Network Meeting

6-7 September 2019

21st Spinal Research Network Meeting

ABSTRACTS
ABSTRACTS
Speakers’ abstracts appear in presentation order, followed by poster abstracts in alphabetical order.

POSTER PRESENTATIONS
Friday 6 September PhD posters 17:45 – 20:00
Saturday 7 September generic poster session 12:45 - 15:15

Scientific Organising Committee

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Mechanisms of axon growth and regeneration

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Almost everybody who has seen neurons under a microscope for the first time is fascinated by their beauty and their complex shape. Early on during development, however, neurons look round and simple without signs of their future complexity. How do neurons develop their sophisticated structure? How do they initially generate domains that later have distinct functions within neuronal circuits, such as the axon? And, can a better understanding of the underlying developmental mechanisms help us in pathological conditions, such as a spinal cord injury, to induce axons to regenerate?

Here, I will talk about the cytoskeleton as a driving force for initial neuronal polarization and axon growth. I will then explore how cytoskeletal changes help to reactivate the growth program of injured CNS axons to elicit axon regeneration after a spinal cord injury. Finally, I will discuss whether axon growth and synapse formation could represent mutually excluding processes. Following this developmental hypothesis helps us to generate a novel perspective on regeneration failure in the adult CNS and to envisage new paths to overcome it. Thus, this talk will describe how we can exploit developmental mechanisms to induce axon regeneration in the adult after a spinal cord injury.

References


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Axon regeneration requires that appropriate cell surface, signaling, cytoskeletal and other molecules are present at the axon tip. They can get there either by transport from the cell body or through local translation. In long-tract CNS axons local translation is limited, so transport has to be a focus. Many molecules associated with growth are present in all axons. However cell surface receptors and some signaling molecules appear to be missing. In sensory axons in the spinal cord it is sufficient to express an appropriate integrin (alpha9, a tenascin/osteopontin receptor) and kindlin-1 an integrin activator. These molecules are transported to the axon tip and drive long-distance spinal cord regeneration. However most CNS neurons undergo a maturation process that leads to highly selective transport. Many molecules are excluded from axons, while others are axon-targeted and many are dendrite-targeted. Integrins, IGF receptors, trkB and other key molecules become excluded from axons. Many of these molecules are transported through the recycling endosome system in Rab11 endosomes, and these endosomes are also excluded from mature axons. Searching for molecules that might link Rab11 to kinesins for anterograde transport we identified protrudin. This scaffolding molecule binds to kinesin, Rab11, endoplasmic reticulum and spastin. A phosphomimetic form of this molecule is a powerful stimulator of regeneration. It enhances Rab11 and integrin transport and also brings endoplasmic reticulum into growth cones. It appears that bringing a relatively small number of molecules into mature CNS axons may be sufficient to facilitate good regeneration.
Screening for promoters of regeneration using human iPSC-derived neurons

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Neurons within the central nervous system have a poor ability to regenerate after injury, leading to permanent functional deficits. Although sensory and motor neurons do retain the ability to regrow after peripheral nerve injury, the functional outcome here is low1,2. Progress in developing novel therapies to promote axon regeneration has been slow, which may be partially contributed to by the lack of robust human-relevant assays suitable for large scale drug screens. We have developed a high throughput axonal growth phenotypic screen using human induced pluripotent stem cell (iPSC)-derived neurons to identify targets for promoting regeneration. We are able to generate large numbers of human derived motor and cortical neurons consistently and efficiently for drug screening. The differentiated neurons undergo an axonal injury surrogate, by re-plating, and are then cultured on chondroitin sulfate proteoglycans – a major component of the extracellular matrix in the injured central nervous system. The bioactive small molecule libraries we use are composed of target-annotated compounds for identifying novel pathways regulating regeneration and for compounds that have potential for drug development. After we identify positive hits, we run secondary screens including laser cutting the axons of a novel human neuron spot culture and study live axon regeneration after drug treatment. The new “injury in a dish” models are suitable for high-throughput drug screening. From a screen of more than 10,000 compounds we have identified some promising hits that dramatically promote axon regrowth in injured human induced neurons. In addition, we have shown the hits significantly promote axon regeneration after sciatic nerve injury in vivo. Our goal is to make progress in finding new therapeutic potentials for promoting axon regeneration.

