurich, Switzerland. We develop innovative therapy solutions working closely with leading clinics and research centres. Our products Lokomat®, Armeo® and Erigo® are applied successfully in renowned clinics and research institutes worldwide in the field of rehabilitation medicine.

Quality in all we do

· Focused on improving functional rehabilitation therapy with innovative solutions

· Product development in close collaboration with leading clinical and scientific partners

· Clinical application workshops and training with certified instructors

· Worldwide service support and maintenance options

For more information please call +41 43 444 2200 oror visit www.hocoma.com

Table of Contents – Presentation Abstracts

Thursday 26th August PM

Neuronal dysfunction in chronic spinal cord injury
Volker Dietz 3

Turning the balance of plasticity to your advantage
Grégoire Courtine 4

Neuromuscular recovery with activity dependent plasticity after neurologic injury
Susan Harkema 5

Advancing the appreciation of segmental changes in SCI
Armin Curt 6

Does “no pain, no gain” apply to sensory-motor recovery after spinal cord injury?
Huub van Hedel 7

From diagnostics to therapy – The possibilities of realtime gait analysis in the rehabilitation of incomplete spinal cord injured subjects
Rüdiger Rupp 8

Friday 27th August AM

Increasing the intrinsic regenerative ability of spinal cord axons
James Fawcett 10

Spontaneous, training- and anti-Nogo-A antibody induced recovery after CNS injury
Martin Schwab 11

PTEN deletion enhances the regenerative ability of adult corticospinal neurons
Zhigang He 12

Molecular target discovery for neural repair in the functional genomics era
Joost Verhaagen 13

Combinatorial approaches to SCI
Mark Tuszynski 14

Therabotics 2030
Heike Vallery 15

Friday 27th August PM

Modeling human spinal cord injury *In Vitro*
Fred Gage 16

Preparation of clinical grade human astrocyte precursors from stem cells
Samuel Pfaff 17

Repair and regeneration of the injured spinal cord: from molecule to man
Michael Fehlings 18

Development of human embryonic stem cells for therapeutic applications
Jane Lebkowski 19

Therapeutic anti-Nogo-A antibodies in acute spinal cord injury – Latest safety and pharmacokinetic data from ongoing first-in-human trial
Klaus Kucher 20

Saturday 28th August AM

Cortical overexpression of neuronal calcium sensor 1 induces functional plasticity in spinal cord following unilateral pyramidal tract injury in rat
Stephen McMahon 21

Studies on pain after dorsal root injury
Phil Waite 22

Electrophysiological assessment of function in animal models of spinal cord injury
John Riddell 23

Mapping of function in the injured human spinal cord by means of functional MRI
Patrick Stroman 24

Advanced techniques for imaging the spinal cord
Spyros Kollias 25

The challence with the balance: wanted versus unwanted treatment effects
Karim Fouad 27

Glial bridges and endogenous cellular repair strategies in spinal cord injury
Lyn Jakeman 28

Session I: Optimising training approaches – Chair: Albert Aguayo

Neuronal dysfunction in chronic spinal cord injury

Volker Dietz

Spinal Cord Injury Center, Balgrist University Hospital, Forchstrasse 340, 8008 Zurich, Switzerland
vdietz@paralab.balgrist.ch

This contribution discusses the spinal neuronal changes that occur after a complete spinal cord injury (SCI) in humans. Early after an SCI, neither locomotor nor spinal reflex activity can be evoked. Once spinal shock has resolved, locomotor activity and an early spinal reflex component reappear in response to appropriate peripheral afferent input. In the subsequent 4–8 months, clinical signs of spasticity appear, largely as a result of non-neuronal (for example, muscular) changes, whereas locomotor and spinal reflex activity undergo little change. At 9–12 months, the electromyographic amplitude in the leg muscles during assisted locomotion declines, accompanied by a decrease in the amplitude of the early spinal reflex component and an increase in the amplitude of a late spinal reflex component. This exhaustion of locomotor activity also occurs in nonambulatory patients with incomplete SCI. Neuronal dysfunction is fully established 1 year after the injury without further alterations in subsequent years. In chronic SCI, the absence of input from supraspinal sources has been suggested to lead to degradation of neuronal function below the level of the lesion or, alternatively, a predominance of inhibitory signaling to the locomotor pattern generator. Appropriate training and/or provision of afferent input to spinal neurons might help to prevent neuronal dysfunction in chronic SCI.

Dietz V (2010) Nature Rev Neurol 6: 167-174
Dietz V & Müller R (2004) Brain 127: 2221-2231

Turning the balance of plasticity to your advantage

Rubia van den Brand, Janine Heutschi, Lucia Friedli, Nadja Kaufmann, Michele Huerlimann, Kay Bartholdi, Nadia Dominici, Pavel Musienko, **Grégoire Courtine**

Neurology Department, University of Zurich, Zurich, Switzerland
gregoire.courtine@bli.uzh.ch

A severe lesion of the spinal cord induces complete and permanent paralysis below the level of the injury. Despite this lack of functional recovery, marked plastic changes take place in spared systems. Indeed, we will provide evidences that a substantial anatomical and functional remodeling of spinal circuitries spontaneously occurs after severe spinal cord damage. These complex and multifaceted changes in the structure and properties of spinal motor systems lead to a progressive degradation of functional capacities in the chronic stages of the injury. Could this plasticity be exploited to improve function? To address this hypothesis, we evaluated the capacity of neurorehabilitation enabled by electrical and pharmacological stimulations to appropriately remodel lumbosacral circuitries and spared intraspinal systems around the lesion site. We will show that use-dependent plasticity allows paralyzed rats to voluntarily control the pharmaco-electrically activated spinal circuitry and to regain the impressive capacity to initiate locomotion, walk freely overground, cross obstacles, climb stairs, and swim. These results highlight the delicate balance between bad and good plasticity after severe spinal cord damage.

Supported by Swiss National Science Foundation, Neuroscience Center Zurich, International Paraplegic Foundation, the National Center of Competence in Research “Neural Plasticity and Repair” of the Swiss National Science Foundation

Session II: Assessment tools – Chair: Robert Grossman

Neuromuscular recovery with activity dependent plasticity after neurologic injury

Susan Harkema, Yury Gerasimenko, Jonathan Hodes, Joel Burdick, Claudia Angeli, Yangsheng Chen, Christie Ferreira, Enrico Rejc, Reggie Edgerton

University of Louisville, Kentucky Spinal Cord Injury Research Center, Frazier Rehab Institute, Abraham Flexner Way Louisville, KY 40202, USA
Susan.harkema@jhsmh.org

After human spinal cord injury, without recover of walking, reconfiguration of spinal interneuronal circuits may occur not only from loss of supraspinal input but also from chronic unloading of the legs. This interneuronal reorganization then routinely results in spasticity and clonic efferent output, rather than more functional tonic efferent out put. This, re-introducing loading of the legs should modulate clonus after SCI, possibly even reducing the amount of clonus. We studied individuals after complete and incomplete spinal cord injury using electromyography from leg flexors and extensors during different loading conditions before and after repetitive stand or step raining. We will present results that show spinal interneuronal circuits can integrate afferent input related to the level of load and rate of stepping and modulate efferent input. We will present evidence that the repetitive afferent input from either stand or step training induces significant and persistent functional reorganization of interneuronal circuits, such that the response to the same afferent input after intensive training is altered. In addition, spinal networks, in the absence of detectable supraspinal input have the capacity to modulate reflexes during stepping. These results support that the functionally isolated human spinal cord maintains specific properties recognized to generate locomotion in other species. These concepts now have been translated into the clinic by the Christopher and Dana Reeve NeuroRecovery Network of seven rehabilitation centers that provide standardized Locomotor Training to individuals with chronic incomplete spinal cord injury. We studied 206 individuals ranging from 0.9 to 26 years post injury with statistically significant improvements of walking and balance after spinal cord injury with 89% showing improvement. However, some of these individuals with incomplete injury and none of those with clinically complete injury have not recovered walking. We hypothesize that a level of central state of excitability is needed for the interaction between sensory and epidural regulation of locomotor circuitry to result in functional walking. Preliminary results suggests that a physiological state can be achieved with epidural stimulation so that the sensory input can effectively control the locomotor circuitry to stand and to step after motor complete human spinal cord injury.

Supported by National Institutes of Health (NINDS P01 NS16333 and R01 NS49209 and NIBIB EB007615) and Christopher and Dana Reeve Foundation

Advancing the appreciation of segmental changes in SCI

Armin Curt

Human Spinal Cord Injury Center, University Hospital Balgrist, University of Zürich
Armin.Curt@balgrist.ch

In clinical trials the outcome of gross body functions (ambulation, independence, and spasticity) are monitored to evaluate the effectiveness of interventions. However, these gross measures abide 2 major shortcomings: 1) they are rather insensitive to provide detailed information about underlying mechanisms of recovery i.e. changes in key functions (motor, sensory, motor control) relevant to recovery, and 2) the clinical value of many interventions will be best evaluated by rather specific or detailed functional outcome measures (hand function, postural stability, sensory feedback etc.) where the level of lesion and segmental deficit is most relevant to overcome specifically addressed impairments.
Therefore, the assessment of segmental function is important for a meaningful stratification of patients and to provide sensitive and detailed outcome measures able to disclose not only subtle changes but also to relate improvements in specific sensor-motor capacity to the recovery of function. Obviously these assessments need to be tailored to the specific goal of an intervention and trial protocols should a priori take into consideration the above mentioned issues. Furthermore, protocols distinguishing outcomes of complex (gross) and specific (detailed) function will provide more sensitive clinical thresholds to identify responders. In this context available data sources from SCI trials (Sygen) and prospective data collections (EMSCI, US System Model) as well as novel assessments have been mined to coach the appropriate stratification of patients, estimating reasonable thresholds of effectiveness and provide sensitive and responsive trial protocols.

The studies are funded by the IFP Zürich, ISRT UK and the NCCR Neural Repair in Zürich

Does “no pain, no gain” apply to sensory-motor recovery after spinal cord injury?

Huub van Hedel

SCI Research, Spinal Cord Injury Center, University of Zurich, Balgrist University Hospital, Forchstrasse 340, CH-8008, Zurich
hvanhedel@paralab.balgrist.ch

This presentation provides an update on the latest investigations in our lab:
(i) The effectiveness of robotic-and weight-supported treadmill training versus lower extremity strength training on walking-related outcomes and other measures in chronic incomplete spinal cord injured (iSCI) subjects. Recent findings from our lab show that strength is predominantly affected in iSCI patients, while accurate muscle activation remains largely unaffected [1,2]. Therefore, iSCI patients might benefit from muscle strength training. As these results were obtained during the performance of single joint movements, we investigated whether this finding applies also to functional tasks, such as walking. In this cross-over randomized single blinded pilot-trial, chronic iSCI subjects performed a 4 week strength training followed/preceded by 4 weeks training with the robotic gait orthosis “Lokomat”. The Lokomat training intensity could be adjusted to the impairments of the patient: (1) 100% guidance force, (2) patient-interaction with the Lokomat and (3) patient-interaction with the Lokomat and treadmill speed. Maximal walking speed (primary outcome measure) was scored by a blinded rater. Preliminary results about various functional and sensory measures will be presented at the joined meeting.

(ii) The responsiveness of the electrical perception threshold (EPT) measure and its ability to predict the occurrence of neuropathic pain and allodynia. The EPT is considered a promising candidate to determine changes in segmental level of lesion in experimental trials that aim to lower the level of lesion by several segments. Previous publications showed its reliability tested in several segments [3] and its ability to determine the sensory level of lesion [4]. The reliability was assessed in 15 healthy subjects (right side dermatomes from C3 to S2) who were measured twice by the same investigator. The average ($\pm$ SD) intra-class correlation coefficient (ICC) over all dermatomes was 0.50 ± 0.26 (range 0 to 0.86). However, low ICC values reflected rather small inter-individual variability than poor test-retest reliability and were therefore complemented with Bland-Altman plots. To determine the responsiveness, the EPT was implemented in 7 SCI centers that participate in the European Multicenter Study for Spinal Cord Injury (EM-SCI). At 1, 3 and 6 months, EPT assessments were performed. First analyses in 32 patients show no significant changes in segmental sensory perception at or just below the level of lesion. In addition, at 6 months, a pain questionnaire was applied to determine whether neuropathic pain and allodynia had developed over time.

(iii) The applicability of patient-reported functional outcome versus objective functional outcome after SCI for clinical trials. An ongoing debate in our field is about the importance of patient-reported (functional) outcome measures in clinical trials. While the importance of some measures (e.g. pain intensity) is given, others such as the perceived interference with daily life activities, is not. Eight EMSCI centers assessed 73 subjects at 1, 3 and 6 months post injury. The objective independence was quantified using the Spinal Cord Independence Measure (SCIM), while the subjective independence was reported by the patient. Both measures were scored between 0 (no independence) to 100 (maximal independence). Initially, both measures were low (around 30). While the SCIM score increased to about 70, the perceived independence score remained stable around 40. Accordingly, correlations between the measures were moderate ($r < 0.41$). In conclusion, the courses of objective and patient-reported independence are different and the measures are influenced by different factors. Based on these results, patient-reported functional outcome might be less favorable to serve as a primary outcome measure for clinical trials.

[1] van Hedel, Wirth, Curt. Ankle motor skill is intact in spinal cord injury, unlike stroke: implications for rehabilitation. Neurology. 2010; 74: 1271-1278. [2] With, van Hedel, Curt. Ankle dexterity remains intact in patients with incomplete spinal cord injury in contrast to stroke patients. Exp Brain Res. 2008; 191:353-61. [3] King et al. J Neurotrauma. 2009:26:1061–1068. [4] Savic et al. Spinal Cord. 2006 Sep;44(9):560-6.
This work was supported by the International Spinal Research Trust (ISRT; Clinical Initiative) and the International Foundation for Research in Paraplegia (IFP) Zurich.

From diagnostics to therapy – The possibilities of realtime gait analysis in the rehabilitation of incomplete spinal cord injured subjects

Rüdiger Rupp, N. Weidner, C. Schuld

Heidelberg University Hospital - Spinal Cord Injury Center, Schlierbacher Landstr. 200a, 69118 Heidelberg, Germany
Ruediger.Rupp@med.uni-heidelberg.de

Introduction
As the therapeutic options for improving locomotor function in incomplete spinal cord injured (iSCI) individuals are growing there is also an increasing demand for quantifying gait under standardized conditions. Specifically, the occurrence of pharmacological interventions calls for quantitative methods in addition to clinical scores for assessing the therapeutic progress. Instrumented gait analysis is the most important procedure for quantifying the effects of walking impairments on the resulting gait pattern. Especially the possibility to assess the dynamics of gait on a treadmill allows for elimination of the speed dependent deviations on the joint angle curves. However a nontrivial problem when it comes to quantifying therapy progress is the question how to compress the information of the large sets of data to a few parameters correlated to gait quality.

Computer based analysis of instrumental gait analysis data
In the past computer based methods have mainly be used for measuring marker trajectories and calculating step normalized gait angle curves. However, little efforts have been undertaken to provide a distance measure which gives a good compromise between being sensitive enough to subtle differences and integral enough in order not to hide the big picture. For this purpose, a novel measure was proposed called the norm distance measure [1]. The basis of this measure consists of weighting the distance of a given joint angle curve to a reference curve by the standard deviation of the normal collective. To obtain a more integral description of an individual's gait pattern, different subsets of joint angles can be summed up and averaged. A reasonable approach for this is to use the six joint angles in the sagittal plane since they have the best signal-to-noise ratio and carry the maximum amount of information. This parameter has been successfully applied to quantification of the course of the gait rehabilitation in iSCI even in cases, in which conventional gait scores show ceiling effects.

Realtime gait analysis systems for movement feedback therapy
Due to the recent technological progress in providing high resolution digital camera systems in combination with high computing power at reasonable prices gait analysis systems can not only be used as diagnostic but also as therapy tools. After setup of a system, which is capable of calculating joint angles and their norm distance separately for swing and stance phase in realtime, a visual feedback in form of graphical symbols (smileys, bars) or number can be provided [2]. This has been shown to facilitate the acquisition of complex motor skills in motor learning and in sports. However, very little is known about the effectiveness of feedback during rehabilitation of movement disorders.
In a feasibility study with four chronic (Ø 2.2 years after SCI) iSCI individuals (Ø 46.25 Lower Extremity Motor Score, sensory accentuated paralysis, WISCI II > 16) with a “stiffed-knee” gait pattern we have been able to show that a normalization towards a physiological gait pattern occurs during feedback training [3]. The norm distance of the knee angle during swing phase has been visualized within two 15 min. sessions with a 5 minute pause between. This therapy scheme has been applied six times once a week with a 2 weeks pause after the first three therapy sessions. Interestingly the study participants have been able to transfer the acquired motor skills to their overground gait pattern without any kind of feedback. This has been confirmed by the fact, that the mean norm distance of the knee angle after the two weeks pause was lower than at the time of first study inclusion.

Conclusion
Instrumental gait analysis methods are a valuable tool for a quantitative and objective assessment of gait. The introduction of the technology for realtime calculation of gait phase related joint angles together with their visual feedback offers new possibilities in the therapy of iSCI patients.

[1] Wolf S., Loose T., Schablowski M., Döderlein L., Rupp R., Gerner H.J., Bretthauer G., Mikut R.: Automated feature assessment in instrumented gait analysis, Gait & Posture 23(3), 331-338, 2006
[2] Schablowski-Trautmann M., Kögel M., Rupp R., Mikut R., Gerner H.J.: From diagnostics to therapy - conceptual basis for real-time movement feedback in rehabilitation medicine, Biomedizinische Technik 51(5-6), 299-304, 2006
[3] Schuld C., Plewa H., Schweidler J., Gerner H.J., Rupp R.: Visuelles Echtzeit-Bewegungsfeedback auf dem Laufband zur Normalisierung des Gangbilds von inkomplett Querschnittgelähmten. In: Schauer T., Schmidt H., Kraft M. (Hrsg.), Automatisierungstechnische Verfahren für die Medizin, Fortschritt-Berichte VDI, Reihe 17 Nr. 274, VDI-Verlag, Düsseldorf, 3-4, 2009

Session III: Regeneration and Plasticity I – Chair: Lorne Mendell

Increasing the intrinsic regenerative ability of spinal cord axons

Melissa Andrews, Richard Eva, Chin-Lik Tan, Elske Franssen, Sunil Kappagantula, **James Fawcett**

Cambridge University Centre for Brain Repair, Cambridge CB2 0PY, UK
jf108@cam.ac.uk

Efforts to stimulate axon regeneration in the damaged CNS have focused mainly on removal of inhibitory molecules from the environment. This approach has had some success, with axon regeneration over 2cm achievable with treatments such as anti NogoA and chondroitinase. However the problem that CNS axons have a low intrinsic ability to regenerate remains to be solved. Some success has been achieved with trophic factors and intervention in signaling pathways such as Rho and cAMP. We have been working on three approaches to enhancing axon regeneration.
Local mRNA translation. Axons in the peripheral nervous system contain many mRNAs, and the machinery to translate these into proteins. This ability is important for axon regeneration, because blocking local translation inhibits axon regeneration. Comparing the mRNAs from embryonic and adult PNS axons there are many changes, including the absence of kinesin mRNAs in adult axons. We find that one of these, kif3C, plays a key role in growth cone regeneration.
Integrins and axon regeneration. In order to grow through the extracellular matrix axons must express appropriate integrins. The main matrix glycoprotein in the damaged CNS is tenascin-C, but tenascin-C binding integrins are lacking. We have transfected alpha9 integrin into neurons, giving them the ability to grow long axons on tenascin in vitro. Transduction of DRG neurons in vivo enhances their ability to regenerate their axons, but only modestly. The problem is that integrin transport into axons is blocked at the axon initial segment. Integrin trafficking relies on Rab11 and Rab coupling protein .
Gangliosides and axon regeneration. Axons contain the membrane enzyme PMGS, which desialyates GD1a ganglioside to produce GM1. We find that axotomy activates PMGS, converting axonal ganglioside to GM1, and that blocking the enzyme inhibits axon regeneration. Retinal axons from the CNS do not convert their surface gangliosides to GM1 after axotomy, but application of an external sialidase causes both ganglioside conversion and promotes axon regeneration. The activation pathway involves Ca2+ and P38.