References

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Recovery of normal breathing following spinal cord injury: novel targets for functional restoration

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There are profound challenges associated with recovering motor function at chronic time points following spinal cord injury (SCI). Critical amongst these is the ability of the paralysed muscle to regain its functional mechanical properties following months of paralysis and potential atrophy. We have previously shown that inducing plasticity within the respiratory motor pools can rapidly induce recovery of diaphragm function at chronic stages post SCI\(^1,2\). However, the degree to which the diaphragm could function after prologued paralysis and how it could rapidly regain activity was not determined. Here, we investigate how diaphragm mechanical properties change six weeks following chronic cervical SCI and after plasticity induced recovery of respiratory motor function\(^3,4\). Collectively, these data show that chronic paralysis of the diaphragm adaptively and plastically alters the muscle’s mechanical performance. However, the muscle retains the capacity for conventional functionality due to the retention of microvascular supply. Importantly, recovery of spinal circuitry mediates a shift back towards optimal working characteristics of the diaphragm muscle. However, oxygen demand in the tissue remains in deficit. These data explain how normal respiratory motor function can be rapidly restored following successful treatment of chronic SCI and provide novel targets for future treatment strategies.

References

Supported by Wings for Life; the European Union (Operational Programme Research, Development and Education) in the framework of the project "Centre of Reconstructive Neuroscience"; and the King’s College London Prize Fellowship
Target reinnervation in a novel human forebrain organoid-spinal circuit assembly

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Restoring spinal cord circuits after spinal cord injury (SCI) and to translate these approaches from mouse models remain a major obstacle. Many strategies have been based on modifying corticospinal axon growth or guidance with variable success. Recent examples show that inducing plasticity within the local spinal neuronal network is also a key to achieve functional recovery. In this respect, glial cells such as astrocytes emerge to the centre stage as targets given the current fresh perspective on their synaptogenic and axon-repair potential. However, an increasingly recognized additional problem that significantly impedes clinical advances is the differences between mouse and human glia-neuron communication and circuit organization. To overcome this limitation, we have recently developed innovative models by generating novel human 3D cerebral and spinal cord organoids from stem cells, mimicking the cytoarchitecture, neuronal networks, transcriptomic profile, cellular interactions and injury responses of the human CNS. This allows for precise mechanistic elucidations and further hypothesis generation for the regulation of human corticospinal and local spinal circuit recovery using single cell RNA-sequencing and cell type-specific gene targeting. Our preliminary work has highlighted new candidates but also indicated that at least some of the regulators of synapse recovery we have previously identified in mouse models, such as thrombospondin-1, are likely to be relevant to human spinal circuit recovery in SCI. Although, further characterization is necessary, we anticipate that our new model system will provide an unprecedented opportunity for discovery and target-validations relevant to therapeutics in human SCI.

References

Supported by the International Spinal Research Trust, Medical Research Council, Wellcome Trust, Isaac Newton Trust
Chondroitin sulphate proteoglycans (CSPGs) are inhibitory extracellular matrix (ECM) molecules which are upregulated in the glial scar after injury in the central nervous system (CNS). In addition, CSPGs are also present in perineuronal nets (PNNs). PNNs are dense pericellular ECM structures that envelop sub-populations of neurones throughout the CNS providing stabilisation of circuitry and thus regulation of plasticity. To enhance recovery after spinal cord injury, therapies aim to promote regeneration of severed axons and the plasticity of surviving circuitry. Enzymatic removal of CSPGs using chondroitinase ABC (ChABC) opens a window of plasticity enhancing functional recovery, particularly in spinal cord injury models. However, there are significant hurdles translating ChABC into clinical use.