Supported by CDRF, ISRT, MRC, Wellcome, EU, Henry Smith Charity

Spontaneous, training- and anti-Nogo-A antibody induced recovery after CNS injury

Martin E. Schwab

Brain Research Institute, University of Zurich, 8057 Zurich, Switzerland
schwab@hifo.uzh.ch

Growth of nerve fibres in the adult mammalian CNS is restricted to very short distances and spatial domains, thereby limiting the capacity of the CNS for plastic rearrangement of fibre connections and repair of large lesions. Nogo-A, several ephrins, the semaphorins Sema4D and 6A, as well as the myelin proteins MAG and OMgp, have all been shown to induce growth cone collapse and arrest of neurite growth in vitro. Unequivocal in vivo evidence for a growth restricting function in the adult injured CNS exists mainly for Nogo-A so far.
In intact animals, injection of function blocking antibodies against Nogo-A into the cerebellum or spinal cord led to transitory sprouting of collaterals and axonal arbors. Nogo-A KO mice exhibit higher levels of growth-associated and cytoskeletal proteins and their mRNAs in the intact spinal cord. DRG neurons dissected from these mice elaborate larger and more dynamic growth cones than wild type neurons. In a classical plasticity paradigm, monocular deprivation, visual cortex plasticity could be induced long after the end of the critical period in Nogo receptor (NgR) KO mice and in Nogo-A/B KO mice (McGee et al., 2005). These results suggest that with the maturation of the CNS and the simultaneous oligodendrocyte differentiation and myelin formation, neurite growth inhibitors, in particular Nogo-A are expressed and function to limit growth and thereby stabilise the highly complex CNS wiring.
Intrathecal infusion of 4 different function blocking anti- Nogo-A antibodies or of reagents blocking the Nogo-NgR interaction or its downstream signaling via Rho resulted in enhanced regenerative sprouting, long distance regeneration and improved recovery of lost functions after spinal cord or brain injury in rats, mice and monkeys. Enhanced sprouting of spared fibres, including fibres from the contralateral intact side in the case of stroke lesions, and an increased overall level of plasticity of CNS fibre connections may contribute in an important way to these reparative processes. Nogo-A therefore appears as a major restricting factor for the spontaneous repair of large spinal cord and brain injuries. Chaotic connections and malfunctions have not been observed so far following treatment with function blocking anti Nogo-A antibodies, neither in the animal models nor in the ongoing clinical trial in paraplegic patients.

PTEN deletion enhances the regenerative ability of adult corticospinal neurons

Kai Liu[1*], Yi Lu[1*], Jae K. Lee[2], Ramsey Samara[2], Rafer Willenberg[3], Ilse Sears-Kraxberger[3], Andrea Tedeschi[1], Kevin Kyungsuk Park[1], Duo Jin[1], Bin Cai[1], Bengang Xu[1], Lauren Connolly[1], Oswald Steward[3], Binhai Zheng[2] , **Zhigang He[1]**

[1]F.M. Kirby Neurobiology Center, Children's Hospital, and Department of Neurology, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA. [2]Department of Neurosciences, University of California San Diego, 9500 Gilman Drive, La Jolla, California, 92093, USA.[3]Reeve-Irvine Research Center, University of California at Irvine College of Medicine; Department of Anatomy & Neurobiology, Neurobiology & Behavior and Neurosurgery, Irvine, California, 92697, USA.
Zhigang.he@childrens.harvard.edu

Despite the essential role of the corticospinal tract (CST) in controlling voluntary movements, successful regeneration of large numbers of injured CST axons beyond a spinal cord lesion has never been achieved. Here we demonstrate a critical involvement of PTEN/mTOR in controlling the regenerative capacity of corticospinal neurons. Upon the completion of development, the regrowth potential of CST axons lost and this is accompanied by a down-regulation of mTOR activity in corticospinal neurons. Axonal injury further diminishes neuronal mTOR activity in these neurons. Forced up-regulation of mTOR activity in corticospinal neurons by conditional deletion of PTEN, a negative regulator of mTOR, enhances compensatory sprouting of uninjured CST axons and even more strikingly, enables successful regeneration of a cohort of injured CST axons past a spinal cord lesion. Furthermore, these regenerating CST axons possess the ability to reform synapses in spinal segments distal to the injury. Thus, modulating neuronal intrinsic PTEN/mTOR activity represents a potential therapeutic strategy for promoting axon regeneration and functional repair after adult spinal cord injury.

Supported by NINDS, Wings for Life, International Spinal Research Trust

Session IV: Regeneration and Plasticity II – Chair: Sue Barnett

Molecular target discovery for neural repair in the functional genomics era

Joost Verhaagen[1], Kasper Roet[1], Ronald E. van Kesteren[2], Koen A. M. Bossers[1], Harold D. MacGillavry[2], Matthew R. Mason[1], August B. Smit[2]

[1]Netherlands Institute for Neuroscience and [2]Neuroscience Campus Amsterdam, The Netherlands
J.Verhaagen@nin.knaw.nl

A comprehensive understanding of the molecular pathways activated by neural trauma is of major importance for the development of treatments for spinal cord and peripheral nerve injury. High-throughput gene expression profiling is a powerful approach to reveal genome-wide changes in gene expression during a specific biological process. Microarray analysis of injured neurons or glial cells would ideally generate new hypotheses concerning the progression or deregulation of injury- and repair-related biological processes. These hypotheses should subsequently be tested experimentally and would eventually provide the molecular substrates for the development of novel therapeutics. Over the last decade, this approach has elucidated numerous extrinsic as well as neuron-intrinsic genes that are regulated following an injury. To date, however, the main challenge is to translate these massive changes in gene expression into a mechanistic framework and understand their functional implications. To achieve this, research on neural repair will have to adopt the conceptual advances and analytical tools provided by the functional genomics and systems biology revolution. Based on progress made in bioinformatics, high-throughput and high-content functional cellular screening and in vivo gene transfer technology, we will discuss a four-step “roadmap” that provides an integrated strategy for molecular target discovery and in vivo functional validation. We used this multi-step screening approach to start to dissect the regeneration-promoting properties of olfactory ensheathing glia (OEC) and to investigate the neuron-intrinsic regeneration-associated gene program. More details on these two projects, that will be discussed during the talk, can be found in the poster abstracts by Kasper Roet et al. and Matthew Mason et al.

Combinatorial approaches to SCI

Mark H. Tuszynski, Paul Lu, Armin Blesch, Ken Kadoya, Ephron Rosenzweig, John Brock, Edmund Hollis, Laura Taylor

University of California, San Diego, 9500 Gilman Drive-MC 0626, B.S.B. 4046, La Jolla, CA 92093
mtuszynski@ucsd.edu

A body of work over the last 30 years has elucidated multiple mechanisms that underlie the inability of the adult CNS to regenerate, including: 1) the absence of cellular or extracellular matrices within lesion sites to support axonal attachment and growth; 2) a failure to express growth factors in appropriate spatial and temporal gradients to stimulate growth; 3) the presence of myelin and ECM-associated molecules that inhibit growth, and 4) a failure to fully upregulate neuron-intrinsic mechanisms to enter a state of active regeneration. In previous experiments we and others have shown that approaches that simultaneously target these multiple mechanisms optimize the growth potential of axons after SCI. In the present set of experiments, we examined whether combinatorial approaches would facilitate axonal regeneration into and beyond sites of complete spinal cord transection in rats. 18 rats underwent T2 complete transections followed by: 1) placement of grafts of syngenic bone marrow stromal cells into the lesion site to reconstitute a permissive matrix for axonal growth; 2) administration of a growth factor (BDNF) using viral vectors within and caudal to the lesion site; and 3) stimulation of the intrinsic growth state of injured reticulospinal neurons using injections of cAMP into the brainstem. Controls received individual therapies, or two of the three therapies.. 3 months later, only subjects that received the full treatment combination exhibited bridging of injured reticulospinal motor axons into and beyond the lesion site. Axons grew 5mm caudal to the lesion, the farthest point of growth factor administration. These findings indicate that treatments targeting multiple mechanisms are required to achieve motor axon regeneration in a severe injury model.

Therabotics 2030

Robert Riener, **Heike Vallery**

Sensory-Motor Systems Lab, Institute of Robotics and Intelligent Systems, ETH Zurich and
Spinal Cord Injury Center, Medical Faculty, Balgrist University Hospital, University of Zurich
riener@mavt.ethz.ch, hvallery@ethz.ch

Robotic technologies and their clinical applications play an increasingly important role in the fields of neurosurgery and neurorehabilitation. Novel robotic applications are being developed or are already in standard clinical use within the different therapeutic phases ranging from theragnostic imaging via surgical technologies to rehabilitation treatments in the acute, subacute, and chronic stage. Robotic technology even extends beyond the clinic to assist the patient in daily life, in the form of mobile or telemedical applications.
MR-compatible robots can support diagnostic and therapeutic procedures and scientific investigations of neurological disorders of the brain. Surgical interventions can be accompanied by minimally-invasive telemanipulators, image-guided systems, as well as robots that support or perform biopsies. In the field of motor rehabilitation, robots can unburden the therapists from their exhaustive and partly monotonous physical labor and allow the patient to perform a longer, more intensive, and more motivating training. Diverse robots support movement of upper and lower extremities, fingers, trunk and neck. Some of the systems can be applied to severely affected inpatients, who require therapeutic training already in the early phase after surgery, injuries like SCI or stroke, or exacerbations of degenerative diseases like multiple sclerosis. Other robots can be used by chronic outpatients, who perform ambulant training with devices installed in the rehabilitation clinic, in order to further improve their movement capabilities and, thus, increase their quality of life. To continuously improve an outpatient's health state, home-based rehabilitation robotic systems can be applied. These systems control and assess the home therapy by a centralized clinical unit, using technologies of tele-monitoring and tele-rehabilitation. Those patients where additional motor training does not lead to further improvements can still benefit from portable or stationary home-based robotic devices that assist during locomotion, manipulation, and other tasks.
In many of these applications, novel human-centered robotic approaches are being developed or already used by the patient and/or the therapist or physician. For instance, a surgeon can be supported by the robotic system in order to perform a smoother, more accurate, and less fatiguing intervention, while still remaining the "master" of the intervention. To support gait or upper-extremity function, so-called patient-cooperative controllers take into account the patient's intention and efforts, rather than imposing any predefined movement. Audiovisual displays in combination with robotic devices can be used to present a virtual environment, where patients perform different movement tasks and activities of daily living. Furthermore, sensors integrated in the robots allow to measure and assess the patient's performance and, thus, evaluate the therapy progress. It is expected that such human-centered robotic approaches can improve the therapeutic quality as well as the patient motivation compared to conventional approaches.
There is already a clear tendency towards increased acceptance and use of robotic technology in neurorehabilitation hospital environments. We conclude that future neurosurgical interventions and neurorehabilitative treatments will further benefit from such novel human-centered robotic technologies. This talk will give a visionary overview about these robotic technologies applied to therapy as we expect them to be prominent in about 20 years from now – that is what we call Therabotics 2030.

Supported in part by the NCCR Neuro, Swiss National Science Foudation, CTI, NIDRR of the US Department of Education, and the Bangerter Rhyner Foundation.

Session V: Stem Cell Treatments – Chair: Jens Zimmer

Modeling human spinal cord injury *In Vitro*

Fred H. Gage

10010 North Torrey Pines Road La Jolla, CA 92037-1099 USA
gage@salk.edu

Current in vitro and in vivo models of spinal cord injury (SCI) have proven valuable for test new methods to induce CNS regeneration and to characterize the cellular, molecular and functional changes that occur following spinal cord injury. Most current models are not suitable for high-throughput screening for small molecules that may protect against damage or induce repair. Furthermore it is not clear how closely the knowledge gained from rodent models of spinal cord injury will translate to human spinal cord injury. We are attempting to develop a flexible, quantitative, and human-based in vitro model of SCI. Injury progression and repair in humans involves many different cell types and physiological processes such as axonal regeneration, neuromuscular junction recapitulation, re-myelination, inflammation, and scar tissue formation. We will make use of human pluripotent cells to generate each of the different cell types and we will present preliminary data on in vitro inflammation, glial responses and re-myeliniation following cell injury. We will discuss assay development considerations, to use this model to screen for drugs that retard injury progression and increase repair.

Supported by Christopher and Dana Reeve Foundation

Preparation of clinical grade human astrocyte precursors from stem cells

Samuel L. Pfaff and the San Diego ALS Disease Team

Howard Hughes Medical Institute and The Salk Institute for Biological Studies,
10010 North Torrey Pines Road, La Jolla CA 92037 USA
pfaff@salk.edu

The transplantation of astrocytes into sites of spinal cord injury (SCI) has shown promising results in animal studies. Nevertheless, the enthusiasm for treating SCI patients with these cells is being approached cautiously because astrocytes represent a diverse cell population. In fact some animal transplant studies using specific subtypes of astrocytes have triggered allodynia whereas other studies have found that astroctyes can strongly repel axons. In addition to the identification of the best astrocyte type for treating SCI, many practical issues need to be resolved before cells can be clinically used, including finding the best source of cells (e.g. primary fetal, iPS-derived, or ES-derived), preparing cells in a GMP facility devoid of adventitious agents, defining the dosing, tumorigenicity, and toxicity parameters, and identification of the best surgical delivery method for the cells. Because the initial human studies with cell-based transplants into the spinal cord will be risky and therefore difficult to receive FDA approval, we have sought to identify a patient population in which exceptional risks are warranted and likely to receive approval for heroic treatments.

Like SCI studies, astroctye transplants have shown good efficacy in treating animal models of amyotrophic lateral sclerosis (ALS). ALS is the most common adult motor neuron disease and is caused by selective dysfunction and death of neurons in the motor pathways. ALS leads to spasticity, hyperreflexia (upper motor neurons), generalized weakness, muscle atrophy, and paralysis (lower motor neurons). Failure of the respiratory muscles is generally the fatal event, occurring within one to five years of onset. Because of the devastating progression of the disease and a lack of effective treatments this patient group can provide a clinical path for obtaining FDA approval for an astrocyte-based investigational new drug (IND) trial.

In collaboration with a team of research scientists at UCSD and Life Technologies Corporation we have recently begun a CIRM funded project to generate clinical grade astroctye precursors from human embryonic stem cells. In this presentation I will describe our team's plan to:
1) generate astrocyte precursors from several different sources of human embryonic stem cell (hESC) lines;
2) identify the hESC line and astrocyte precursors combination that has the best characteristics of minimal toxicity, best efficiency in generating astrocytes and reducing disease phenotypes in vivo in a rat model of ALS;
3) manufacture the appropriate cell banks in a GMP facility;
4) work with our established clinical team to design a Phase I safety trial; and
5) submit an IND within the next four years.

Session VI: Clinical Trials – Chair: Armin Curt

Repair and regeneration of the injured spinal cord: from molecule to man

Michael G. Fehlings

University of Toronto
Michael.Fehlings@uhn.on.ca

The last two decades have witnessed remarkable advances in our understanding of the pathophysiology of spinal cord injury. It is now recognized that SCI involves the primary mechanical injury followed by a complex series of secondary injury events which are triggered by ischemia, inflammation, glutamatergic excitotoxicity, and oxidative cell injury.

The current lecture will summarize the clinically relevant advances in our understanding of the pathophysiology of spinal cord injury and will focus on translationally relevant therapeutic advances including neuroprotective strategies, novel surgical approaches, and the use of neural stem cells and biologics such as Cethrin and Anti-Nogo to stimulate regeneration and plasticity.

The lecture will conclude with a summary of current and ongoing clinical trials in the area of acute traumatic spinal cord injury and provide perspectives on the challenges related to the treatment of chronic spinal cord injury.

References

1. Karimi-Abdolrezaee S, Eftekharpour E, Wang J Schut, Fehlings MG: Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord, Journal of Neuroscience 2010 Feb 3:30(5)1657-76
2. Salewski RP, Eftekharpour E, Fehlings MG: Are induced pluripotent stem cells the future of cell based regenerative therapies for spinal cord injury. Journal of Cellular Physiology 2010; Mar222(3):515-521
3. Furlan JC, Noonan V, Cadotte DW, Fehlings MG: Timing of decompressive surgery of spinal cord after traumatic spinal cord injury: an evidenced based examination of pre-clinical and clinical Studies.J Neurotrauma, 2010 Mar 4.
4. Liu Y, Figley S, Spratt SK, Lee G, Ando D, Surosky R, Fehlings MG: An engineered transcription factor which activates VEGF-A enhances recovery after spinal cord injury, Neurobiology of Disease 2010 Feb:37(2):384-93
5. Baptiste DC, Fehlings MG: update on the treatment of spinal cord injury, progress and brain research 2007;161:217-33

Development of human embryonic stem cells for therapeutic applications

Jane S. Lebkowski

Senior Vice President & Chief Scientific Officer, Regenerative Medicine, Geron Corporation, 230 Constitution Drive, Menlo Park, CA 94025 USA
jlebkowski@geron.com

HESC-based regenerative cell therapies require 1) evidence for reliable production and quality control of product manufacturing, 2) rigorous safety testing in preclinical models, and 3) the design of clinical trial protocols that assess the safety and benefit of the therapy in appropriate patient populations. GRNOPC1 is a population of allogeneic cells containing oligodendrocyte progenitors derived from characterized, dedicated, human embryonic stem cell banks. GRNOPC1 induces myelination of axons in rats with spinal cord injuries and in Shiverer mice, which lack compact myelin, and also produces numerous neurotrophic factors such as midkine, BDNF, and activin. Extensive preclinical studies were performed to determine the distribution of GRNOPC1 as well as any potential toxicities after injection near the thoracic injury epicenter. Pending clearance from the FDA, a Phase I clinical trial to assess the safety of GRNOPC1 in patients with subacute, complete ASIA A, thoracic injuries whose last fully preserved neurological level is T3 to T10 will be conducted.

Therapeutic anti-Nogo-A antibodies in acute spinal cord injury – Latest safety and pharmacokinetic data from ongoing first-in-human trial

Klaus Kucher

Novartis Pharma AG, CH-4002 Basel, Switzerland
klaus.kucher@novartis.com

Objectives. Spinal cord injury (SCI) leads to malfunction of motor, sensory and autonomic functions below the lesion. Regeneration and plasticity in the adult central nervous system (CNS) of mammals are extremely restricted, accounting for the low degree of recovery following SCI and brain injury [1]. The molecular impediments that form the basis of this phenomenon are proteins expressed in CNS myelin which inhibit neurite growth after CNS injury. One of the most potent is Nogo-A, a membrane protein comprising multiple inhibitory domains that activate independent receptors. Currently there is no cure for SCI. Monoclonal antibodies against Nogo-A have been shown to neutralize the inhibitory activity of purified or recombinant Nogo-A, oligodendrocytes and CNS myelin *in vitro* [2, 3] and, more importantly, anti-Nogo-A antibody treatment facilitates neuroregeneration at the anatomical level in a non-human primate model of SCI [4]. The ongoing *first-in-human*, feasibility study aims to investigate whether a safe, intrathecal administration of the anti-Nogo-A antibody ATI355 to patients with very recent spinal cord injuries is technically feasible.
Methods. An open-label, multi-center, multiple cohorts study to assess feasibility, acute safety, and pharmacokinetics of a continuous i.t. infusion administered by an external pump as well as repeated manual bolus injections in acute SCI paraplegic and tetraplegic patients is currently ongoing. Patients must have neurologically complete thoracic or cervical lesions (C5 ≤ lesions ≥ T12) and treatment with ATI355 must begin within 4 to 28 days post injury. Patients of the first four cohorts received ATI355 by continuous intrathecal infusion, treatment duration lasted from 1 to 28 days. Patients of the fifth cohort received 6 i.t. bolus injections with 22.5 mg per injection and patients of the sixth cohort are receiving 6 i.t. bolus injections with 45 mg per injection.
Results. To date a total of 48 acute SCI patients were treated with ATI355. Twenty-three of those patients received continuous i.t. infusions and 25 had repeated i.t. bolus injections. All patients tolerated ATI355 well, the observed adverse events and serious adverse events were a consequence of the injury or caused by the infusion mode of administration or by concomitant medication. In the continuous i.t. infusion group three cases of catheter-related technical complications occurred and one patient suffered from bacterial meningitis. In contrast, repeated i.t. bolus injections were well tolerated and did not cause technical complications to date. Otherwise, no clinically relevant deviations in vital signs, ECGs or laboratory parameters were observed. ATI355 concentrations were measured in serum and CSF, both after infusions and injections. The decline in serum was slow with a terminal half-life of about 2-3 weeks. The half-life in CSF is estimated to be approximately 2-3 days.
Conclusions. No ATI355-related safety concerns were reported to date. Continuous infusion mode of administration is challenging due to infection risks and device related complications. Pharmacokinetic data are supportive to switch to repeated i.t. bolus injections, which produce relevant CSF exposure during the treatment period. The current study is suited to define the most appropriate treatment regimen for intrathecal application of the ATI355 antibody in acute SCI patients.

References:

[1] Schwab ME (2004); Nogo and axon regeneration. Curr Opin Neurobiol; 14:118-24.
[2] Chen MS, Huber AB, van der Haar ME, et al (2000); Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature; 403(6768):434-9.
[3] Walmsley AR and Mir AK (2007); Targeting the Nogo-A signaling pathway to promote recovery following acute CNS injury. Current Pharmaceutical Design; 13:2470-2484.
[4] Freund P, Schmidlin E, Wannier T, et al (2009); Anti-Nogo-A antibody treatment promotes recovery of manual dexterity after unilateral cervical lesion in adult primates – re-examination and extension of behavioral data. Eur J Neurosci; 29(5):983-96.

Supported by Novartis Pharma AG, Basel, Switzerland.

Session VII: Physiology of the Injured Human Spinal Cord I – Chair: Peter Ellaway

Cortical overexpression of neuronal calcium sensor 1 induces functional plasticity in spinal cord following unilateral pyramidal tract injury in rat

Stephen B. McMahon

Neurorestoration Group, Wolfson CARD, King's College London, Guy's Campus, London, United Kingdom.
Stephen.mcmahon@kcl.ac.uk

Following trauma to the central nervous system, neurons show very little capacity to re-grow their axons, which can lead to a permanent loss of function in the damaged regions. Here we show that this failure for axon re-growth is due to low intracellular levels of a small molecule called neuronal calcium sensor-1 (NCS1). We modified a non-reproducing virus in two ways so as to increase the level of NCS1 in neurons while simultaneously labeling them with a green fluorescent protein, which allowed us to track neuronal growth. Using this virus to increase the level of NCS1 in corticospinal tract neurones that communicate between the brain and spinal cord, we show that new axonal growth occurred in the spinal cord with or without injury to the neurons. Electrophysiological assessments demonstrated that these new processes formed functional connections in the spinal cord, and behavioral experiments revealed that this recovery also improved locomotor function. Furthermore, an increase in NCS1 protected these neurons, so that fewer of them died after the injury. These findings demonstrate that increasing the intracellular levels of NCS1 in neurons can aid in the recovery from central nervous system injury, and can help improve behavioral function.

Studies on pain after dorsal root injury

Wu A[1], Lauschke JL[1], Gorrie CA[1], Hayward I[2], Mackay-Sim A[2], Morris R[1], **Waite PME[1]**

[1]Neural Injury Research Unit, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052
[2] National Centre for Adult Stem Cell Research, Griffith University, Brisbane, QLD 4111, Australia
P.Waite@unsw.edu.au

Pain is a common consequence of dorsal root injury (Parry, 1980). Here we compare 2 rat models of cervical root injuries, one which eliminates all sensory input from the forepaw (unilateral crush of roots C5-8), the other a partial forepaw denervation (crush of roots C7,8 only). Under anaesthesia (ketamine and xylazine, 100 and 10 mg/kg, ip, respectively) the roots on the side of the preferred paw were exposed and crushed with fine forceps (No 5, 10s, repeated 3 times). Skilled reaching and ladder walking tasks were assessed 6 weeks after dorsal root injury (DRI). Mechanical sensitivity to von Frey hairs and withdrawal latency to thermal stimulation were monitored weekly for 6 weeks.
Deficits in performing the skilled reaching and ladder walking tests were seen in both groups, with the degree of impairment dependent on the lesion severity. 2-root lesioned animals developed a persistent mechanical allodynia and thermal hyeralgesia in the affected paw, whereas after 4-root lesions, reduced sensitivity occurred.

The efficacy of transplants of olfactory ensheathing cells (OECs) to modify skilled motor tasks and forepaw sensitivity was tested after 2-root lesions. OECs were prepared as previously reported (Deng et al., 2006) and genetically modified to express GFP. The cells (60 $x10^3$ in 0.5µl medium) were injected into the dorsal horn ipsilateral to the crush at C7 and C8. Injections were carried out 1 week after DRI; control animals received a similar volume of medium only.
The delayed OEC injections had no effect on the performance of skilled reaching or ladder walking. However, in OEC injected animals, the extent of allodynia and hyperalgesia was reduced from week 3 onwards compared to control animals ($p<0.05$). Histology and immunohistochemistry on the cord showed no change in CGRP, IB4 or VGLUT1 labelling as a result of OEC injection. This suggests that the anti-nociceptive effect of OECs may be independent of changes in sprouting of spared afferents from adjacent roots. Other potential mechanisms include modified dorsal horn excitability and changes in the inflammatory responses within the dorsal horn.

Electrophysiological assessment of function in animal models of spinal cord injury

John Riddell, Andrew Toft, Tao Meng, Susan Barnett, Rab Prinja & Dugald Scott

College of Medical,Veterinary and Life Sciences, University of Glasgow, Glasgow, UK
John.Riddell@Glasgow.ac.uk

Reliable and sensitive methods for assessing function in animal models of spinal cord injury are essential to the process of developing safe and effective therapies. Behavioural tests provide a useful "global" indication of function but choice of test and quantification can be problematic. In addition, they do not provide information on specific pathways or on the mechanisms underlying functional changes and cannot distinguish between mechanisms of recovery involving the brain and spinal cord. For these reasons we have explored the use of electrophysiological approaches in recent studies of experimental therapies.

Spontaneous plasticity and effect of olfactory ensheathing cell (OEC) transplants and myelin inhibitors in a cervical dorsal column transection model

Rats were subjected to a lesion of the dorsal columns at the C4/5 level interrupting the main component of the corticospinal tract and most ascending dorsal column fibres. Changes in function of the corticospinal system and sensory circuits with input from forelimb sensory afferents were assessed by recording cord dorsum potentials (CDPs) after stimulation in the pyramids (corticospinal) or of the radial nerve (sensory fibres). To investigate spontaneous plasticity electrophysiology was performed in normal animals and at different time points after a dorsal column lesion. Both corticospinal and sensory fibre systems showed modest spontaneous plasticity following a dorsal column lesion. Plasticity at the terminations of axotomised fibres occurred relatively rapidly (within one week) while plasticity in spared systems passing the injury level occurred more slowly.

To investigate whether OEC transplants enhance plasticity after spinal cord injury, OECs were transplanted so that they were distributed within the spinal cord for several mm either above or below the lesion. Electrophysiological methods were then used, as above, to investigate whether transmission in corticospinal and sensory systems was improved in transplanted animals compared to lesioned controls examined 3 months after injury. Neither corticospinal nor sensory CDPs above or below the lesion showed any enhancement in OEC transplanted animals so that OECs appear unlikely to support recovery by promoting plasticity in the spinal cord after injury.

To investigate whether anti-NOGO or anti-MAG treatment enhances plasticity following spinal cord injury, antibodies were delivered intrathecally via implanted osmotic minipumps over a period of six weeks following a cervical dorsal column lesion and electrophysiology carried out four weeks later. Vehicle treated and normal animals were investigated for comparison. The presence of the cannula and/or vehicle alone had a detrimental effect on corticospinal function above the lesion. Treatment with anti-Nogo antibody targeting the amino-Nogo terminal enhanced transmission of corticospinal actions both above and below the lesion as well as the actions of sensory afferent fibres below the lesion. In contrast, treatment with anti-MAG had no effect on either corticospinal or sensory-evoked activity in the spinal cord.

Electrophysiological assessment following contusion injuries

Certain therapeutic targets (e.g. neuroprotection of white or grey matter, demyelination) cannot be investigated in partial transection models. We have therefore designed electrophysiological tests of function around two contusion models of spinal cord injury, one at a thoracic level (T9) and the other at a cervical (C6) level. These have been designed to enable us to test i) different aspects of the pathology of spinal cord injury (effects on white matter and effects on grey matter), ii) different aspects of spontaneous repair (remyelination and plasticity) and iii) different mechanisms of therapeutic action (neuroprotection, remyelination and plasticity). Preliminary studies show the tests are sensitive to injury severity. Appropriately designed electrophysiological tests are therefore a powerful tool for the quantitative assessment of functional changes in the injured spinal cord.

Supported by the ISRT, Wellcome Trust, University of Glasgow and GSK

Session VIII: Imaging and Characterization of the Injured Human Spinal Cord – Chair: Charles Tator

Mapping of function in the injured human spinal cord by means of functional MRI

Patrick W. Stroman

Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada
stromanp@queensu.ca

Functional MRI of the human spinal cord (spinal fMRI) has been shown to be a sensitive method for mapping function non-invasively, and has the potential to become a valuable clinical tool. A significant challenge for all MRI methods applied to the spinal cord is the poor field homogeneity caused by magnetic susceptibility differences, and this is further confounded by metallic fixation devices used to stabilize the spine after trauma. Another challenge for such clinical applications of fMRI is to be able to develop a method that is practical in a clinical setting, both in how it can be applied and the time required, while ensuring that it provides sufficient information for diagnostic purposes.
Here, we present a novel method of stimulating multiple sensory dermatomes simultaneously, in order to map function on both the right and left sides of the cord, as well as above and below the injury level. Thermal stimuli are used because they are passive, and involve spinal cord pathways involved with sensation, pain responses, and a component of motor responses. The stimuli are applied in distinct block paradigms that are linearly independent to permit detection of distinct responses to each of the stimuli. Data are presented from cases of spinal cord injured patients to demonstrate the clinical value, and practicality.

Advanced techniques for imaging the spinal cord

Spyros Kollias

University Hospital of Zurich
kollias@dmr.usz.ch

The past ten years have witnessed a revolution in the diagnosis and management of spinal disorders. MR imaging has quickly emerged as the study of choice for virtually all disorders of the spine. With the inherent contrast sensitivity, the high spatial and temporal resolution, the multiplanar sampling of anatomy, the reliable differentiation between normal and pathologic tissue and the lack of irradiation hazards of MR, the morphology of the spinal cord, and nerve roots but also of the vertebrae, intervertebral disk, epidural space, can be visualized with striking clarity. The use of paramagnetic contrast agents became well established in a variety of disorders allowing definition of abnormal vessels, leptomeninges, and disrupted blood-cord barrier. State-of-the-art MR has increased the specificity of diagnosis of spinal disease, aided earlier diagnosis of spinal lesions and increased the anatomical precision of disease localization. It is now possible to diagnose processes that were previously only inferred from imaging studies. Diagnostic innovations have been followed by considerable therapeutic advances. But also therapeutic advancements particularly in the field of regeneration of the neural tissue in spinal cord injury are driving technological developments in imaging. Despite this progress, the modality remains in an evolution stage, with almost unlimited room for improvement. New imaging methodologies have been developed over the last years that are used to clarify not only morphological changes but also the physiology and pathophysiology of neural tissue.

High-resolution Magnetic Resonance (MR), diffusion-weighted (DWI) and diffusion-tensor (DTI) imaging, functional MR imaging (f-MRI) and MR spectroscopy (MRS) have evolved into important research tools for examining the structural and functional nature of neurological pathology, in both animal and human tissue. Applications in the brain have already gained widespread clinical acceptance however, imaging the spinal cord places additional demands on imaging, due to its fine structure and its elasticity, the requirement for high in-plane resolution and avoidance of artefacts arising from cord and CSF motion, respiratory motion, and swallowing. Optimization and application of these non-invasive MR techniques on studying the human spinal cord can potentially provide new morphological, physiological and functional information in vivo and eventually, important insight into a variety of disease processes affecting the spinal cord and its functional recovery after injury improving the specificity of conventional imaging approaches.

Presently, application of advanced imaging methodologies for in vivo imaging of the human spinal cord gain widespread acceptance for potential use in clinical studies. These include high resolution differentiation between spinal cord grey and white matter using high field (3-T) MR systems, evaluation of microstructural changes in the integrity of white matter using DTI, mapping functional activity of the spinal sensorimotor neurons using fMRI, as well as metabolical imaging of the spinal cord tissue using MR spectroscopy. First clinical applications in patients with demyelinating disease, (i.e., multiple sclerosis), spinal cord injury (SCI), neoplastic processes etc, indicate that these techniques provide better demonstration of the structural damage and understanding of its functional consequences and its evolution in the human spinal cord. Quantitative imaging parameters can be used as surrogate markers of disability for determining prognosis and for following up rehabilitation and pharmacologically induced recovery. With the progression of regeneration enhancing treatment for spinal cord injury form basic research to patient trials, these new diagnostic tools for the clinical assessment, including prognosis and post-treatment follow-up, become of utmost importance for assessing with increased specificity and sensitivity the structural, physiological and functional status of the human spinal cord in vivo and in the clinical setting.

It must be always remembered, however, that the body has a limited range of responses to an apparently infinite variety of insults from infectious, inflammatory, traumatic, and neoplastic entities. Images are often sensitive but not specific, and a logical pathologic differential diagnosis must be given. Further, many morphologic derangements can be demonstrated in asymptomatic individuals, which further complicates the concept of abnormality. In certain situation there may only be a moderate correlation between the imaging evidence of morphologic alteration and the presence of symptoms. These facts emphasize that

we need to concentrate more effort on determining the significance of the morphological changes we can now so exquisitely demonstrate, and the central importance of both the clinical and electrophysiological evaluation in the work-up of patients with spine disorders. Imaging is an intermediate test that must be integrated into, rather than isolated from, the clinical and neurophysiological evaluation. The management of patients with spinal disorders must begin and end with a thorough clinical assessment and imaging findings must be correlated and validated with clinical and electrophysiological parameters.

Session IX: Physiology of the Injured Human Spinal Cord II – Chair: Geoffrey Raisman

The challence with the balance: wanted versus unwanted treatment effects

Karim Fouad

University of Alberta, Faculty of Rehabilitation Medicine, 3-48 Corbett Hall, Edmonton, Canada, T6G 2G4
Karim.fouad@ualberta.ca

Over the last years the paradigm that the central nervous system (CNS) and especially the spinal cord is hard wired has been replaced with the view that following injury adaptations occur at various anatomical and physiological levels. Following spinal cord injury (SCI) this plasticity has frequently been related to functional recovery but also to emerging complications as neuropathic pain, autonomic dysreflexia or spasticity. A current goal towards a meaningful treatment of SCI is to harness plasticity and promote the mechanisms that could be considered self repair without augmenting unwanted plasticity. Over the last years we approached this by studying injury induced plasticity in motor systems. We found that spared and injured descending fibers respond to injury by sprouting new or alternate branches, a process that we recently attempted to enhance using the application of neurotrophic factors. These factors were administered at the level of the cell bodies and/or areas we considered to be meaningful targets (e.g., brainstem nuclei). Results from these studies are encouraging, demonstrating that we can promote substantial plasticity; however our results also made it very clear that there is a lot to learn in the design of meaningful treatments. An important finding was that the control of spinal cord networks, which are innervated by excitatory, inhibitory and neuromodulatory systems, has to be carefully regulated since a treatment can easily unbalance these networks. This is challenging as treatments designed to target certain descending tracts (e.g., corticospinal tract) frequently also influence others systems as serotonergic fibers, well known for their responsiveness to treatments. Together these changes might have positive effects on certain tasks but also negatively influence others, or even promote unwanted side effects. For example we find that attempts to promote recovery can partially restore motor function but can also unbalances the delicate control between exaction and inhibition of spinal networks. Thus, frequently we find that in our hands plasticity promoting treatments, as simple as rehabilitative training can promote recovery in one task but hamper the performance in another, or neurotorphic factors that promote the exaggerated recovery of motoneuron activity.
In conclusion, it appears that the injured spinal cord is in a fairly well balanced state, and any treatment effectively manipulating this system has to carefully examine the global effects on different pathways, motor, sensory and autonomic function. The suggestion that recovery and side effects may go hand in hand is supported by our recent finding that the mechanism responsible for motoneuron recovery following SCI contributes to functional recovery, but is also involved in the occurrence of exaggerated reflex responses. Therefore, it will be a challenge for the future to promote plasticity in a controlled and balanced manner, and it has to be considered that treatments might frequently come with a dark side.

These studies were supported by: The International Spinal Research Trust, Wings for Life, Alberta Innovaties Health Solutions, and the Canadian Institute for Health Research.

Glial bridges and endogenous cellular repair strategies in spinal cord injury

L. B. Jakeman[1]; *R. E. White[1], M. Rao[3,4]; B. K. Kaspar[3,4]

Departments of [1]Physiology and Cell Biology, [2]Neuroscience, and, [3]Pediatrics; The Ohio State Univ., Columbus, OH and [4]Res. Institute at Nationwide Children's Hospital, Columbus, OH
Jakeman.1@osu.edu

Astrocytes and glial progenitor cells provide a permissive terrain for axon growth during development and after spinal cord injury in lower vertebrates and very young mammals. After injury in most adult mammals, however, these cells migrate to the border and upregulate expression of growth inhibitory molecules to form a physical and chemical scar that walls off the injury site. This restricts the invasion of inflammatory cells, but also serves as a barrier to axonal regeneration. For smaller spinal cord lesions, it may be sufficient to promote sprouting and plasticity of spared circuitry in order to restore function. However, in order to repair the site of injury after more severe injuries, it will be essential to address the scar and develop bridges that are conducive to regeneration of centrally derived axons. Endogenous glial cells are not always inhibitory to axon growth; adult axons are able to regenerate along glial bridges that can be formed from endogenous astrocytes or grafted cells. We have proposed that targeting endogenous astrocytes and progenitors with an appropriate stimulus might enhance their capacity to provide an intrinsic CNS bridge that is supportive of axonal growth following spinal cord injury. Intrathecal administration of transforming growth factor α (TGFα) to the injured mouse spinal cord increased the proliferation and infiltration of astrocytes into center of a spinal cord contusion injury, accompanied by increased axon growth at the injury site. However, intrathecal administration has several caveats, including widespread activation of nonspecific targets. To provide an intraparenchymal source of TGFα, we constructed an adeno-associated virus (AAV) (serotype 1) that infects neurons and astrocytes and induces chronic production of human TGFα adjacent to the injury site. Mice injected with TGFα-AAV exhibited a less-defined glial scar at the lesion site at 10 days post injury than mice injected with a green fluorescent protein (GFP)-AAV. Using in vitro assays, we have demonstrated that TGFα is a potent, dose-dependent mitogen for NPCs, while administration of TGFα to NPC-derived astrocytes resulted in transformation to an elongated phenotype that is highly permissive for axonal growth. Together, these results demonstrate that localized administration of factors targeting the glial response to injury may be a promising step toward promoting endogenous repair after spinal cord injury.