We have recently repurposed a small molecule, perineuronal net inhibitor (PNNi), which provides a non-invasive strategy in down-regulating CSPGs in the CNS. Daily oral administration of PNNi successfully down-regulates CSPGs in spinal cords and enhances the recovery of locomotor functions when combined with rehabilitation. We are currently exploiting the use of PNNi in enhancing recovery in chronic spinal cord injury.

Supported by the International Spinal Research Trust, Wings for Life, Medical Research Council
**Metabolic pathology after spinal cord injury is comparable to eating a high fat diet**

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Spinal cord injury not only interferes with nerve pathways controlling motor and sensory function. It also disrupts innervation to and regulation of all body organs below the level of the injury. This results in metabolic disturbances that contribute to the elevated levels of diabetes, cardiovascular disease and shortened lifespan in the SCI population. Our prior work showed that SCI in rodents causes similar pathologies commonly noted in SCI subjects, including fatty livers, hyperlipidemia, and insulin resistance. These are central components of Metabolic Syndrome (MetS) indicating that SCI induces the development of MetS in rodents. To test how comparable SCI is to a standard model of MetS, we performed a study in which rats were divided into two groups, one receiving standard chow and one receiving a high fat diet. After 8 weeks, half the rats in both groups were given a moderate T8 contusion and maintained on the same diets for another 8 weeks. Thus, we had four groups: naïve rats, naïve rats fed a high fat diet, SCI rats and SCI rats fed a high fat diet. Results revealed that rats with SCI fed standard chow had the same level metabolic pathology as uninjured obese rats fed the high fat diet. This included elevations in circulating triglycerides, free fatty acids, insulin and glucose. They also had comparable levels of endotoxemia, suggesting bacterial translocation from the gut occurs to a similar extent in models of SCI and obesity. Both groups showed evidence of marked liver inflammation and fat accumulation. Thus, SCI alone is as potent at inducing MetS as consuming a high fat diet. Notably, almost all outcome measures were even worse in rats with SCI plus a high fat diet, revealing that the combination of these two “insults” potentiates the deleterious effects of each one. Notably, SCI rats fed a high fat diet also had poorer motor recovery. Overall these results reveal that SCI causes marked metabolic pathology and that our rodent models mimic many of the systemic problems observed in SCI individuals.

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Overcoming immune-mediated mechanisms underpinning age-dependent axonal regenerative failure

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Axonal regeneration is limited or absent following injury in the peripheral or central nervous system (PNS or CNS) respectively, undermining functional recovery. The axonal regenerative ability and recovery potential further decline with ageing, which is an increasing risk factor for axonal injuries and disability (DeVivo and Chen, 2011; Singh et al., 2014). The knowledge of the cellular and molecular substrates responsible for this decline is very sparse. Previous studies showed that an age-related impairment in de-differentiation and activation of schwann cells (SCs) limits axonal regrowth in the injured PNS (Painter et al., 2014). Following spinal cord injury, deletion of PTEN can partially limit the age-dependent regenerative decay in the CNS (Geoffroy et al., 2016). Here, by RNAseq experiments in dorsal root ganglia (DRG), we found that 18-24 month old mice display age-dependent stark alterations in immune and cell-cell communication signalling pathways in comparison with 2 months old young mice both preceding and following a sciatic nerve injury. The main age associated molecular signature was represented by an increase in T cell activation and signalling, which was reduced by exposing mice to environmental enrichment (EE) before an injury. EE reversed the regenerative decline in aged mice back to the level of the young and offered an opportunity to identify molecular signalling that reverses age-dependent regenerative failure. Mechanistically, we found that an age-dependent increase in inflammatory cytokines activates NFkB in DRG neurons, which act as antigen presenting cells (APCs) and express NFkB-dependent chemokine that recruits T cells in proximity of neurons. Activated T cells in turn repress axonal regeneration of sensory DRG neurons by inhibiting regenerative signals. Remarkably, in vivo antibody-mediated specific T cell depletion or chemokine neutralization restores axonal regeneration of sensory neurons to the level of the young following sciatic nerve and spinal cord injury alike. These data propose a novel mechanism restricting the axonal regenerative ability in the old. They also suggest that antibody-mediated manipulation of neuron-immune cell communication might be a clinically promising avenue to counteract regenerative decline.

Reference


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Fecal transplant prevents gut dysbiosis and anxiety-like behaviour after spinal cord injury in rats

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Secondary manifestations of spinal cord injury beyond motor and sensory dysfunction can negatively affect a person’s quality of life. For example, spinal cord injury is associated with an increased incidence of depression and anxiety; however, the mechanisms of this relationship are currently not well understood. Human and animal studies suggest that changes in the composition of the intestinal microbiota (dysbiosis) can cause mood disorders. The objective of this study was to establish whether a cervical contusion spinal cord injury in rats triggers anxiety like behavior, and to determine whether the gut microbiota plays a role in the observed behavioural changes. We found that even a small cervical spinal cord injury caused dysbiosis and increased symptoms of anxiety-like behaviour. Treatment with a fecal transplant prevented both spinal cord injury-induced dysbiosis as well as the development of anxiety-like behaviour. These results indicate that an incomplete unilateral cervical spinal cord injury can cause affective disorders and intestinal dysbiosis, and that both can be prevented by treatment with fecal transplant therapy.

This study is supported by the Craig Neilsen Foundation
Relevance and underlying mechanisms of the systemic SCI-induced Immune Deficiency Syndrome (SCI-IDS)

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The immune system was discovered by its capacity to protect the body against viruses and bacteria, foremost as a defense mechanism without harming the body. Requiring permanent attendance the immune system is not only a defensive but also to maintenance mechanism of the body challenged by many perturbations. A titrated, adequate immune response to those perturbations can only occur if being orchestrated all the time. This requires integration of feedback into a processing system, the immune system, to keep the inflammatory response in check doing the right thing at the right time. Main aspects on how the immune system becomes defective resulting in maladaptive (skewed, ectopic and ineffective) immune responses will be illustrated. We will focus on a recently discovered maladaptive sympathetic-neuroendocrine adrenal reflex mediating systemic immunosuppression after SCI (SCI-induced immune-deficiency syndrome). This underlying pathological reflex represents a mechanistic target to develop non-antibiotic treatment strategies in order to prevent infections and attributed mortality, impaired neurological recovery and disability (1-6).

References


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J.M.S. is a Discovery Theme Initiative Scholar (Chronic Brain Injury) of The Ohio State University
A gut metabolite promotes axonal regeneration

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Spontaneous functional mammalian axonal regeneration after injury fails. High density and fast axon regeneration across distances are needed for efficient repair in humans. These are likely influenced by complex neuronal intrinsic and extrinsic metabolic and signalling mechanisms. Environmental factors such as exercise and diet have been shown to affect metabolism and signalling promoting health and repair processes in several diseases. Intermittent fasting (IF) has been shown to increase synaptic plasticity and neurogenesis that partially share molecular mechanisms with axonal regeneration. We recently discovered that IF promotes axonal regeneration after sciatic nerve crush in the mouse. Interestingly, we found that IF selectively modifies the gut metabolism leading to the specific increase in a gut bacteria-derived metabolite. Feeding mice with the metabolite increased sciatic axonal regeneration after injury phenocopying IF. Further, RNaseq transcriptomic analysis from dorsal root ganglion neurons suggests a role for the immune system in the regenerative phenotype. Currently, we are further investigating whether this metabolite affects axonal regeneration after spinal injury, which could offer a novel translational approach for nerve repair and functional recovery after nervous system injury.