Supported by NINDS NS043246

Table of Contents – Poster Abstracts

Timing of decompression in acute traumatic central cord syndrome associated with spinal stenosis
Bizhan Aarabi 32

The effect of non-integrating lentiviral expression of GM-CSF in the rodent central nervous system
Caroina Acosta-Saltos 33

Combined light stimulation of Channelrhodopsin-2 and Chondroitinase ABC treatment restores respiratory activity in chronically C2 hemisected rats and reveals plasticity of spinal cord circuitry
Warren J. Alilain 34

Assessing transport of integrins in adult CNS axons *in vivo*
Melissa R. Andrews 35

CNS injury: development of a novel *in vitro* model
Sue C. Barnett 36

Characterisation of the stem cell-like population found within human olfactory mucosa biopsies
Sue C. Barnett 37

Characterising functional, anatomical and electrophysiological changes from acute to chronic stages of spinal contusion injury
Katalin Bartus 38

Measuring CNS Plasticity
Karen Bosch 39

Neurotrophic factors restore locomotion in the untrained adult spinal rat
Vanessa S. Boyce 40

Experience-dependent plasticity and modulation of growth regulatory molecules at central synapses
Daniela Carulli 41

Significance of Motor Evoked Potentials in the Abductor Digiti Minimi (ADM) muscle in the foot in incomplete Spinal Cord Injury
Bernard A Conway 42

Electrical perceptual threshold: reliability and validity of a test for cutaneous sensation in spinal cord injury
Peter H. Ellaway 43

Virtual Reality for Motor Rehabilitation and functional Pain Treatment in Incomplete SCI Patients
Kynan Eng 44

Corticomotor representation to human arm muscle changes following cervical spinal cord injury
Patrick Freund 45

Investigating corticospinal tract integrity using diffusion tensor MRI following spinal cord injury
Patrick Freund 46

Disability, cortical reorganization and atrophy following spinal cord injury
Patrick Freund 47

Changes in trans-cranial MEPs and SSEPs in association with cellular injections into porcine spinal cord injury epicenters after SCI
James Guest..... 48

The effects of eicosapentaenoic acid delivered as dietary treatment after spinal cord injury
Jodie C.E. Hall 49

Tropism of adeno-associated virus 8 for large diameter sensory neurons of dorsal root ganglia after direct injection or intrathecal delivery
Steven J Jacques 50

Multiple Intrinsic and Extrinsic Factors Restrict Sensory Axon Regeneration in Chronic Spinal Cord Injury
Ken Kadoya 51

Development of a myelination assay of human neurons generated from HESCs
Bilal E. Kerman 52

Electrophysiological properties of bilateral VPL neurons after spinal cord hemisection injury
Li Liang 53

Delivery of Decorin to acute dorsal column lesion sites suppresses inflammation, scar formation and angiogenesis
Daljeet Mahay..... 54

In vitro assay to measure inflammatory response in human glial cells after injury
Maria Carolina Marchetto 55

Deciphering the regeneration-associated gene expression program: gene expression profiling of axotomized facial motor neurons in conditional c-Jun knockout mice
Matthew R.J. Mason 56

Specific and synergistic functions of monoaminergic receptors in the control of spinal locomotion
Pavel Musienko 57

Local translation of MAP2K7 allows JNK-dependent neurite outgrowth
Olivier Pertz. 58

Investigating the reactivation of structural plasticity after digestion of Chondroitin Sulfate Proteoglycans with Chondroitinase ABC
Oliver Raineteau 59

A multi-step screening approach successfully uncovers novel genes involved in the regeneration-promoting properties of olfactory ensheathing glia cells
Kasper C.D. Roet..... 60

Optimized diffusion MRI protocols for estimating axon diameter with known fibre orientation
Torben Schneider 61

Microstructural spinal changes detected by diffusion tensor imaging in chronic spinal cord injury
Torben Schneider 62

Ketogenic diet improves gross forelimb function and fine-motor skills after incomplete cervical SCI
Wolfram Tetzlaff..... 63

Transplantation of skin-derived precursors differentiated into Schwann cells at eight weeks after spinal cord contusion
Wolfram Tetzlaff..... 64

Neurochemical biomarkers concentrations in the CSF of patients with traumatic spinal cord injury
Henk van de Meent 65

The effect on migration of primary Schwann cells and SCTM41 cells expressing a modified chondroitinase ABC enzyme
Philippa M. Warren 66

Promoting the survival, migration and integration of Schwann cells after transplantation into spinal cord by overexpression of polysialic acid
Yi Zhang 67

Timing of decompression in acute traumatic central cord syndrome associated with spinal stenosis

Bizhan Aarabi

University of Maryland, Baltimore, USA
baarabi@smail.umaryland.edu

Background: Preliminary analysis of STASCIS III database has indicated a modest positive effect on AIS of earlier (within 24 hours) spinal cord decompression in patients with cervical spinal cord traumatic injury. The efficacy of surgical decompression in helping functional recovery in acute traumatic central cord syndrome (ATCCS) due to spinal stenosis is even less clear.
Object: Testing the safety, feasibility and efficacy of a prospective randomized trial of early (within 5 days) and late (within 6 weeks) spinal cord decompression in ATCCS due to spinal stenosis.
Methods: In an extensive 100-month preliminary study of 42 patients with ATCCS due to spinal stenosis, timing of decompression within and after 48 hours of injury was ineffective in 4 domains of outcome: Follow-up ASIA motor score p=0.47, ; Functional Independence Measure p=0.88; Manual Dexterity p=0.9; and dysesthetic pain p=0.19.
From May 2007-May 2009, 77 patients with ATCCS were screened for enrollment in a prospective randomized trial to study the effectiveness of decompression of spinal cord within 5 days or after 6 weeks of ATCCS due to spinal stenosis and hyperextension. Injury was due to fall in 41, MVC in 24 and sports in 7. Injury was varied in another 5 subjects. Mean age was 56.3 and 59 patients were male. Injury was due to hyperextension in 45, fracture subluxations in 19, disc protrusion in 7 and SCIWORA in 6. Only 63 patients were eligible for surgery of which 16 were fit for enrollment in the present study. Seven patients signed legally informed consent: 2 in late and 5 in early decompression. One of 7 died 6 months after surgery and six patients were followed for at least one year.
Results: Admission ASIA motor score in five early surgical patients was 85 and in 2 late surgeries 50.5. One patient in the early surgical group died at 6 months and the mean ASIA motor score for 4 remaining patients was 100 at the end of one year of follow-up. In the late group one patient had an AMS at 1 year of 100 and another one 37. The latter had an admission AMS of 31.
Conclusions: Study of early (within 5 days) versus late (within 6 weeks) effects of spinal cord decompression in ATCCS due to spinal stenosis, though difficult, it is not impossible. A multicenter study of the subject is recommended.

The effect of non-integrating lentiviral expression of GM-CSF in the rodent central nervous system

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

[1] Perinatal Brain Repair Group, Institute for Women's Health, UCL
[2] Molecular Immunology Unit, Institute of Child Heath, UCL
[3]Department of Anatomy and Developmental Biology, UCL
francia.acosta-saltos.09@ucl.ac.uk

Neurons projecting axons through the injured PNS mount a competent regenerative response, characterised by neuronal expression of regeneration-related proteins and activation of resident microglia around the axotomised neuronal cell bodies. In contrast spinal cord injury does not trigger such a response by corticospinal neurons and their neighbouring microglia. Activated microglia are known to produce a host of neurotrophic factors and neuroinflammation around neuronal perikaria has been associated with increased neurite outgrowth.

To test the effect of increasing microglial activation on the neuronal regenerative response after spinal cord injury, we have created a non-integrating lentiviral vector (NILV) expressing the potent microglia mitogen and activator, granulocyte macrophage colony-stimulating factor (GM-CSF), under the control of the spleen focus-forming virus (SFFV) promoter. The viral vector is pseudotyped with the vesicular somatitis virus envelope glycoprotein (VSV-G), which ensures predominantly neuronal expression of the viral genome. In addition, to identify transfected neurons and their axons our NILV encodes for enhanced green fluorescent protein (eGFP), whose translation is initiated by the X-linked inhibitor of apoptosis (XIAP) internal ribosome entry site (IRES).

When we tested our virus in vitro, supernatant from HEK-293T cells infected with increasing titres of our GM-CSF/eGFP NILV caused a dose dependent increase in cell density of the murine microglial BV-2 cell line, with half maximal increase at 1:300 dilution. We then tested the effect of the virus in vivo by stereotactically injecting it in rat motor cortex and striatum. 14 days after injection, CNS areas treated with GM-CSF/eGFP NILV revealed extensive local trauma, as well as widespread activation of microglial cells and the entry of small rounded macrophages, using immunohistochemistry for alphaM and IBA-1. Areas injected with the control NILV only expressing the EGFP construct revealed little trauma and activation of microglia.

Since working with mice provides the advantage of widely available transgenics, we also investigated GMCSF effect in the mouse motor cortex, starting with the CD1 mice, 1, 2, 4, 7, 14 and 28 days after injections. However, the level of microglia activation here appeared to be just slightly more than that seen in eGFP-only NILV injections. Since mouse strains differ widely in many respects particularly in their immune responses, we are currently also screening different mouse strains to see whether this low susceptibility is general, or whether there are strains with a clear and strong microglial response as that seen in rats.

In summary, overexpression of GMCSF using non-integrating lentiviral constructs in rat CNS produces a highly reliable, mciroglia/macrophage-driven inflammatory response that we are currently testing for possible effects on central regeneration. In addition, we are also screening for mouse strains with similar susceptibility which would allow the use of this vector in transgenic animal models.

Combined light stimulation of Channelrhodopsin-2 and Chondroitinase ABC treatment restores respiratory activity in chronically C2 hemisected rats and reveals plasticity of spinal cord circuitry

Warren J. Alilain*, Xiang Li, Thomas E. Dick, Stefan Herlitze, Jerry Silver

*Department of Neurosciences, 2109 Adelbert Road, Room E-658, Case Western Reserve University School of Medicine, Cleveland, OH USA
warren.alilain@case.edu

Spinal cord injury (SCI) is often at the cervical level and can lead to respiratory complications and death. Diminished respiratory capacity is usually due to impairment or paralysis of the diaphragm muscle. To study these complications, the changes which take place in the spinal cord after injury, and potential therapeutic strategies, we utilize the C2 hemisection model of SCI, which disrupts bulbospinal inputs to the phrenic motor nucleus and results in paralysis of the ipsilateral hemidiaphragm. While most of the SCI community is at chronic post-injury states, there are few reparative strategies that have been successful at lengthy times after injury. In our prior studies, we have shown that immediately following C2 hemisection there is a dramatic increase of the perineuronal net and chondroitin sulfate proteoglycans around denervated phrenic motor neurons. Degradation of these plasticity inhibiting extracellular matrix molecules with Chondroitinase ABC (ChABC) can lead to a partial restoration of hemidiaphragm activity. Additionally, in another set of experiments, we have shown that expression and photostimulation of the light sensitive cation channel channelrhodopsin-2 (ChR2) can induce long lasting recovery of the paralyzed hemidiaphragm at acute stages after C2 injury. Restoration of rhythmic respiratory activity was achieved through expression of a unique form of respiratory plasticity not described before, possibly induced through a spinal respiratory central pattern generator. We hypothesized that after infecting rat spinal neurons in and around the phrenic motor pool to express ChR2, light stimulation would restore respiratory motor function in chronic C2 hemisected adult rats. Our results show that expression of ChR2 ipsilateral to the lesion and subsequent photostimulation can lead to fragmented inspiratory activity/breaths contralateral to the lesion but with a modest return of function ipsilaterally. However, when ChR2 is expressed contralateral to the chronic lesion and then light stimulated, a higher level of restored respiratory activity ipsilateral to the lesion is achieved. When combined with ChABC treatment, a significant amount of recovery is obtained. These results strongly suggest considerable remodeling of respiratory circuitry in the chronically injured animal and the presence of functionally critical contralateral projecting interneurons. Additionally, these experiments show that inhibitory factors are present in the chronically injured animal and that removal is necessary to induce significant recovery. Overall, we demonstrate that ChR2 and light stimulation can be used to induce recovery in the chronically C2 hemisected animal; as well as reveal the changes which take place after injury and the capacity for plasticity at both the circuit and systems level.

References:
Alilain WJ, Li X, Horn KP, Dhingra R, Dick TE, Herlitze S, Silver J. Light Induced Return of Motor Function After Spinal Cord Injury. *J. Neuroscience.* 28(46): 11862-11870. 2008.

Alilain WJ and Silver J. Shedding light on restoring respiratory function after spinal cord injury. *Frontiers in Molecular Neuroscience.* 2(18): 1-6. 2009.

Funded by the International Spinal Research Trust (WJA) and NIH Grant NS060767 (JS)

Assessing transport of integrins in adult CNS axons *in vivo*

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

[1]University of Cambridge, Centre for Brain Repair, Robinson Way, Forvie Site, Cambridge, CB2 0PY, UK;
[2]Ecole Polytechnique Fédérale de Lausanne, Brain Mind Institute, EPFL SV BMI LEN, AI 1207, Lausanne, Switzerland
mra36@cam.ac.uk

As the central nervous system (CNS) matures, the regenerative ability of neurons and axons decrease. Several proteins found within the developing nervous system become downregulated or silenced, including many transmembrane receptors such as integrins which bind to extracellular matrix molecules present in the CNS. In previous studies, reintroduction of integrin receptors in adult neurons *in vitro* successfully enhanced neurite outgrowth on inhibitory substrates. We have demonstrated that *in vivo* forced expression of the alpha9 (α9) integrin subunit (tenascin-C receptor) promotes axon regeneration after dorsal rhizotomy or dorsal column crush injury for short distances. An important finding in our study was the disparity observed between the substantial *in vitro* effect and the modest *in vivo* result. Therefore, in the current study we are evaluating the *in vivo* localization/transport of different integrin subunits within CNS axons. To perform these studies we have ectopically expressed an eYFP-tagged integrin using lentivirus injected into uninjured adult rat sensorimotor cortex. In intact cortex, results with integrin alpha 6 (laminin receptor, α6) show that α6-eYFP remains mostly in neuronal cell bodies with some observed within the immediate processes. Even upon addition of a cervical spinal injury, in an attempt to stimulate transport, we observed no difference in the localization of α6-eYFP or α9-eYFP beyond the cell body. In contrast, evaluation of the same paradigm in developing rat cortex (P0 aged rat pups) has revealed striking differences. We observe localization of α9-eYFP in the neuronal cell body with evidence of integrin transport into axons of the corpus callosum and/or internal capsule. We are now examining differences that may exist between adult and developing CNS neurons which may shed light on the disparity we observe in integrin transport. We believe these findings will further our understanding of the distinct cellular mechanisms associated with the inability of CNS neurons to regenerate.

CNS injury: development of a novel *in vitro* model

S.D. Boomkamp, L. Ross[†], M. Riehle[†] **Sue C. Barnett**

Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8QQ
[†] Centre for Cell Engineering, University of Glasgow, Glasgow G12 8QQ
s.barnett@clinmed.gla.ac.uk

Damage to the central nervous system (CNS) significantly impacts the ability of nerves to communicate and often leads to loss of neuronal function. Currently there are few *in vitro* models to evaluate the pathological consequences of CNS injury. Despite this, research into the repair of spinal cord injury (SCI) has seen significant progress and it is now believed a combination of treatments may promote functional regeneration, such as cell transplantation, growth factors and reagents that manipulate and reduce the inhibitory environment of the spinal cord**.** To reduce animal load and get a more rapid screen of a range of compounds we have developed an *in vitro* model of SCI using a previously described CNS mixed cell culture system. These cultures produce a carpet of spinal cord axons when plated on a monolayer of neurosphere-derived astrocytes which become myelinated by oligodendrocytes which form internodes of myelin and nodes of Ranvier. We can cut these axons and follow axonal outgrowth and myelination over time. Following establishment of the base-line culture status after axotomy, a combination of reagents will be introduced to assess their affect on axonal outgrowth and myelination. After axotomy, areas of demyelination and axonal damage could be observed using a panel of neural markers. Areas in close proximity to the cut areas were cell-free for 5-10 days, whereas the cells adjacent to the lesion remained viable. Over time the gap around the lesion filled with phase-bright cells, some expressing GFAP, suggesting the ingrowth of astrocytes. Interestingly these cells appeared reactive and upregulate markers of astrocytic reactivity, including nestin, CSPGs. Axons align up to the lesion and do not cross the area filled with astrocytes. Preliminary data has shown that the addition of inhibitors of the Rho GTPase pathway, Y-27632 (a Rho-kinase inhibitor) and a cell permeable Rho inhibitor (C3, ADP-ribosyltransferase) appear to promote axonal regeneration and myelination demonstrating their known therapeutic potential in the treatment of SCI and supporting the validity of the developed *in vitro* model of SCI.
In order to achieve a more organized topology allowing easier measurement of axonal outgrowth, micro-engineered biodegradable constructs are currently being developed to align axons prior to axotomy. In summary, we hope this *in vitro* cellular model of CNS injury will allow the assessment of the cellular changes occurring during neuronal degeneration and regeneration following introduction of combined therapeutics.

Characterisation of the stem cell-like population found within human olfactory mucosa biopsies

Lindsay SL, Clark L*, Sheikh S*, **Sue C. Barnett**

Address Division of Clinical Neurosciences, GBRC, Glasgow University, Glasgow, G12 8TA *ENT Department, Southern General Hospital, Glasgow, G51 4TF.
s.barnett@clinmed.gla.ac.uk

The peripheral olfactory system undergoes continual neurogenesis throughout life, making it a unique tissue. It is comprised of the olfactory mucosa (OM) which resides in the PNS and the olfactory bulb (OB) which resides in the CNS. The olfactory mucosa (OM) consists of the olfactory epithelium (OE) and the underlying lamina propria (LP). The capability of the OM to repair has been attributed in part to the multipotential stem cells present in the basal layer of the OE. However, we have recently shown that embryonic rat OM contains two stem cell-like populations; one originating from the OE and the other from the LP. Since transplants of human OM are already ongoing in clinical trials, it is necessary to assess the potential of such tissue as a source of stem cells and focus on whether human OM has similar stem cell populations as described for the rat. Human olfactory biopsies were obtained with ethics approval from nasal/polypectomy surgery. Samples were processed for immunofluorescence or cultured in neurosphere media to promote stem cell-like proliferation. The staining profile of human and rat mucosa was compared using a panel of markers and similar patterns were found. Culture of human OM resulted in either the generation of neurospheres, akin to that found in the rat and/or a monolayer of cells. The majority of neurospheres were tightly packed with a defined membrane expressing cytokeratin positive cells. In addition, other spheres were found to contain cells positive for nestin, SMA, Stro-1, GFAP and Thy1.1. Cells derived from spheres were found to undergo differentiation into osteogenic and adipogenic lineage when placed into the appropriate induction medium, suggesting they have mesenchymal stem cell-like properties. Cells from monlayers had a mixed phenotype expressing both OE and LP markers. Preliminary data using FACS analysis has shown it is possible to isolate Stro-1 positive cells from these mixed populations. We can also purify these MSC-like cells using magnetic beads. These results suggest that there are similarities between human and rat OM and they each contain distinct cell populations with stem-cell like properties. Interestingly, there may be a mesenchymal stem-cell like population which is likely to originate from the LP. These findings highlight the cellular complexity of olfactory tissue as a potential source of stem cells.

Supported by Chief Scientists office

Characterising functional, anatomical and electrophysiological changes from acute to chronic stages of spinal contusion injury

Nick James**, Katalin Bartus**, Daire Rowlands, John Grist, Stephen McMahon, Elizabeth J. Bradbury

The Wolfson Centre for Age-Related Diseases, Neurorestoration Group, King's College London, London SE1 1UL
elizabeth.bradbury@kcl.ac.uk

Traumatic spinal cord injury (SCI) generally results in severe motor, sensory, and autonomic deficits below the level of the injury. As contusion injuries are the most common form of SCI in humans, an animal model of spinal contusion injury provides a clinically relevant tool for studying pathological changes that occur following SCI and to assess the efficacy of potential therapeutic interventions. We have performed a detailed characterisation of some of the physiological changes that occur from acute to chronic stages post injury in this model, using a novel electrophysiological technique to assess axonal conduction through the lesion over time. Adult rats received a 150kD (Infinite Horizons) contusion injury and electrophysiological recordings were performed at a number of time points (1, 7, 28 and 84 days) post injury. Acutely (1 day) post-injury there was a complete absence of conduction across the contusion site. This increased slightly in the sub-acute stage (1 week), with the percentage of axons conducting across the injury remaining at low levels as the injury progressed to chronic stages (4 – 12 weeks). The electrophysiological data exhibited a similar pattern to the behavioural assessments (BBB locomotor scale and ladder walking) which showed an initial severe functional deficit, with some improvement over the sub-acute stage, but no further recovery into the chronic stages. Anatomical characterisation was also performed at the different injury time points, to assess the degree of tissue loss, cell death, glial scarring and demyelination. Further characterisation at the electron microscopic level will provide a valuable anatomical correlate to the assessments of conduction properties of surviving axons following contusion injury. Thus, we have provided a detailed characterisation of changes over time in a clinically relevant spinal injury model. Such thorough assessments, which combine electrophysiological and behavioural function with anatomical measures, could prove invaluable to furthering our understanding of mechanisms underlying the pathological events that occur following spinal cord injury, and for assessing potential therapies aimed at promoting repair.