Supported by the International Spinal Research Trust
Targeted neurotechnologies for spinal cord injury

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Over the past 15 years, my research team have developed a multipronged intervention that reestablished voluntary control of paralyzed legs in animal models of spinal cord injury, and recently in humans. This intervention acts over two time-windows. Immediately, electrical and chemical stimulations applied to the lumbar spinal cord reactivate lumbar execute centers located below the injury that coordinate leg movements, enabling voluntary control of paralyzed muscles during locomotion. In the long term, will-powered training regimens enabled by these electrochemical stimulations and cutting-edge robotic assistance promote the reorganization of residual connections that restores voluntary movements without stimulation. We recently exploited these neurotechnologies to target the sympathetic circuitry, which allowed us to develop a neuroprosthetic baroreflex that precisely controls hemodynamic instability after severe SCI in preclinical models and humans. During my talk, I will discuss the technological and conceptual development of these interventions in preclinical models, how we translated these developments in humans with SCI, and how we envision the next steps to establish a clinically viable treatment.
Neuroprosthetics to facilitate recovery of locomotion after spinal cord injury

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Spinal cord injuries (SCI) are the second leading cause of paralysis after stroke. The ability to walk is often lost after SCI, reducing quality of life and increasing healthcare costs. Current rehabilitation interventions to restore walking use intensive training protocols with or without electrical stimulation of the spinal cord. These protocols directly target the spinal cord circuits responsible for leg muscle activation, but ignore engaging brain structures that are essential for initiating, planning, and producing voluntary movements. Our lab recently developed the first neurostimulation strategy that allows stimulating specific area of the brain during ongoing behaviour. When applied to the motor cortex of intact rats during walking, stimulation immediately allows modulating and tuning ongoing locomotion. In rats with SCI, cortical stimulation immediately alleviates deficits, reproducing the natural dynamics of muscular activation to produce walking. When applied chronically after SCI, neurostimulation improved voluntary control of movements and the performance was retained long after therapy was discontinued, providing the proof of principle that the benefit of neurostimulation is maintained even when stimulation is discontinued. Our results highlight the motor cortex as a critical target for rehabilitation of walking.

Supported by the Craig H. Neilsen Foundation, the Natural Sciences and Engineering Research Council of Canada, and Fonds de recherche du Québec – Santé (M.M. salary)
Epidural Stimulation for the restoration of function following severe spinal cord injury

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We have previously shown that chronic, motor complete SCI individuals can progressively recover voluntary movement and standing ability when lumbosacral spinal cord epidural stimulation (scES) is applied with task- and individual-specific parameters (1-3). The aim of this study was to investigate the effects of two different activity-based training paradigms with scES on standing and stepping ability, and to determine whether standing and stepping can be concurrently trained without limiting the recovery of standing in individuals with chronic complete SCI using scES. Eight individuals with chronic, motor complete spinal cord injury (SCI) were implanted with a spinal cord epidural stimulation unit. All research participants received an implant of a 16-electrode array on the dura (L1-S1 cord segments, T11-L1 vertebrae). Four individuals performed approximately 80 sessions of stand training with scES (5 days/week; 1 hour per session) followed by 80 sessions of step training with scES (Group 1). Four other individuals (Group 2) performed an interleaving stand-step training with scES, which consisted of stand training and step training that alternated every session until achieving approximately 160 sessions (5 days/week; 1 or 2 hours per day). Interleaving stand-step training with scES promoted significant recovery of standing ability in all four individuals with chronic complete SCI and seemed more effective than the previous paradigm in which stand training was completed prior to step training. Additionally, all individuals trained with the interleaving stand-step protocol regain the ability to take independent steps on the treadmill. Two out of the four individuals in this group recovered the ability to walk over-ground with an assistive device. These results indicate that the human spinal circuitry can learn to generate motor patterns effective for standing and stepping, while retraining both tasks with task-specific scES. For complex tasks such as stepping and walking, the human lumbosacral circuitry was transformed into functional states that generated independent steps when optimized epidural stimulation was present and task specific proprioception for stepping was ongoing. However, independent stepping only occurred when the individual was driving specific intent for walking. This unexpected finding showed that de novo functional supraspinal connections had emerged to reestablish control of aspects of locomotion in individuals who had been clinically diagnosed with motor complete spinal cord injury. The spinal circuitry has potential to drive recovery after severe spinal cord injuries if provided with the appropriate retraining when in a primed central state of excitability.