Measuring CNS Plasticity

Karen Bosch, Franziska Denk, James Fawcett, Stephen McMahon

Neurorestoration Group, Wolfson CARD, King's College London, Guy's Campus, London, SE1 1UL, United Kingdom
karen.bosch@kcl.ac.uk

Current treatment for peripheral nerve injury is surgical repair but recovery is often disappointing (Jaquet et al, 2001) because topographical accuracy of re-innervation is poor (Nguyen et al, 2002). Using electrophysiological techniques we found robust differences in ulnar nerve reflex responses to median or radial nerve stimulation in naïve rats. By creating animals with different degrees of inaccurate re-innervation (cut and direct repair vs. cut and crossover repair) we aim to establish what level of compensation the normal animal can make for inaccurate re-innervation, using these reflex measures. We will then ask whether intraspinal chondroitinase ABC, an experimental treatment that may promote CNS plasticity, can encourage central compensation for disrupted reflexes. We will use behavioural as well as electrophysiological outcome measures. To investigate anatomical changes we have constructed two lentiviruses that express tagged proteins that localise at synapses, synaptophluorin and synaptophysin. We will inject these constructs into the cortex to transfect corticospinal tract neurons and thereby reveal the synaptic organisation of this projection in the spinal cords of both normal and experimental rats. We hypothesise that enhanced plasticity in the spinal cord will allow compensation for inaccurate wiring in the periphery by amplifying desirable adaptive changes.

Jaquet JB, Luisterburg AJ, Kalmijn S, Kuypers PD, Hofman A, Hovious SE (2001) Median, ulnar and combined median-ulnar nerve injuries: functional outcome and return to productivity. J Trauma 51:687-692.
Nguyen QT, Sanes JR, Lichtman JW (2002) Pre-existing pathways promote precise projection patterns. Nat Neurosci 5:861-867 .

This work is supported by Spinal Research.

Neurotrophic factors restore locomotion in the untrained adult spinal rat

Vanessa S. Boyce[1], Jihye Park[1], Sandeep S. Reddy[1], Kathleen P. Thomas[1], Fred H. Gage[2] and Lorne M. Mendell[1]

[1] Department of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook NY
[2] Salk Institute, La Jolla, CA
lmendell@notes.cc.sunysb.edu

A combination of the neurotrophic factors (NTFs) BDNF and NT-3 was previously shown to restore locomotor function in the adult spinal cat (Boyce et al, 2007). Here we examine the effect of each factor on locomotor recovery in the untrained adult spinal rat and provide electrophysiological analysis of their possible mode of action.
Female adult rats received complete spinal cord transection injuries at T10-11 (Tx) and BDNF or NT-3 were administered at the injury site via adeno associated viral vectors (AAV). Control rats received AAV-GFP or injury alone. Animals were housed singly and received no step training. At 2, 4 and 6 weeks post -Tx locomotor function was assessed via treadmill and footprint analysis. Over-ground locomotion and sensitivity to noxious thermal stimuli (Hargreaves test) were also assessed at 7 weeks post -Tx.
During terminal electrophysiological experiments (6-9 weeks post –Tx), intracellular measurements of rheobase (Rh), input resistance (R_n) and afterhyperpolarization depth (AHP) were made from antidromically identified medial gastrocnemius (MG) and lateral gastrocnemius soleus (LGS) motoneurons. The amplitude of the segmental (sEPSP) and central (cEPSP) synaptic responses was also determined by electrical stimulation of the MG or LGS nerves or by stimulation of the ventrolateral funiculus respectively.
Kinematic analysis of ***treadmill locomotion*** showed that control rats never regained locomotor ability. However, at 2 weeks post –Tx, BDNF rats exhibited plantar stepping while NT-3 rats recovered stepping ability by the 4th week post-Tx. There was no significant difference between these groups at 6 weeks post-Tx. Interestingly, in tests of ***over-ground locomotion*** only BDNF treated rats could generate volitional plantar weight bearing steps. Increased thermal sensitivity was observed in BDNF rats only.
Motoneurons were significantly more excitable in BDNF- treated rats (Mean Rh: 5.3 nA) than in NT-3- treated counterparts (10.3 nA). Control rats exhibited Rh midway between these two values. Mean R_n was lower and mean sEPSP tended to be larger in NT-3 rats compared to BDNF rats. Conversely AHP depth was significantly larger in BDNF versus NT-3 treated animals. cEPSP was not significantly different between groups.
These results demonstrate that either BDNF or NT-3 could restore locomotion in untrained adult spinal rats. The electrophysiological analysis suggests that the mechanisms by which this occurs differs between the NTFs. We speculate that BDNF increases excitability in motoneurons, and perhaps in trkB- expressing interneurons to allow intrinsic spinal stepping patterns to be expressed during both ***treadmill*** and ***over-ground locomotion***. In contrast, NT-3 reduces AHP depth thereby increasing the discharge rate of motoneurons when they reach threshold. This may be important in ***treadmill locomotion*** where spindle activity driven by the action of the moving treadmill on the limb joints would be sufficient to discharge motoneurons despite the elevated threshold and contribute to stepping (Pearson, 2004). The latter is reduced in ***over-ground*** locomotion and so the effect of reduced AHP depth would be minimized. Finally, these results lend support to the translational potential of BDNF and NT-3 in restoring locomotor function after spinal cord injury.

References
Boyce VS, Tumolo M, Fischer I, Murray M, Lemay MA (2007). Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats. J Neurophysiol 98:1988-1996.
Pearson KG (2004). Generating the walking gait: role of sensory feedback. Prog Brain Res 143:123-129.

Supported by the Christopher and Dana Reeve Foundation and the National Institutes of Health.

Experience-dependent plasticity and modulation of growth regulatory molecules at central synapses

Foscarin S.[1], Ponchione D.[1], Leto K.[1], Pajaj E.[1], Gawlak M.[2], Wilczynski G.[2], Rossi F.[1], **Daniela Carulli**[1]

[1] Scientific Institute of the Cavalieri-Ottolenghi Foundation, Neuroscience Institute of Turin (NIT), University of Turin, Orbassano (Turin), Italy
[2] Dept. of Neurophysiology and Molecular Morphology, Nencki Institute of Experimental Biology, Warsaw, Poland
daniela.carulli@unito.it

The decline in CNS plasticity that occurs during development is partly due to the appearance of extracellular matrix aggregates around some kind of neurons, so-called perineuronal nets (PNNs; Pizzorusso et al. 2002). Nonetheless, neurite remodeling can occur in the adult, particularly after exposure to increased environmental stimulation (Nithianantharajah and Hannah, 2006). To unravel some of the mechanisms that underlie experience-dependent plasticity, we studied the effects of 1 month of enriched environment (EE) on Purkinje cell (PC) axons, which contact deep cerebellar nuclei (DCN) neurons. Moreover, we assessed the effects of EE on plasticity of transgenic PCs overexpressing the growth-associated protein GAP-43, which show enhanced plastic abilities after injury compared to wild-type (WT) mice (Buffo et al. 1997).
We found that in the DCN of enriched animals PC axon terminals increased in size, glutamatergic terminals increased in number and, in parallel, PNNs around DCN neurons were reduced. These effects were more pronounced in GAP-43 mice. When we analyzed mutant mice with reduced PNNs due to the lack of a key PNN component, cartilage link protein 1, we found that PC terminals of these mice were bigger than in WT mice, suggesting that PC plasticity after EE may depend on a reduction of inhibitory signals conveyed by PNNs. To elucidate whether PNN reduction after EE is due to a decrease in the synthesis of PNN components, we examined the expression of key PNN molecules by *in situ* hybridization and real time PCR in WT and GAP-43 mice. The mRNA levels for cartilage link protein 1 and aggrecan decreased after EE in the DCN of WT mice but not of GAP-43 mice. To assess whether increased matrix degradation contributes to PNN reduction, we investigated the activity of matrix metalloproteinases (MMPs) by in situ zymography. After EE, MMP enzymatic activity was increased in PCs. Moreover, it was higher in DCN neurons that bore reduced PNNs, particularly in enriched GAP-43 mice. Finally, to see whether EE-dependent PNN decrease is linked to increased activity of DCN neurons, we removed part of the GABAergic inputs of DCN neurons by chemically depleting PCs of a restricted cortical region. PNNs in partially deafferented DCN showed a reduction in their staining intensity similarly to what we observed after EE. These results indicate that the synthesis of PNN components is regulated by an interplay between pre- and postsynaptic partners, which is strongly influenced by experience. In this way, external stimuli modulate growth-regulatory mechanisms in order to fine-tune the plastic capabilities of neural circuits and connections.

Buffo A, Holtmaat AJ, Savio T, Verbeek JS, Oberdick J, Oestreicher AB, Gispen WH, Verhaagen J, Rossi F, Strata P (1997) Targeted overexpression of the neurite growth-associated protein B-50/GAP-43 in cerebellar Purkinje cells induces sprouting after axotomy but not axon regeneration into growth-permissive transplants. J Neurosci 17:8778-8791.
Nithianantharajah J and Hannan AJ. (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 7:697-709.
Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002) Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248-1251.

Supported by International Foundation for Research in Paraplegia (IRP)

Significance of Motor Evoked Potentials in the Abductor Digiti Minimi (ADM) muscle in the foot in incomplete Spinal Cord Injury

Galen SS, Catton CJ, Allan DB, **Bernard A Conway**

Bioengineering Unit, University of Strathclyde, Glasgow, & Queen Elizabeth National Spinal Injuries Unit, Southern General Hospital, Glasgow, UK
b.a.conway@strath.ac.uk

Motor Evoked Potentials (MEP) produced through Transcranial Magnetic Stimulation (TMS) has been used to assess the function of motor pathways following spinal cord injury. The MEP recordings in the ADM muscle in the upper limb have been shown to be highly predictive of the recovery of hand function in incomplete Spinal Cord Injury (ISCI). However to date the relationship between the MEP recordings in the lower limb ADM muscle and recovery of ambulatory function has not been investigated. In this study we have used TMS to record MEP in lower limb muscles, prior to and following an intensive 6 –week locomotor training programme using the lokomat®. Eighteen subjects (13 acute and 5 Chronic) with ISCI participated in this study (Age range: 26-63 years). MEPs were recorded in the Quadriceps, Tibialis Anterior, Gastrocnemius and ADM muscles. MEP responses in the ADM muscle was elicited in over 80% of the acute subjects prior to the lokomat training who were classified as functional walkers following the lokomat training. All Chronic subjects who were classified as functional walkers also had a response elicited in the ADM prior to lokomat training. Presence of MEP in ADM following ISCI may be closely related to locomotor recovery.

Electrical perceptual threshold: reliability and validity of a test for cutaneous sensation in spinal cord injury

Peter H. Ellaway[1], A. Kuppuswamy[1], A.V. Balasubramaniam[1], R. Maksimovic[1], C.J. Mathias[1], A. Gall[2], M.D. Craggs[2]

[1]Division of Experimental Medicine, Imperial College, London W6 8RP, UK. [2]The London Spinal Cord Injuries Centre, Royal National Orthopaedic Hospital NHS Trust, HA7 4LP, UK.
p.ellaway@imperial.ac.uk

The ability to detect physiological change associated with rehabilitation or treatments to effect axonal regeneration in spinal cord injury (SCI) will be challenging using the widely employed American Spinal Injuries Association (ASIA) impairment scales (AIS) for sensory and motor function. Despite many revisions to the AIS standard neurological assessment, there remains a perceived need for more sensitive, quantitative and objective outcome measures.
Our aim in Stage 2 of the ISRT Clinical Initiative was to validate and test the reliability of developed and improved physiological tests. As a treatment that was expected to improve functional outcome, repetitive (5Hz) transcranial magnetic stimulation (rTMS) was applied to the motor cortex in 15 stable (chronic) cervical spinal cord injury subjects and compared with sham stimulation. The aim was to see whether any functional changes were correlated with physiological changes to sensory, motor or autonomic systems.
The rTMS produced only modest group improvements in hand function (Action Research Arm test) that did not reach statistical significance. There were no changes in level of SCI (ASIA) or in AIS sensory or motor scores. Two of the subjects showed sustained (up to 120 hours) increases in cutaneous sensitivity within cervical and T1 dermatomes as measured by the electrical perceptual threshold (EPT) test (Savic et al, 2006). Although the other subjects showed no changes to either rTMS or sham stimulation, it was recognised that repeated measures of EPT provided a further opportunity to examine the reliability of the technique. King et al (2009) had previously established good inter and intra-rater repeatability for EPT in SCI subjects. In this rTMS study, highly significant Pearson's product moment correlations were evident when comparing baseline with post treatment EPT measures for C3 to T1 dermatomes. Correlation coefficients ranged from 0.62 to 0.95 ($P<0.001$) for 11 of the 13 subjects who showed no change to rTMS/sham treatment, and were 0.37 ($P<0.05$) and 0.12 ($P>0.05$) for the other two subjects.
An additional result from this study provided circumstantial evidence to support the notion (Leong et al, 2009; Kramer et al, 2010) that EPT tests the dorsal (posterior) column pathway for light touch rather than the anterolateral spinothalamic pathway for pain and temperature sensibility. Subjects showed an association between impaired AIS scores for light touch and raised EPT but dissociation between AIS pin prick scores and EPT. For example, one subject (ASIA D, level C4) had EPT values within the normal range on the right side for all dermatomes tested (C3 - T1) and for all but dermatomes C5 and C6 on the left. AIS light touch scores were also normal for all dermatomes on the right and abnormal over dermatomes C5 to C8 on the left. In contrast, pin-prick scores on the right side were grossly abnormal over dermatomes C6 -C8 at which levels both light touch and EPT values were normal.
The high levels of correlation on repeating the EPT measures emphasise the reliability of the technique for providing a quantitative measure of cutaneous sensibility at multiple sites in SCI. Indirect evidence suggests that EPT tests the dorsal column afferent projection pathway.

References
N.K. King, G. Savic, H. Frankel, A. Jamous, P.H. Ellaway. 2009. Reliability of cutaneous electrical perceptual threshold in the assessment of sensory perception in patients with spinal cord injury. J. Neurotrauma. 26, 1061-8.
J.K. Kramer, P. Taylor, J.D. Steeves, A. Curt. 2010. Dermatomal Somatosensory Evoked Potentials and Electrical Perception Thresholds During Recovery From Cervical Spinal Cord Injury. Neurorehabil. Neural Repair. 24, 309-17.
G.W. Leong, C.A. Gorrie, K. Ng, S. Rutkowski, P.M. Waite. 2009. Electrical perceptual threshold testing: a validation study. J. Spinal Cord Med. 32, 140-6.
G. Savic, E.M. Bergström, H.L. Frankel, M.A. Jamous, P.H. Ellaway, N.J. Davey. 2006. Perceptual threshold to cutaneous electrical stimulation in patients with spinal cord injury. Spinal Cord. 44, 560-566.

Virtual Reality for Motor Rehabilitation and functional Pain Treatment in Incomplete SCI Patients

Michael Villiger, Jeremy Spillmann, Bruno Meilick, Pawel Pyk, Edith Chevrier, Natalia Estevez, Marie-Claude Hepp-Reymond, Armin Curt, Daniel Kiper, Spyros Kollias, Sabina Hotz-Boendermaker, **Kynan Eng**

Institute of Neuroinformatics, Uniklinik Balgrist and Institute of Neuroradiology
University of Zurich and ETH Zurich
kynan@ini.phys.ethz.ch

Spinal cord injury (SCI) causes both long-lasting lower limb sensorimotor dysfunction and, in 69% of cases, associated pain of which about 40% is of neuropathic origin. Currently, although these two conditions share related cortical mechanisms, pharmacological interventions are mostly used for neuropathic pain and physiotherapy for sensorimotor dysfunction. Interventions which directly address both neuropathic pain and motor dysfunction may thus bring substantial benefits to incomplete SCI (iSCI) patients.

We are creating the first virtual reality (VR) training system for lower limb movements combining action observation and execution using motivating games. It is known that execution and observation of goal-directed actions activate overlapping cortical networks ("mirror system", (Rizzolatti and Craighero 2004), even in chronic SCI patients. There is also some evidence that neuropathic pain in iSCI patients can be reduced by viewing a virtual illusion of walking (Moseley 2007). Therefore, with intensive training using entertaining games on our VR system, we may be able to reshape cortical networks to simultaneously improve motor function and reduce neuropathic pain in iSCI patients.

Our project combines technology development, clinical testing, and neuroimaging studies. To interface to the VR system we are developing size-adjustable wearable shoe sensors to measure both foot pressure distribution and leg/foot angles. Clinical assessment before and after treatment will test the hypothesis that that training with our VR system reduces neuropathic pain and improves lower limb function in chronic and acute iSCI patients. In addition, neuroimaging studies will examine the relationships between goal-directed observation, imagination during observation, and imitation in healthy volunteers and iSCI patients. We expect to see training-related changes in cortical activation in sensorimotor areas corresponding to a reduction in neuropathic pain and improvements in motor function.

So far in this project, we have produced a first prototype of the VR system and completed the fMRI study in the healthy control group. The neuroimaging study is the first to show fMRI activation resulting from combined observation and imagination of lower limb movements. In future activities we will test SCI patients using the same paradigm. We will also begin usability and clinical testing of the VR system in iSCI patients and further refine the design of the VR system.

Moseley, G. L. (2007). Using visual illusion to reduce at-level neuropathic pain in paraplegia. Pain 130: 294-298.
Rizzolatti, G. and Craighero, L. (2004). The Mirror-Neuron System. Annual Reviews in Neuroscience 27: 169-192.

Supported by the International Foundation for Research in Paraplegia

Corticomotor representation to human arm muscle changes following cervical spinal cord injury

Patrick Freund [1, 2, 3, 4], J. Rothwell [5], M. Craggs [4], A. Thompson [1], S. Bestmann [5]

[1] Department of Brain Repair & Rehabilitation, UCL Institute of Neurology, London, UK; [2] Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, London, UK; [3] Swiss Paraplegic Research, Nottwil, Switzerland; [4] Spinal Cord Injury Centre, Royal National Orthopaedic Hospital, UCL, London, UK; [5] Sobell Department of Motor Neuroscience, UCL Institute of Neurology, London, UK
p.freund@ion.ucl.ac.uk

Background Spinal cord injury (SCI) induces reorganization of cortical muscle maps. Cervical rewiring and cortico-cortical remodeling are potential neuronal substrates. We used navigated transcranial magnetic stimulation (TMS) to non-invasively measure changes of cortical map reorganization to an arm muscle in primary motor cortex (M1) following human SCI.
Method Nine chronic cervical SCI participants with impaired lower and upper limb function and thirteen controls participated. All participants underwent a T1-weighted anatomical scan prior to the TMS experiment. Functional impairment was clinically assessed and spinal cord cross sectional area (SCA) measured at cervical level C2. The motor thresholds of the extensor digitorum communes (EDC) were defined and its cortical muscle representation mapped. The centre of gravity (CoG), the cortical silent period (CSP) and active motor thresholds (aMT) were measured. Linear regression analysis investigated relationships between SCI induced spinal changes and TMS parameters.
Results SCA was significantly reduced in SCI participants when compared to controls (52.19 mm^2 vs. 79.98 mm^2, $p<0.05$). SCI subjects had increased aMT ($p<0.05$) and increased CSP duration ($p<0.05$). The CoG of the EDC-MEP map shifted posterior towards the anatomical hand representation of M1 in SCI participants when compared to controls ($p<0.05$). SCA was negatively associated with aMT and CSP duration ($r^2 \geq 0.25$, $p<0.05$).
Conclusion Cortical muscle representations reorganize and corticospinal (CS) integrity is reduced following SCI in man. Cortical motor representations may reorganize to maximize output to muscles of the impaired upper limb following SCI. Degeneration of CS neurones due to trauma might drive this reorganisation. Another mechanism could be associated to changes in afferent feedback from the periphery.

This study was supported by Swiss National Science Foundation (NCCR), Schweizerische Stiftung für Medizinisch-Biologische Stipendien (SSMBS), Swiss Paraplegic Research (Nottwil) and Biotechnology and Biological Sciences Research Council (BBSRC).