References

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Targeted walking in incomplete spinal cord injury: a new method to assess corticospinal control?

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After an incomplete spinal cord injury (iSCI), the affected individuals have the potential to regain walking function depending on the severity of the injury. However, the mechanisms explaining the recovery are not fully understood in humans. As walking is a complex motor task that requires proficient coordination and balance, locomotor centers at all levels – remnant ascending and descending pathways and integrating spinal circuits and supraspinal centers (brain stem nuclei, the cerebellum, basal ganglia networks and the cortex) – play crucial roles in locomotor recovery. To assess the functional recovery during the rehabilitation clinical gait analysis has been established but unfortunately fails to differentiate between the role of spinal and supraspinal networks during recovery. In this talk a new functional assessment will be presented that has the potential to discriminate between spinal and supraspinal integrity in SCI patients. There is evidence that corticospinal control is enhanced during targeted walking, where the foot must be continuously placed on an unpredictable visual stimulus. Therefore, a targeted walking task for cats has been translated into an assessment that can be used in a clinical setting in humans. This presentation demonstrates the potential of targeted walking in the functional assessment of corticospinal integrity. Data of control participants and individuals with chronic iSCI that performed normal and targeted walking on a treadmill (while muscle activity and kinematics were recorded) will be presented. While the overall kinematic walking pattern was comparable between walking conditions, controls showed distinct changes in muscle activity (EMG pattern as well as EMG frequencies) which was disturbed in individuals with iSCI according to the severity of the lesion. The targeted walking task holds potential as a research tool to reveal further insights into the neuromuscular control of locomotion.

References

Supported by the Clinical Research Priority Program for NeuroRehab of the University of Zurich. Movement analysis was supported by the Swiss Center for Clinical Movement Analysis, SCMA, Balgrist Campus AG, Zürich
Prediction models in acute SCI: advanced stratification for clinical trials

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As spinal cord injury is a highly heterogeneous syndrome, focusing on only one outcome measure as traditionally performed in clinical trials is unlikely to reflect the full complexity and diverse nature of recovery. Therefore, several variables must be considered in a comprehensive manner to measure the SCI syndrome and to evaluate therapeutic efficacy. Therefore, we propose prediction models based on a non-unbiased statistical approaches to address the multidimensional composite scores which are sensitive to spontaneous recovery and are useful for the evaluation of clinical trials (and may or may not be useful for clinical practice). The talk will focus on the outcome of upper limb motor sores as a template example and alludes about the importance to reveal underlying conditions that affect recovery and outcomes. Data (i.e., neurological and functional) are retrieved from the EMSCI project, to capture and characterize the full spectrum of recovery after spinal cord injury and focuses on cervical SCI (C0 –T1).
Neurotrophic factor gene therapy with a first generation “stealth” gene switch

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Gene therapy is a powerful strategy to delivery therapeutic genes to the injured spinal cord. The ability to control therapeutic gene expression is important for three reasons. First, inducible expression can be employed to study the effect of the timing and dose of the therapeutic gene on the regeneration process. Second, controlled expression would be essential to prevent unwanted side effects that occur as a result of persistent overexpression, e.g. neuropathic pain or entrapment of regenerating axon. Finally, regulatable gene expression is a safeguard against unexpected effects of the transgene in clinical studies.