Investigating corticospinal tract integrity using diffusion tensor MRI following spinal cord injury

Patrick Freund [1,2,3,4 *], C. Wheeler-Kingshott [5], Z. Nagy [2], N. Weiskopf [2], K. Friston [2], A. Thompson [1] C. Hutton [2]

[1] Department of Brain Repair & Rehabilitation, UCL Institute of Neurology, UCL, London, UK; [2] Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, UCL, London, UK; [3] Spinal Cord Injury Centre, Royal National Orthopaedic Hospital, UCL, London, UK; [4] Swiss Paraplegic Research, Nottwil, Switzerland; [5] Department of Neuroinflammation, UCL Institute of Neurology, UCL, London, UK
p.freund@ion.ucl.ac.uk

Background Traumatic spinal cord injury (SCI) leads to disintegration of axonal architecture resulting in tissue loss, impeded information flow between the brain and spinal cord and clinical impairment. We set out to determine whether tissue loss and clinical impairment predict changes in axonal integrity of the corticospinal tract (CST) using diffusion tensor imaging (DTI).
Method Cervical injured volunteers with bilateral motor impairment and healthy controls were studied. Structural MRI and DTI of the brain assessed axonal disintegration over the entire CST. Voxel based diffusometry assessed changes to axonal integrity and associations between structure and function.
Results Two DTI parameters, fractional anisotropy (FA) and axial diffusivity, both measures of neuroaxonal integrity, were significantly reduced in the CST when compared to controls. For FA these areas comprised the pyramids ($p=0.005$), posterior limb of the internal capsule ($p=0.010$), hand ($p= 0.042$) and leg ($p=0.027$) area of M1. AD was reduced in the right pyramid ($p=0.044$) M1 hand area ($p=0.038$). FA correlated negatively with cord area in the posterior limb of the internal capsule and with clinical measures of dexterity in the M1 hand area, pyramids and posterior limb of the internal capsule.
Conclusion Reduced FA and AD in cranial regions of the CST are likely to reflect axonal degeneration due to distal axotomy. DTI indices, in particular FA, appear sensitive to structural damage following SCI that is associated with atrophy and clinical disability.

This study was supported by Swiss National Science Foundation (NCCR), Schweizerische Stiftung für Medizinisch-Biologische Stipendien (SSMBS), Swiss Paraplegic Research and Wellcome Trust.

Disability, cortical reorganization and atrophy following spinal cord injury

Patrick Freund [1,2,3,4 *], N. Weiskopf [2], C. Hutton [2], M. Craggs [3], K.Friston [2] and A.J. Thompson [1]

[1] Department of Brain Repair & Rehabilitation, UCL Institute of Neurology, UCL, London, UK; [2] Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, UCL, London, UK; [3] Spinal Cord Injury Centre, Royal National Orthopaedic Hospital, UCL, London, UK; [4] Swiss Paraplegic Research, Nottwil, Switzerland
p.freund@ion.ucl.ac.uk

Background The impact of traumatic spinal cord injury (SCI) on the structural integrity of the nervous system and functional impairment is variable and may depend on a balance between local damage and the capacity for plastic reorganization.
Methods We addressed the effects of SCI using morphometric MRI measures of the cervical cord and brain in SCI subjects and controls. Morphometric changes were assessed using cross sectional cord area, voxel-based morphometry (VBM) and voxel based cortical thickness of T1-weighted images in 10 SCI and 16 healthy subjects. SCI subjects were clinically assessed and compared with controls. In SCI subjects, regression analysis was used to determine associations between morphometric changes and disability.
Results Subjects with SCI had impaired upper and lower limb function and reduced cord area, when compared to controls (53·2mm² vs. 79·2mm², >30%, $p<0·001$). VBM analysis revealed reduced white matter volume in the brainstem, left cerebellar peduncle and reduced grey matter (GM) volume and cortical thinning in the leg region of primary motor cortex (M1) and primary sensory cortex. In SCI subjects, cord area was negatively associated with upper limb impairment ($r^2>0·36$, $p<0·05$). GM volume in the cortical leg area of M1 was negatively associated with upper limb function ($p\leq0·002$).
Conclusion SCI leads to spinal and cortical atrophy. Cord atrophy predicts disability. Structural cortical correlates of disability indicate cortical hand reorganization into the leg area of M1. Thus, relative changes in GM volume at cortical level in SCI subjects may reflect reorganization that allows compensatory recovery of upper limb function.

This study was supported by Swiss National Science Foundation (NCCR), Schweizerische Stiftung für Medizinisch-Biologische Stipendien (SSMBS), Swiss Paraplegic Research and Wellcome Trust.

Changes in trans-cranial MEPs and SSEPs in association with cellular injections into porcine spinal cord injury epicenters after SCI

James Guest, Francisco Benavides, Juan Solano, Howard Levene

University of Miami, Neurological Surgery and the Miami Project to Cure Paralysis
jguest@med.miami.edu

Introduction. Typically, the perioperative effects of experimental spinal cord injections are determined by observing changes in behavioral tests and terminally by examination of histological sections that are compared to controls. Relatively large injections are likely to be necessary in order to deliver sufficient cells to elicit repair after spinal cord injury (SCI). The safety of such injections relies on several factors amongst which the dose, volume, and method of delivery are important. These variables contribute to the maximum tolerated dose (MTD), which is that dose, above which irreversible additional spinal cord damage occurs.
MTD may be examined in several ways: surgical observation, behavioral changes, and imaging. Neurophysiologic measures performed during spinal cord surgery and injections may also be useful. Because it is established that certain reductions or losses in MEPs or SSEPS predict clinically significant neurological deficits it may be true that maintenance or loss of conduction during spinal cord injections is a useful safety parameter.
Methods. Adult pigs undergo anesthesia and leads are placed for transcranial evoked motor evoked EMG potentials (tc-MEP) and tibial-nerve evoked SSEPS. For tc- MEPs, the upper extremities serve as circuit controls while for SSEPs the median and sciatic nerves are used for control values and to calculate central conduction times. The MEPS and SSEPS are recorded prior to a T9/10 contusive lesion, following it, and then two weeks later prior to spinal cord injection, during, and after injection. Injections of 50-100 μl are placed into the injury epicentre. Correlations are being sought between changes in MRI signal, locomotor function, histologic volumes and loss or retention of MEP and SSEP signal.
Observations. Of 12 pigs studies thus far, only 4 have had measurable MEPs and SSEPs 2 weeks post –injury. Of these 2 had a partial loss of conduction associated with a 100 μl injection. One of these had a clear reduction in locomotor function after surgery.
Conclusion. In the presence of retained spinal cord conduction, large injection volumes may be associated with neurological injury, and this may be detected during the injection procedure using MEP and SSEPs. These findings may be of clinical importance.

The effects of eicosapentaenoic acid delivered as dietary treatment after spinal cord injury

Jodie C.E. Hall, J. V. Priestley and A. Michael-Titus

Centre for Neuroscience & Trauma, Blizard Institute of Cell & Molecular Science, Barts and The London School of Medicine and Dentistry, 4 Newark Street, Whitechapel, LONDON E1 2AT
j.c.e.hall@qmul.ac.uk

Neuroprotective effects of the omega-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and α–linolenic acid (ALA) have been reported in animal models of spinal cord injury (SCI) and stroke, when given intravenously or in the diet (Nguemeni et al., 2010; Huang et al. 2007; Lauritzen et al., 2000). We sought to determine the effects of eicosapentaenoic acid (EPA) dietary enrichment on outcome after SCI. Female adult Sprague-Dawley rats (n=6 per group) received compression SCI at vertebral thoracic level 12, followed by control or EPA-enriched diet (150 mg/kg/day) for four weeks. Outcome measures included locomotor function (BBB score) and bladder functional recovery, development of hind limb mechanical hypersensitivity and histological assessment. Surprisingly, the group receiving the EPA diet had the worst locomotor recovery out of the two groups. Although both groups recovered bladder function by 8 days, the EPA diet group revealed a significantly higher retention of urine and a permanent increase in bladder width by 4 weeks. Mechanical withdrawal threshold decreased significantly from baseline in both groups, but there was no significant difference between the groups. Histological assessment of specific areas in the epicentre and 5 mm rostral to the injury site revealed no significant difference between groups in cavity size, NeuN labelled neurones in the dorsal horn, SMI32 labelled non-phosphorylated neurofilament, APC labelled oligodendrocytes, or ED1 and Iba1 labelled macrophages/microglia. Further studies are required to characterize the mechanisms underlying the apparent beneficial effect of acute i.v. EPA on functional recovery after SCI (Lim et al., 2008) and the detrimental effect of an EPA-enriched diet.

Tropism of adeno-associated virus 8 for large diameter sensory neurons of dorsal root ganglia after direct injection or intrathecal delivery

Steven J Jacques, Anna Forbes, Michael Douglas, Zubair Ahmed, Martin Berry, Ann Logan

Molecular Neuroscience Group, Neuropharmacology and Neurobiology, School of Clinical and Experimental Medicine, University of Birmingham, UK
sjj894@bham.ac.uk

Background: Adeno-associated viruses (AAVs) display a natural tendency to transduce neurons. Within dorsal root ganglia, AAV serotypes 6 and 8 target dorsal root ganglion neurons (DRGN), with undetectable transduction of non-neuronal cells. This study aimed to: (i) compare the tropism of AAV8 within the dorsal root ganglion (DRG) after direct and intrathecal (IT) injection, with particular emphasis on DRGN sub-populations; (ii) assess the usefulness of GFP delivered by AAV8 for tracing the projections of DRGN to the spinal cord; and (iii) examine the effects of AAV8 transgene delivery on spinal cord glia and peripheral nervous system inflammatory cells.
Methods: The left L4 and L5 DRGs of 6 adult male Sprague-Dawley rats were surgically exposed and injected with $AAV8_{gfp}$ (10^9 viral genomes). Another group of 6 rats received IT injection of $AAV8_{gfp}$ (10^{12} or 10^9 genomes). Rats were left 30d and then killed, their DRGs, spinal cords and brains harvested and processed allowing frozen sections to be prepared for immunohistochemistry.
Results: GFP+ DRGN were clearly visible in the DRG after each method of delivery, with minimal contralateral labeling after direct injection. No GFP+ non-neuronal cells were observed in either the DRG or spinal cord after either method of delivery. After direct injection, 10-12% of DRGN were transduced in total; 32% of large diameter DRGN were GFP+ compared with 2% of small diameter DRGN. Central axonal projections of DRGN were clearly visible in the dorsal root, at the dorsal root entry zone and within the spinal cord. IT injection gave a lower overall transduction rate (1.5%), but the same tropism for large diameter DRGN was seen (17% of large diameter DRGN were GFP+). The same pattern of central projections was seen after IT injection as after direct DRG injection. Furthermore, 42% of the transduced large diameter DRGN were parvalbumin (PV) positive, consistent with them being muscle spindle afferents. No cells in the spinal gray matter were transduced after either method of delivery. **Conclusions**: AAV8 preferentially transduces large diameter, PV+ DRGN, labeling their central projections within the spinal cord. This finding has important implications for directed therapies for spinal cord injuries. Additionally, AAV8 has been shown to be a potentially useful vector for gene delivery to sensory ganglia where trophic stimulation of nociceptive afferent neurons needs to be avoided.

Multiple intrinsic and extrinsic factors restrict sensory axon regeneration in chronic spinal cord injury

Ken Kadoya[1], Mark H. Tuszynski[1,2]

[1]Department of Neurosciences, University of California – San Diego, CA;
[2]Veterans Administration Medical Center, La Jolla, CA.
kkadoya@ucsd.edu

Previous studies showed a marked reduction in regenerative capacity of chronically injured spinal cord axons compared to acutely injured axons, an effect likely due to a combination of intrinsic and extrinsic factors. We examined the effects of acute and chronic C3 dorsal column lesions (DCL) in conjunction with peripheral nerve conditioning lesions (CL) on 1) sensory axon regeneration into a marrow stromal cell graft in the lesion site and 2) the intrinsic growth capability of sensory axons. Additionally, we examined differences in extrinsic factors such as glial scarring and inflammation between acute and chronic injuries. Following DCL, a cell graft was performed either immediately (acute) or 15 months (chronic) post injury. CL was applied 1 week before cell graft in both groups. We traced dorsal column sensory axons by CTB injections into sciatic nerves. Robust sensory axon regeneration was observed in the cell graft in acutely treated subjects, whereas few axons were found in the lesion site in chronically treated subjects. To identify possible intrinsic and extrinsic factors contributing to this reduced regeneration in chronic SCI, we assessed the effects of DCL on axon retraction (intrinsic), neurite outgrowth in culture (intrinsic), glial scarring (extrinsic), and inflammation (extrinsic). Compared to acute injuries, chronic injuries resulted in significantly more axon retraction, greater concentration of glial scarring around the lesion site, and less inflammation, whereas cultured sensory neurons showed equivalent neurite outgrowth between groups. To determine the relative contribution of these factors to reduced regeneration in chronic SCI, the same treatments were applied to a fresh C7 lesion site 15 months after initial DCL lesion at C3. In this fresh C7 lesion site, there was little axonal retraction, no glial scarring, and no lack of inflammation. Notably, the proportion of chronically injured axons regenerating into the C7 lesion site, 15 months after the initial C3 lesion, increased by 460% when compared to the number of axons regenerating into the C3 chronic lesion site. Nonetheless, significantly fewer axons regenerated into the C7 chronic lesion site than an acute C7 lesion site. These results suggest that both intrinsic and extrinsic mechanisms inhibit the regeneration of chronically injured sensory axons. Ongoing studies are examining the molecular and cellular mechanisms underlying this reduced regeneration in chronic SCI.

Supported by the Veterans Administration, NIH, and the International Spinal Research Trust.

Development of a myelination assay of human neurons generated from HESCs

Bilal E. Kerman, Ariana Mei, Stephane Genoud, Ahmet M. Denli, Francesco Paolo Di Giorgio, Fred H. Gage

10010 North Torrey Pines Road La Jolla, CA 92037-1099 USA
bkerman@salk.edu

Myelination of axons by oligodendrocytes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system (PNS) is critical for neuronal function and survival. Local loss of myelin, as occurs in many neurological disorders and as a consequence of spinal cord injury (SCI), leads to disruption of electrical impulse conductivity, atrophy of neurons and permanent functional deficits. Moreover, re-growth of transected nerve fibers is impaired in part by myelin associated neurite growth inhibitors such as Nogo-A. Therefore, increased understanding of oligodendrocyte biology in humans may yield valuable treatments for recovery of neural function of patients with SCI. Currently, the myelination (as well as re-myelination) process in human neurons has only been studied in postmortem tissues or in byproducts (in cerebral spinal fluid, for example). Human embryonic stem cell (HESC) technology enables us, for the first time, to study the process of myelination in human neurons in real time. Our intend is to establish an in vitro system to study the processes of myelination and re-myelination on the developing human neuron, using HESCs as a starting point to produce both functional neurons and oligodendrocyte progenitors. Main advantages of such an in vitro system are that it will be robust, reliable, quantitative, and open to modification. It will also enable dissection of myelination process and study myelin formation progress in real time. Our preliminary results using ESC-differentiated motor neurons (MNs) indicate that we are able to obtain oligodendrocyte progenitors ensheathing neurons with myelin basic protein. Moreover, we are using our in vitro assay system to understand functional importance of human HRP3, a neuron derived factor that was discovered in our lab through a microarray analysis to uncover the neuron-derived signals that mediate myelination in mouse CNS in regulating the process of myelination and re-myelination.

Supported by Christopher Dana Reeve Foundation, Sanofi Aventis

Electrophysiological properties of bilateral VPL neurons after spinal cord hemisection injury

Li Liang, Lorne Mendell

Department of Neurobiology and Behavior, SUNY at Stony Brook, USA
lilliang@notes.cc.sunysb.edu

Neuropathic pain commonly occurs after spinal cord injury (SCI). The thalamus, especial ventroposterolateral (VPL) nucleus, a key nucleus in the lateral system of ascending pain pathways, is believed to play an important role in the processing of somatosensory and nociceptive information. to examine this simultaneous bilateral extracellular multi unit recordings of nociceptive and multi receptive neurons were made in rostral VPL of adult male Sprague-Dawley rats with hemisection (HX) at the T10 thoracic level. Four different preparations were studied: intact rats, the same rats after acute hemisection at T10, chronically hemisected rats 2 weeks and 4 weeks after T10 Hx. Graded mechanical stimuli were delivered to contralateral hindlimb receptive fields of these neurons.

A total of 581 neurons were recorded from VPL nuclei on both sides. In intact preparations under these conditions, most cells were multireceptive; only a small minority (<10%) were nociceptive specific. Acute left HX reduced the number of multireceptive cells in right VPL which likely converted to nociceptive- specific due to loss of left dorsal column input. Four weeks after Hx we observed a return of multireceptive neurons to the right VPL, presumably due to increased responsiveness of dorsal horn cells in the left lumbar cord projecting to the right VPL. Surprisingly, the proportion of multireceptive and nociceptive- specific cells in left VPL was unchanged throughout this period.

More striking was the change in firing rate of cells on both sides of VPL. After acute Hx cells in left VPL exhibited a large decrease in spontaneous firing rate as well as a decreased response to brush, tap and pinch. Cells in right VPL displayed an increase in spontaneous firing rate but little change in firing rate from intact preparations. These differences in firing rate were exaggerated in chronically Hx rats because the firing rate, both spontaneous and stimulus- driven (from the opposite limb), increased substantially more in right VPL than in left VPL. In addition, a bilateral increase was observed in burst activity in VPL after chronic Hx.

Behavioral studies in these animals revealed that mechanical threshold (Von Frey stimulation) for hindlimb withdrawal decreased considerably for the left hindlimb by 4 weeks after left Hx but increased for the right hindlimb. Thermal threshold (Hargreaves) to left hindpaw stimulation was reduced, but only slightly elevated from the right hindpaw. These results are consistent with the electrophysiological changes observed in VPL. Behavioral changes at 2 weeks were less consistent perhaps because the differences in firing rate of left and right VPL had not yet been fully established.

These studies indicate that VPL may be a useful target system to study pain mechanisms after spinal injury. The use of simultaneous bilateral recording provides clear indication of the laterality of changes after Hx and differences in plastic changes that develop after chronic Hx.

Supported by CDRF and NIH

Delivery of Decorin to acute dorsal column lesion sites suppresses inflammation, scar formation and angiogenesis

Daljeet Mahay, Martin Berry, Ann Logan

Molecular Neuroscience group, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK
d.mahay@bham.ac.uk

Following spinal cord injury (SCI), the terminals of the long tract axons become arrested in the walls of a dense glial scar. Astrocytes conspire with meningeal fibroblasts to lay down a basal lamina of a glial limiting membrane and an extracellular matrix (ECM) comprising of laminin, fibronectin, collagen and chondroitin sulphate proteoglycans (CSPG) such as NG2. In order to achieve optimal axon regeneration and functional repair after SCI, we hypothesise that prevention of scar deposition is required to promote axon regeneration through the CSPG-rich inhibitory barrier of the wound site by suppression of transforming growth factor beta-1 and 2 (TGF-β1/2) activities using the TGF-β antagonist, Decorin. The anti-fibrotic and neuritogenic activity of Decorin was first investigated *in vitro* by: 1), evaluating the anti-fibrogenic bioactivity of Decorin through monitoring expression of NG2 in TGF-β2 stimulated primary rat meningeal fibroblasts; and 2), monitoring the neuritogenic activity of Decorin in primary cultures of rat dorsal root ganglion neurons (DRGN). *In vitro* Decorin suppressed TGF-β2-stimulated NG2 expression by meningeal fibroblasts. Furthermore, the administration of recombinant Decorin to primary adult rat DRGN cultured on meningeal fibroblasts pre-treated with TGF-β2 increased neurite outgrowth. The same activities of Decorin were apparent *in vivo*. After implantation of a collagen matrix pellet containing recombinant Decorin into an acute dorsal funicular lesion of adult rats we observed: 1), reduced lesion size by greater than 50%; 2), suppressed inflammation, marked by a 52% reduction in ED1-positive cells; 3), reduced both fibronectin and laminin deposition, e.g. laminin deposition was marked by a 60% reduction in coverage of the lesion margins and a 90% reduction in basal lamina thickness within the glia limitans; and 4), suppressed angiogenesis, marked by a 61% reduction in vascular basal lamina; compared to the PBS controls. Together, our results demonstrate that delivery of recombinant Decorin protein to the site of an acute SCI significantly reduces lesion volume, inflammation, angiogenesis and scarring. We are now well placed to investigate the effects of Decorin in a chronic model of SCI.

Supported by ISRT

In vitro assay to measure inflammatory response in human glial cells after injury

Maria Carolina Marchetto, Fred H. Gage

Salk Institute, 10010 North Torrey Pines Road La Jolla, CA 92037-1099 USA
marchetto@salk.edu

A strong neuroinflammatory response is one of the most prominent features following SCI. Spinal contusion and compression injury cause acute central hemorrhagic necrosis in all mammals followed by prominent glial activation, cytokine production and leukocyte infiltration. Here we present preliminary data on optimization of an in vitro detection system to quantitatively identify inflammatory response in human glial cells after injury. We used a highly sensitive multiplex image detection system (Sector Imager 6000, Meso Scale Discovery) to quantitatively measure a set of inflammatory markers on conditioned media derived from human glial cells (spinal primary astrocytes). For the preliminary experiments, we “injured” the glia using glass-pipette scratching and measured the levels of several inflammatory cytokines on the media over time. We detected a clear quantitative inflammatory response that peaked 24h after the injury. We now plan to evaluate the inflammatory response after injury in the presence of drugs with anti-inflammatory properties. Ultimately we would like to perform drug screen assays using this system and search for compounds that alleviate the inflammatory response and promote repair.

Supported by Christopher and Danna Reeve Foundation

Deciphering the regeneration-associated gene expression program: gene expression profiling of axotomized facial motor neurons in conditional c-Jun knockout mice

Matthew R.J. Mason[1], Susan van Erp[1], Koen Bossers[1], Mariya Hristova[2], Axel Behrens[3], Genna Raivich[2], Joost Verhaagen[1]

[1]Netherlands Institute for Neuroscience, Amsterdam, The Netherlands, [2]Dept. of Obstetrics and Gynaecology, Institute for Women's Health, University College London, [3]Mammalian Genetics Laboratory, Cancer Research UK, Lincoln's Inn Fields Laboratories, London, UK.
J.Verhaagen@nin.knaw.nl

Successful regeneration requires the up-regulation of a large number of regeneration-associated genes (RAGs). The transcription factor c-Jun has been shown to be functionally important for axonal regeneration but it is not known what its contribution is to regulation of the RAG expression program. We have conducted gene expression profiling on axotomized facial motor neurons from nestin-cre/floxed c-Jun animals (in which c-Jun is deleted in the CNS) and litter-mate controls, at 0, 1, 4 and 14 days after injury. The facial nucleus was isolated by laser dissection microscopy. Upregulation of many RAGs is diminished in mutant animals but not abolished. We identify overrepresented gene ontology (GO) classes of genes upregulated in a c-Jun-dependent manner. These include known functions of c-Jun, such as apoptosis and cell-cycle control, as well as a number of metabolic processes and other classes linked to regeneration processes, such as cell motility and neuropeptide signaling. Genes annotated to muscle contraction and muscle specific cell compartments form a new class of c-Jun dependent RAGs, supporting a role for stretch-sensing in regeneration. Interestingly, genes are also identified which are repressed in a c-Jun dependent manner, most prominently a number of ion transporters, probably related to the reduced requirement for neurotransmission. Surprisingly, in the mutants, a large number of genes are upregulated after axotomy compared to the controls, suggesting activation of an alternative gene expression program. GO analysis of these genes reveals metabolic and transcriptional classes but also classes related to synaptic plasticity and axon guidance. We also identify a number of genes that are candidate c-Jun targets and confirm the neuronal expression of some of these by in situ hybridisation and immunohistochemistry.

Specific and synergistic functions of monoaminergic receptors in the control of spinal locomotion

Pavel Musienko[1,5,6], R. van den Brand[1], Y.P. Gerasimenko[2,5], R.R. Roy[2,4], V.R. Edgerton[2,3,4] and G. Courtine[1,6]

[1]Department of Neurology, Experimental Neurorehabilitation Laboratoty, University of Zurich, CH 8008, Switzerland; [2]Department of Physiological Science, University of California, Los Angeles, CA 90095, USA; [3]Department of Neurobiology, University of California, Los Angeles, Los Angeles, CA 90095, USA; [4]Brain Research Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA; [5]Pavlov Institute of Physiology, St. Petersburg, 199034, Russia; [6]Rehabilitation and Technology Platform Zurich (RITZ), Zurich, Switzerland
pavel.musienko@bli.uzh.ch

Following a complete spinal cord transection, descending serotonin (5-HT), noradrenaline (NA) and dopamine (DA) containing fibers are interrupted, and the capacity to step is lost. Since post-synaptic receptors remain present on spinal neurons, they can be stimulated pharmacologically, encouraging locomotion in paralyzed subjects. However, their respective function in the modulation of stepping remains poorly understood. Here, using specific agonists and antagonists, we studied the contribution of various monoamine receptor subtypes to the modulation of stepping patterns facilitated by epidural electrical stimulation in adult spinal rats *in vivo*. Detailed kinematics, kinetic, physiological and statistical analyses revealed functional links between various monoaminergic receptors and distinct gait modulations, which we visualized in receptor-specific maps. Next, we showed the capacity to manipulate multiple receptors simultaneously in order to synergistically encourage stepping, including full weight bearing locomotion with the appropriate patterns of coordination in paralyzed spinal rats. These findings provide further evidence to the view that the spinal motor infrastructure is composed of distributed and heterogeneous circuits and receptors that each underlie specific motor functions. Our results suggest that tailored combinations of pharmacological stimulations could improve the neurorehabilitation of severely paralyzed patients.

Supported by: IRP P106, University of Zurich, NCCR Neural repair and plasticity

Local translation of MAP2K7 allows JNK-dependent neurite outgrowth

Daniel Feltrin[1], Erika Fluri[1], **Olivier Pertz[1].**

[1] Institute of Biochemistry and Genetics, Department of Biomedicine, University of Basel
Olivier.pertz@unibas.ch

One important aspect of the regulation of neuronal development or in the mature brain is the transport and local translation of a subset of mRNAs to neuronal processes such as axons and synapses. However, the existence and significance of such molecular events has not yet been addressed during the early neuronal differentiation process, e.g. the initial extension of a neurite. We have performed a genome-widescreen of mRNAs that are localized to the neurite. Our working hypothesis is that some of these neurite-enriched mRNAs are locally translated to regulate the outgrowth process. Using the N1E-115 neuroblastoma cell line as model system, we have used a siRNA approach to test the functional requirement of some of these mRNAs in the neurite outgrowth process. Knockdown of the MAP2K7, an mRNA encoding for a MAP kinase kinase that is directly involved in the regulation of the JNK pathway, leads to a total loss of neurite outgrowth. A direct target of MAP2K7 is the signaling molecule JNK2, a well known key regulator of apoptosis/cell survival during cellular stress. Importantly, proteins belonging to the JNK family have also been recently involved in the regulation of microtubule dynamics during dendrite formation (Podkowa M, Mol Cel Bio, 2010) and axonal regeneration (Stone MC, Mol Cel Bio, 2010). Consistently, we show local activation of MAP2K7 and its target JNK2 in the neurite. Most likely MAP2K7 transcript gets localized to the growth cones of protruding neurites were it might get locally translated, allowing a direct, quick and confined action on its downstream targets. Thus, JNK signaling could be biased to the regulation of cytoskeletal events in the neurite rather than regulation of cellular stress in the nucleus. To prove local mRNA translation and validate our hypothesis, several imaging approaches will be used. Moreover, to prove the importance of mRNA localization and local translation, MAP2K7 knockdown N1E-115 cells will be functionally rescued transfecting MAP2K7 cDNA with or without the correct 3'UTR. Finally the action of MAP2K7 will be further explored by analysing its effect of other hypothetical direct or indirect targets, such as microtubule associated proteins (MAPs).

References:
Podkowa M, Zhao X, Chow CW, Coffey ET, Davis RJ, Attisano L. "Microtubule stabilization by BMP receptor-mediated scaffolding of JNK promotes dendrite formation", Mol Cell Biol. 2010 Feb 22
Stone MC, Nguyen MM, Tao J, Allender DL, Rolls MM. "Global up-regulation of microtubule dynamics and polarity reversal during regeneration of an axon from a dendrite", Mol Biol Cell. 2010 Mar;21(5):767-77

This work was supported by grants from the International Foundation for Research in Paraplegia and the Swiss National Science Foundation.

Investigating the reactivation of structural plasticity after digestion of Chondroitin Sulfate Proteoglycans with Chondroitinase ABC

Orlando C.[1], Matus A.[2], Fawcett J.W.[3], **Oliver Raineteau[1]**

[1]: Brain Research Institute, University of Zurich/ETHZ, Zurich, Switzerland
[2]: Friedrich Miescher Institute, Basel, Switzerland
[3]: Brain Repair Institute, Cambridge, UK
raineteau@hifo.uzh.ch

During early postnatal life, the central nervous system undergoes a critical period for plasticity. The closure of this critical period is characterized by the accumulation of Chondroitin Sulphate Proteoglycans (CSPGs) that aggregate in perineuronal nets (PNNs) surrounding parvalbumin-positive inhibitory interneurons. An increasing number of studies have shown that degradation of CSPG sulfate chains by Chondroitinase ABC (ChABC) reactivates plasticity. However, the mechanisms mediating this reactivation remain largely unclear.
In this study we investigated the reactivation of plasticity induced by ChABC treatment in a slice culture model. Considering that dendritic spines develop, grow, change shape, and retract in response to synaptic input and to the overall neuronal activity, we focus on dendritic spine dynamics as a measure of structural plasticity. Live imaging was performed on mature hippocampal slice culture (> 3 weeks) prepared from transgenic mice expressing YFP in CA1 pyramidal neuron. Hippocampal slice cultures develop PNNs with a spatio-temporal pattern identical to the *in vivo* situation. Overnight incubation with ChABC resulted in a rapid and complete digestion of CSPG sulfate chains, as revealed by disappearance of WFA-specific binding sites. Following treatment, proximal and distal segments of CA1 pyramidal cells apical dendrite were imaged over a period of 20 minutes. Density, turnover, shape factor and motility of spines were measured over the imaging period. Results revealed an increased turnover of dendritic spines in ChABC-treated compared to control slices, as well as a more pronounced spine motility.
Taken together, our results show that ChABC treatment results in rapid changes of dendritic spines dynamics. Future experiments will aim at understanding whether these structural changes are due to a digestion of the CSPGs around dendritic spines or to a more global network effect resulting from a change of properties of PNNs surrounded parvalbumin-positive interneurons.

A multi-step screening approach successfully uncovers novel genes involved in the regeneration-promoting properties of olfactory ensheathing glia cells

Kasper C.D. Roet[1], Nitish Fagoe[1], Elske H.P. Franssen[1], Anke H.W. Essing[1], Sjirk-Jan J. Zijlstra[1], Freddy M. de Bree[1], Ronald E. van Kesteren[2], August B. Smit[2], Joost Verhaagen[1]

[1]Netherlands Institute for Neuroscience and [2]Neuroscience Campus Amsterdam, Amsterdam, The Netherlands
J.Verhaagen@nin.knaw.nl

Olfactory ensheathing glia cells (OEC) are a specialized type of glia that promote axon regeneration. The biological basis for these beneficial effects is largely unknown. We used a multi-step screening approach to dissect the regeneration-promoting properties of OEC. First, a microarray analysis was performed on cultured OEC and on OEC in the olfactory nerve layer of the lesioned, regenerating olfactory nervous system. This resulted in the identification of a set of approximately 1000 genes regulated in culture or regulated after injury. Second, by combining the microarray data with a gene ontology analysis and literature study a “top”-list of 118 candidate target genes potentially involved in, but not previously linked to, axon regeneration was selected and contained genes associated with cell adhesion, migration or extracellular matrix. Third, siRNA-mediated knock-down of each of these 118 target genes in OEC co-cultured with E15 DRG neurons revealed that knock-down of least 15 of the OEC targets resulted in diminished neurite outgrowth of E15 dorsal root ganglion neurons. Lentiviral vectors encoding these and a number of additional target genes were created to examine the effect of overexpression on neurite outgrowth. LV-mediated overexpression of three of the genes (scavenger receptor class B, member 2 [Scarb2], S100A9 and Neuroserpin) in primary skin fibroblasts significantly increased neurite outgrowth of embryonic DRG neurons co-cultured on these fibroblasts. To further corroborate these effects the experiment was repeated with adult DRG neurons. We found that Scarb2, S100A9 and Neuroserpin also significantly promoted neurite outgrowth of adult DRG. Experiments are ongoing to determine the effects of overexpression of the other target genes. Finally, the regeneration promoting effects of Scarb2, S100A9 and Neuroserpin on axons are currently investigated using ex vivo delivery of these genes in transected Fischer 344 rat thoracic dorsal columns. In conclusion, our multi-step screening approach has successfully identified potential novel molecular targets for neural repair. Moreover, we have shown that this multi-step screening and functional validation strategy can be used to unveil the regeneration-promoting properties of OEC.

Optimized diffusion MRI protocols for estimating axon diameter with known fibre orientation

Torben Schneider[1], Daniel C Alexander[2], Claudia AM Wheeler-Kingshott[1]

University College London, [1]UCL Institute of Neurology [2]UCL Department of Computer Science
Department of Neuroinflammation, UCL Institute of Neurology, Queen Square, London, WC1N 3BG
t.schneider@ion.ucl.ac.uk

Diffusion MRI provides a probe into tissue microstructure by imaging the mobility of water molecules. From measurements of the dispersion pattern, we can infer features of the tissue microstructure like cell size and packing density. Previous studies demonstrated that diffusion MRI can measure axon diameter and density of white matter fibres, e.g., in excised spinal cord (Assaf *et al*, 2008) and *in-vivo* rat brain (Barazany *et al*, 2009). However, the proposed imaging protocols require very high gradient strengths and long acquisition times and are therefore infeasible in a clinical setting. Recently an approach has been proposed that optimizes protocols to be acquired under the constraints of clinical systems (Alexander, 2008). The method determines a small set of measurements that maximize information about the tissue microstructure and allows reducing scan time to under 1h. However, the algorithm assumes no information about the organization of fibres and therefore requires a fixed set of diffusion encoding directions.
We adapt the optimization approach to exploit known single fibre orientation characteristic that are specific to the spinal cord. To improve axon diameter and density measurements in those structures, we optimize the diffusion directions for each acquisition set in addition to the original approach. Computer simulations show that our protocols can significantly increase the precision of parameter estimations in single fibre structures especially when the data is very noisy. We also show that the modified protocols outperform the orientation independent protocols even if the assumed fibre direction deviates slightly from the real fibre orientation. Finally we proof the validity of our protocols under moderate signal-to-noise ratio by applying the protocol on a fixated monkey spinal cord using an 4.7T experimental high field scanner. We are able to discriminate differences in axon diameter and density in several tracts of the spinal cord using the optimized single fibre protocols.

Assaf, Y., Blumenfeld-Katzir, T., Yovel, Y., Basser, P.: AxCaliber: A method for measuring axon diameter distribution from diffusion MRI. MRM 59 (2008)
Barazany, D., Assaf, Y., Basser, P.: In vivo measurement of axon diameter distribution in the corpus callosum of rat brain. Brain 132 (2009)
Alexander, D.C.: A general framework for experiment design in diffusion MRI and its application in measuring direct tissue-microstructure features. MRM 60 (2008)

Microstructural spinal changes detected by diffusion tensor imaging in chronic spinal cord injury

Torben Schneider[1*], Patrick Freund[2,3,4,5*], Zoltan Nagy[3], Claudia Wheeler-Kingshott[1], Alan Thompson[2]

1 Department of Neuroinflammation, UCL Institute of Neurology, UCL, London, UK; 2 Department of Brain Repair & Rehabilitation, UCL Institute of Neurology, UCL, London, UK; 3 Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, UCL, London, UK; 4 Spinal Cord Injury Centre, Royal National Orthopaedic Hospital, UCL, London, UK; 5 Swiss Paraplegic Research, Nottwil, Switzerland
* The first two authors contributed equally;
p.freund@ion.ucl.ac.uk

Background Traumatic spinal cord injury (SCI) leads to disintegration of axonal architecture. Consequently, tissue loss occurs and information flow between the brain and spinal cord is impeded; responsible for the ensuing clinical impairment. Diffusion tensor imaging (DTI) allows non-invasive quantification of microstructural and orientational features of white matter in the central nervous system.
The sensitivity to microscopic axonal changes makes DTI a useful imaging tool in the assessment of SCI.
Objective We investigate how DTI measures of high cervical cord in SCI differ from controls and correlate with clinical impairment.
Methods We studied nine cervical injured volunteers with bilateral motor impairment and 10 healthy controls. DTI of the whole spinal cord area between C2-C4 assessed axonal and myelin disintegration. We present a novel automated SC analysis method that reduces segmentation inconsistencies between subjects and minimizes partial volume contributions from surrounding cerebrospinal fluid. Multiple regressions of DTI indices are used to test for associations between structure and disability.
Results Fractional anisotropy (FA) is reduced and radial diffusivity (RD) increases in the cervical cord distal to the site of injury when compared to controls. FA correlates positively with cord area and impaired upper limb function.
Conclusion Lower FA and higher RD, both measures of neuroaxonal integrity, are most likely explained by axonal degeneration and demyelination due to distal axotomy. DTI indices, in particular FA, appear sensitive to structural damage following SCI that is associated with atrophy and clinical disability.

This study was supported by The International Spinal Research Trust, Swiss National Science Foundation (NCCR), Schweizerische Stiftung für Medizinisch-Biologische Stipendien (SSMBS), Swiss Paraplegic Research and Wellcome Trust.

Ketogenic diet improves gross forelimb function and fine-motor skills after incomplete cervical SCI

F. Streijger, W.T. Plunet, , J. H.T Lee, J. Liu, C.K. Lam, K. Matheson, B.J. Hilton, C.M. Yuan, S. Park, P. Assinck, **Wolfram Tetzlaff**

International Collaboration on Repair Discoveries (ICORD), Blusson Spinal Cord Centre, 818 West 10th Avenue, Vancouver, BC
tetzlaff@icord.org

In this study, we investigated the preclinical use the ketogenic diet (KD), a high fat diet devoid of carbohydrates, as a potential treatment for acute spinal cord injury. We observed improved behavioral recovery after a unilateral crush of the cervical spinal cord in animals fed a KD with a 7:1 ratio of fat to carbohydrate plus protein. KD treated animals showed a significant ~50% reduction in the percentage of footslips while crossing an irregularly spaced horizontal ladder, as well as improved usage of the injured forelimb during vertical exploration.
However, the 7:1 ratio is higher than the ratio used clinically, which ranges from 2:1 to 4:1 due to higher protein requirements for body growth. Therefore, we also tested the effectiveness of the more clinically relevant 3:1KD and compared it to a standard control diet (SD) following cervical spinal cord contusion. By two weeks of 3:1KD treatment, we observed 3-fold more frequent usage of the affected paw while rearing compared to SD animals. Furthermore, during face grooming ~66% of the KD animals could reach up to eye level whereas most of the SD animals (88%) could only reach as far up as the nose.
After surgery, extension and flexion of the arm and digits and pronation and supination of the paw were dramatically affected in both SD and KD group. However despite severe deficits in supination, KD animals nonetheless were capable of compensatory grasping and supination movements as observed with the single pellet-reaching test. Improved pellet retrieval success in the Montoya staircase test further reiterates that the KD benefits the skilled reaching task.
Histologically, KD treatment showed some ***neuroprotection*** as observed in smaller lesion size at the epicenter and increased grey matter sparing.
Our results suggest that KD not only benefits gross forelimb movement but also improves recovery in fine-motor skills after incomplete cervical SCI. The latter is relevant since skilled hand and arm movement are a priority for the majority of SCI patients. Since KD is already a well-established therapy for epilepsy and ketogenic formulas have already been developed for clinical use, this diet could be readily translated into the clinical setting of spinal cord injury.

Supported by: Craig H. Neilsen Foundation, Christopher and Dana Reeve Foundation and CIHR of Canada

Transplantation of skin-derived precursors differentiated into Schwann cells at eight weeks after spinal cord contusion

P. Assinck[1], S. Dworski[2], J. S. Sparling[1], G. J. Duncan[1], Y. Jiang[1], A. Skinner[1], D.L. Wu[1], C.M. Yuan[1], R. Church[1], J. Liu[1], F. D. Miller[2], **Wolfram Tetzlaff[1]**

[1]ICORD, University of British Columbia, Vancouver, BC, CANADA, [2] Hospital for Sick Children, University of Toronto, Toronto, ON, CANADA
tetzlaff@icord.org

Cell transplantation has emerged as a promising candidate therapy for spinal cord injury. Transplanted cells may provide neuroprotective factors, modulate inflammation, replace lost myelinating cells, and bridge the lesion cavity to promote axonal re-growth. However, the best candidate cell for a transplantation-based treatment of SCI remains a matter of intense debate. Our laboratories have previously shown that Schwann cells differentiated from skin-derived precursors (SKP-SCs), when transplanted 7 days after contusion injury, promote neuroprotection, bridge the lesion, and improve functional recovery in rats. SKPs are potentially suitable for autologous transplantation but the therapeutic potential of SKP-SCs in the chronic injury environment has not yet been investigated. Here, we transplanted one million cells into the lesion site of rats at 8 weeks post T9/T10 contusion injury and allowed survival until week 29. Behavioral data indicate that SKP-SC transplantation prevented the decline of forelimb and hindlimb stride length (Catwalk) and elicited a trend towards higher BBB scores, which reached significance in week 17 and 19 after injury (one tailed t-test). SKP-SC survived for 21 weeks post injury in all transplanted animals albeit to various degrees. The amount of tissue sparing was not significantly different between groups as expected in a chronic transplantation paradigm. Qualitative observation indicates that SKP-SC transplantation facilitates an increase in the presence of endogenous Schwann cells in the injured cord, and there appears to be less astrocyte hypertrophy in areas containing the transplanted SKP-SCs versus endogenous Schwann cells. Cellular bands of SKP-SCs bridged the lesion in predominantly rostro-caudal orientation and were massively filled with axons ensheathed by P0-positive (Schwann cell) myelin of endogenous as well as transplant origins. Only a small fraction of these axons stained positive for CGRP, indicative of their peripheral origin. Numerous tyrosine hydroxylase positive axons of presumed brainstem origin entered into the transplants and several crossed the caudal transplant/spinal cord interface consistent with a re-entry into the host spinal cord. Further quantitative histological analyses are underway. In conclusion, SKP-SCs transplantation may become a viable approach to promote repair of the chronically injured spinal cord.

This work was supported by grants from the Stem Cell Network (Canada), the Canadian Institutes of Health Research (CIHR) and the Rick Hansen Institute.

Neurochemical biomarkers concentrations in the CSF of patients with traumatic spinal cord injury

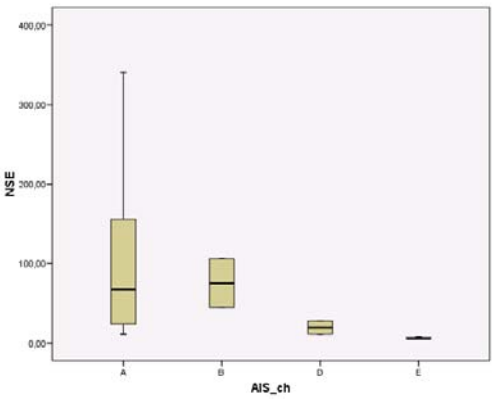
Martin Pouw, Allard Hosman, **Henk van de Meent**

Spine Study Group Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands
H.vandemeent@reval.umcn.nl

Study Design: Prospective multi-center cohort study.
Objectives: To describe the preliminary results of neurochemical biomarkers in the cerebrospinal fluid (CSF) of patients with traumatic spinal cord injury (SCI). The final purpose is to investigate whether biomarkers can be used as a diagnostic test to discriminate between patients who improve vs patients who do not improve after neuroprotective interventions in the acute phase.
Methods: Patients were included in the study based on the following inclusion criteria: 1) 18 years or older; 2) traumatic SCI between C1 and T12; 3) informed consent and ability to undergo CSF investigation and neurological examination according to the ASIA standards within 24 hours post-injury. CNS-specific proteins S100B and enolase (NSE) were measured and descriptive analysis was performed comparing S100B and NSE CSF concentrations with ASIA outcome data.
Results: From the 22 included patients samples, 13 are currently analysed on S100B and NSE concentrations. The mean NSE concentrations in group with AIS A (n=6) was 111.1 µg/l (SD 123.2), in group AIS B (n=2) 75.6 µg/l (SD 43.1), in group AIS D (n=2) 19.7 µg/l (SD 11.7) and in group AIS E (n=3) 6.5 µg/l (SD 1.2) see figure 1. The mean S100B concentrations in SCI group AIS A was 585.7 µg/l (SD 684.3), in group AIS B 197.1 µg/l (SD 260.5), in group AIS D 20.7 µg/l (SD 24.5), and group E 7.8 µg/l (SD 9.3) see figure 2.
Conclusion: These preliminary results show that subjects with motor complete SCI (AIS A and B) have higher CSF concentrations of NSE and S100B compared to subjects with motor incomplete SCI (AIS D and E). Further extension of patient numbers is necessary to increase the power of the study before valid conclusions can be drawn.

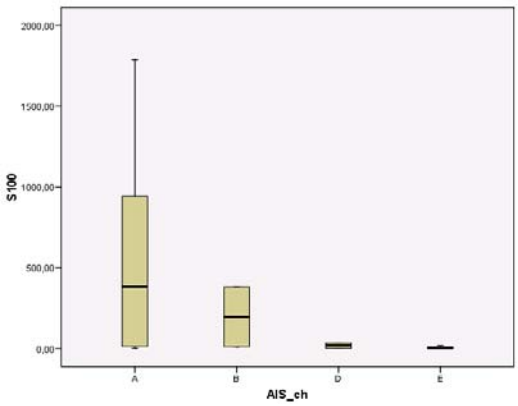
Supported by IFP

Figure 1: Boxplot of the initial (within 24 hours post-injury) NSE concentrations (µg/l) in CSF plotted against the 12 months post-injury AIS.



Abbreviations: Neuron specific enolase; NSE, cerebrospinal fluid; CSF, ASIA impairment scale 12 months post-injury; AIS_ch

Figure 2: Boxplot of the initial (within 24 hours post-injury) S100B concentrations (µg/l) in CSF plotted against the 12 months post-injury AIS



Abbreviations: Cerebrospinal fluid; CSF, ASIA impairment scale 12 months post-injury; AIS_ch

The effect on migration of primary Schwann cells and SCTM41 cells expressing a modified chondroitinase ABC enzyme

Philippa M. Warren[1]., Muir, E[2]., Rogers, J[2]., Keynes, R[2]. & Fawcett, J.W[1].

1: Cambridge Centre for Brain Repair, E.D. Adrian Building, Forvie Site, Robinson Way, Cambridge, CB2 0PY, UK.
2: Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK.
pmw44@cam.ac.uk

Transplantation of Schwann cells into the lesion site following spinal cord injury (SCI) is a promising treatment strategy as the cells are able to remyelinate demyelinated CNS axons and fill cystic cavities. In addition they facilitate axonal growth by providing a good cell surface environment for axon growth and by expressing neurotrophic factors while shielding axons from the inhibitory environment of the central nervous system. However, following transplantation Schwann cells show limited migratory ability and they are unable to intermingle with host astrocytes. This leads to formation of a sharp boundary and an abrupt transition between the Schwann cell graft and the host tissue, preventing regenerating axons from exiting the graft. Nonetheless, it has been shown that application of bacterial chondroitinase ABC at the host-graft interface facilitates Schwann cell integration into host tissue and permits many more regenerating axons to exit the grafts into CNS territory where they may form functional connections. The objective of this study is to determine if Schwann cell migration on an inhibitory substrate may be improved by the cells secreting a 'mammalian' chondroitinase ABC construct. Through modifying multiple glycosylation sites on the enzyme we have produced a form of chondroitinase ABC that can be synthesized in mammalian cells and secreted in a functionally active state. We report the enzymatic activity of this mammalian chondroitinase (*m*ChABC). Using *in vitro* assays of cell migration, we show that primary Schwann cell and Schwann cell line (SCTM41 cell) migration is inhibited by aggrecan, and that this chondroitin sulphate proteoglycan-mediated inhibition of Schwann cell migration is overcome by application of bacterial chondroitinase ABC. Further to this, we demonstrate that expression of *m*ChABC by primary Schwann cells and SCTM41 cells enables substrate inhibition to be overcome leading to enhanced migration.

Supported by the International Spinal Research Trust

Promoting the survival, migration and integration of Schwann cells after transplantation into spinal cord by overexpression of polysialic acid

Juan Luo, Xuenong Bo, Dongsheng Wu, John Yeh, Peter M. Richardson, **Yi Zhang**

Barts and the London School of Medicine and Dentistry, Queen Mary, University of London
yi.zhang@qmul.ac.uk

Schwann cells (SCs) are attractive candidates for cell therapy of spinal cord injury. However, transplantation studies of the cells in animal models have revealed several drawbacks such as poor survival, limited migration and integration of the transplanted SCs. To overcome such drawbacks we genetically modified SCs to overexpress polysialic acid (PSA) by lentiviral vector (LV) delivery of polysialyltransferase (PST). PSA expression on SCs was shown to modestly but significantly enhance their survival after transplantation into the spinal cord. Several potential factors that might contribute to the cell death were tested *in vitro* and it was found PSA could partially protect SCs death from serum and growth factor withdrawal.

In contrast to implanted GFP/SCs that caused stress response of host astrocytes demonstrated by sharp boundary formation, increased expression of glial fibrillary acidic protein (GFAP) and chondroitin sulfate proteoglycan (CSPG) in the host spinal cord, transplanted PST/SCs intermixed well with the host cells and did not cause obvious stress responses of the host tissues. Using an *in vitro* SCs/astrocytes confrontation assay, we found that significantly more PST/SCs crossed the SCs/astrocytes boundary than GFP/SCs. By measuring the sizes of individual astrocytes at the boundary it was shown that GFP/SCs caused significant hypertrophy of astrocytes than PST/SCs.

Previously, we observed that both PST/SCs and GFP/SCs did not migrate from the transplantation site after being implanted into intact spinal cord. To investigate SCs migration in injured spinal cord, a crush injury was created in adult rat spinal cord and PST/SCs or GFP/SCs were transplanted into the dorsal column 1.5mm caudal to the lesion. Three weeks later, GFP/SCs were observed to spread only a short distance around the injection site; while PST/SCs showed enhanced migration towards the lesion site along the degenerating dorsal column, and a few PST/SCs were found within the lesion cavity. However, when transplantation of PST/SCs was combined with LV/PST injection around the lesion site to induce PSA expression in the host spinal cord, more PST/SCs penetrated the glial scar and migrated into the lesion cavity. Similarly, induced PSA expression in spinal cells also promoted the migration of GFP/SCs along the PSA^+ pathway towards the lesion site, but the migration distance was shorter than PST/SCs in combination with LV/PST injection. These results demonstrate that expression of PSA on SCs causes significantly less stress response in astrocytes, which in turn leads to increased integration of SCs with host tissues. Induced PSA expression in the host tissues modifies the CNS environment and provides a pathway for SCs migration and facilitate SCs penetration of the glial scar into the lesion site.

Taken together, our results show that genetic modification of SCs to overexpress PSA enhances their survival, migration and integration after transplantation into the injured spinal cord. Such modification would make SCs more feasible for potential clinical application in the treatment of spinal cord injury.

Supported by ISRT

Delegates	Location	E-mail
Bizhan Aarabi	Baltimore	baarabi@smail.umaryland.edu
Carolina Acosta-Saltos	London	francia.acosta-saltos.09@ucl.ac.uk
Albert Aguayo	Montreal	albert.j.aguayo@mcgill.ca
Warren Alilain	Cleveland	warren.alilain@case.edu
David Allan	Glasgow	spinalunit@sgh.scot.nhs.uk
Aileen Anderson	Irvine	aja@uci.edu
Melissa Andrews	Cambridge	mra36@hermes.cam.ac.uk
Mark Bacon	Bramley	mark@spinal-research.org
Sue Barnett	Glasgow	s.barnett@clinmed.gla.ac.uk
Katalin Bartus	London	katalin.bartus@kcl.ac.uk
Jesus Benito	Barcelona	jbenito@guttmann.com
Xuenong Bo	London	x.bo@qmul.ac.uk
Karen Bosch	London	karen.bosch@kcl.ac.uk
Vanessa Boyce	Stony Brook	vboyce@notes.cc.sunysb.edu
Elizabeth Bradbury	London	elizabeth.bradbury@kcl.ac.uk
Anita Buchli	Zürich	buchli@hifo.uzh.ch
Daniela Carulli	Turin	daniela.carulli@unito.it
Daniel Chew	Cambridge	dc501@cam.ac.uk
Bernard Conway	Glasgow	b.a.conway@strath.ac.uk
Gregoire Courtine	Zürich	gregoire.courtine@bli.uzh.ch
Armin Curt	Zürich	armin.curt@balgrist.ch
Volker Dietz	Zürich	vdietz@paralab.balgrist.ch
Jean-Jacques Dreifuss	Tartegnin	JeanJacques.Dreifuss@unige.ch
Isabelle Dusart	Paris	isabelle.dusart@snv.jussieu.fr
Peter Ellaway	London	p.ellaway@imperial.ac.uk
Kynan Eng	Zürich	kynan@ini.phys.ethz.ch
Vieri Failli	Salzburg	vieri.failli@wingsforlife.com
James Fawcett	Cambridge	jf108@cam.ac.uk
Michael Fehlings	Toronto	Michael.Fehlings@uhn.on.ca
Linard Filli	Zürich	filli@hifo.uzh.ch
Karim Fouad	Edmonton	karim.fouad@ualberta.ca
Ralph Frankowski	Houston	Ralph.F.Frankowski@uth.tmc.edu
Patrick Freund	London	Patrickfreund1@gmail.com
Fred Gage	La Jolla	gage@salk.edu
Robert Grossman	Houston	rgrossman@tmhs.org
James Guest	Miami	jguest@med.miami.edu
Jodie Hall	London	j.c.e.hall@qmul.ac.uk
Susan Harkema	Louisville	susan.harkema@jhsmh.org
Jim Harrop	Philadelphia	James.harrop@jefferson.edu
Zhigang He	Boston	Zhigang.He@childrens.harvard.edu
John Hick	London	jwahick@waitrose.com
Susan Howley	Short Hills, NJ	showley@christopherreeve.org
Ronaldo Ichiyama	Leeds	r.m.ichiyama@leeds.ac.uk
Steve Jacques	Birmingham	sjj894@bham.ac.uk
Lyn Jakeman	Columbus	Jakeman.1@osu.edu
Ken Kadoya	La Jolla	kkadoya@ucsd.edu
Yorck-Bernhard Kalke	Ulm	yorck-bernhard.kalke@rku.de
Naomi Kleitman	Bethesda	KleitmaN@ninds.nih.gov
Spyros Kollias	Zürich	kollias@dmr.usz.ch
Timea Konya	Bramley	timea@spinal-research.org
Jiri Kriz	Prague	Jiri.Kriz@fnmotol.cz
Klaus Kucher	Basel	Klaus.kucher@novartis.com
Jane Lebkowski	Melon Park	JLebkowski@Geron.com
Roger Lemon	London	rlemon@ion.ucl.ac.uk
Li (Jack) Liang	Stony Brook	llianggz@hotmail.com
Juan Luo	London	juan.luo@qmul.ac.uk
Daljeet Mahay	Birmingham	d.mahay@bham.ac.uk
Doris Maier	Murnau	dmaier@bgu-murnau.de

Didier Martin	Liège	didier.martin@chu.ulg.ac.be
Stephen McMahon	London	stephen.mcmahon@kcl.ac.uk
Lorne Mendell	Stony Brook	lorne.mendell@notes.cc.sunysb.edu
Adina Michael-Titus	London	a.t.michael-titus@qmul.ac.uk
Pavel Musienko	Zürich	pavel.musienko@bli.uzh.ch
Clara Orlando	Zürich	orlando@hifo.uzh.ch
Matt Pankratz	La Jolla	mpankratz@salk.edu
Olivier Pertz	Basel	Olivier.Pertz@unibas.ch
Samuel Pfaff	La Jolla	pfaff@salk.edu
Tiffany Poon	La Jolla	poon@salk.edu
John Priestley	London	j.v.priestley@qmul.ac.uk
Olivier Raineteau	Zürich	raineteau@hifo.uzh.ch
Geoffrey Raisman	London	G.Raisman@ion.ucl.ac.uk
Gennadij Raivich	London	g.raivich@ucl.ac.uk
Louis Reichardt	San Francisco	louis.reichardt@ucsf.edu
John Riddell	Glasgow	j.riddell@bio.gla.ac.uk
Frank Röhrich	Halle	frank.roehrich@bergmannstrost.de
Jane Roskams	Vancouver	roskams@zoology.ubc.ca
Ferdinando Rossi	Turin	ferdinando.rossi@unito.it
Rüdiger Rupp	Heidelberg	Ruediger.Rupp@ok.uni-heidelberg.de
Torben Schneider	London	t.schneider@ion.ucl.ac.uk
Jan Schwab	Berlin	jan.schwab@charite.de
Martin Schwab	Zürich	schwab@hifo.uzh.ch
Christopher Shaffrey	Charlottesville	CIS8Z@virginia.edu
Prithvi Shah	Los Angeles	pkshah@ucla.edu
Michelle Starkey	Zürich	starkey@hifo.uzh.ch
Andreas Steck	Basel	asteck@uhbs.ch
Patrick Stroman	Kingston	stromanp@queensu.ca
Charles Tator	Toronto	charles.tator@uhn.on.ca
Wolfram Tetzlaff	Vancouver	tetzlaff@icord.org
Mark Tuszynski	La Jolla	mtuszynski@ucsd.edu
Heike Vallery	Zürich	heike.vallery@mavt.ethz.ch
Henk Van de Meent	Nijmegen	h.vandemeent@reval.umcn.nl
Huub van Hedel	Zürich	hvanhedel@paralab.balgrist.ch
Joost Verhaagen	Amsterdam	J.Verhaagen@nin.knaw.nl
Phil Waite	Sydney	p.waite@unsw.edu.au
Philippa Warren	Cambridge	pmw44@cam.ac.uk
Claudia Wheeler-Kingshott	London	c.wheeler-kingshott@ion.ucl.ac.uk
Yi Zhang	London	yi.zhang@qmul.ac.uk
Rongrong Zhao	Cambridge	rz239@hermes.cam.ac.uk
Min Zhuo	Toronto	min.zhuo@utoronto.ca
Jens Zimmer	Odense	jzimmer@health.sdu.dk

Public Transport Kartause Ittingen

Departure Timetables from Kartause Ittingen to Zurich Airport Saturday

Station	Travel by		Time	Platform	Comments
Kartause Ittingen	10min walk to Warth				See map
Warth Kreuz	Bus 823 or 825	Dep	11:26/ 11:47/ 12:26/ 12:47/ 13:26/ 13:47		
Frauenfeld		Arr	11:37/ 11:58/ 12:37/ 12:58/ 13:37/ 13:58		
	Train	Dep	11:42/ 12:12/ 12:42/ 13:12/ 13:42/ 14:12	1	
Zurich Airport		Arr	12:08/ 12:38/ 13:08/ 13:38/ 14:08/ 14:38	4	

Organised Transport Kartause Ittingen

Seiler Transport - Bus to Zurich Airport from the Monastery at 12.30 and 13.00 on Saturday 28th August, and 07.00 on Sunday, 29th August. tel ++41 52 722 10 11

Taxi from Kartause Ittingen or Frauenfeld to Airport Zurich

Ilg Taxi	tel ++41 52 720 44 44

Important contacts

Kartause Ittingen	tel ++41 52 748 44 11
Hotel Domicil, Frauenfeld	tel ++41 52 723 53 53
Timea Konya	mobile ++44 787 155 24 95