The doxycycline-inducible gene switch is one of the most promising systems for regulating therapeutic gene expression because doxycycline is a widely used, safe antibiotic. Unfortunately, an immune response directed against the foreign bacterial transactivator protein (TA), that binds to a doxycycline responsive DNA-element and activates transcription in the presence of doxycycline, hampers translational studies. During evolution, many viruses have developed effective immune-evasive mechanisms. A glycine-alanine repeat (GAR) in the Epstein-Barr virus nuclear-antigen protein results in evasion of cytotoxic T-cell-mediated tissue-destruction by preventing the formation of MHCI-directed antigenic peptides and/or by the modulation of protein translation and stability.

We took advantage of this mechanism to create an immune-evasive - stealth – version of the doxycycline-inducible TA (designated GAR-TA). GAR-TA and TA were embedded in a lentiviral vector encoding the reporter gene luciferase (Luc). We show that GAR-TA retains its transactivator function and allows reliable (re)induction of transgene expression in the spinal cord for a period of one year, whereas transgene expression regulated by the classical transactivator TA starts to die down around week 15. In a bioassay for human antigen presentation, GAR-TA exhibited a significant immune-evasive advantage over TA. To investigate the immune response in the intact rat spinal cord induced by GAR-TA and TA, a FACS analysis was performed on spinal tissue 7 weeks after injection of lentiviral vectors expressing TA, GAR-TA or an empty lentiviral vector. A significant induction of CD8+ T-cells was observed in rats expressing TA but not in rats expressing GAR-TA. Taken together these observations show that the GAR-TA based system has unique immune-evasive – “stealth” – properties and can be used to reliably control transgene expression in the spinal cord.

We tested and applied a doxycycline inducible lentiviral vector stealth gene switch for GDNF (dox-i-GDNF) in a spinal ventral root avulsion lesion (Eggers at al 2019). Time-restricted GDNF expression (1 months) using the immune-evasive gene switch was sufficient to promote long-term motor neuron survival for a post-lesion period of 24 weeks, enhanced a limited degree of axon regeneration into the distal nerve and facilitated the recovery of compound motor axon potentials (CMAPs). In contrast, persistent GDNF expression impaired axon regeneration by inducing axon entrapment. In conclusion, we present a novel first generation immune-evasive inducible lentiviral vector system for therapeautic gene regulation in the injured spinal cord.

References

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Axon regeneration across SCI lesions

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This talk will explore the questions of (i) why do axons fail to regrow directly across SCI lesions in adults, and (ii) what are the cellular and molecular requirements to achieve axon regrowth across SCI lesions. Evidence will be presented that in contrast with long held beliefs, astrocyte “scars borders” are not a primary cause for the failure of axon regrowth and instead are supportive of axonal regrowth when it is induced by combinatorial stimulation strategies. In addition, evidence will be presented showing that three mechanisms, which are essential for developmental axon growth but are attenuated or lacking in adults, (i) neuron intrinsic growth capacity, (ii) growth-supportive substrate and (iii) chemoattraction, are all individually required and are in combination sufficient to stimulate robust axon regrowth across anatomically complete SCI lesions in adult mice and rats.

References


Supported by NIH-NINDS NS084030; Dr. Miriam and Sheldon G. Adelson Medical Foundation; Paralyzed Veterans Foundation of America; Wings for Life
Recovery from spinal cord injury – and its attendant neurodegenerative processes – can follow a complicated trajectory spanning several years after trauma, where the ensuing diaschisis (meaning “shocked throughout”) affects the entire neuroaxis. With potential treatments targeting repair of the injured spinal cord, there is an imperative to improve clinical trial design and efficiency, optimise patient stratification in the context of disease heterogeneity and identify potential trial outcome measures.

The ability to track trauma-induced structural changes across the neuroaxis provides the opportunity to quantify pathological processes driving diaschisis and recovery-related plasticity. During my talk I will present evidence of preserved tissue bridges and their role in recovery by means of conventional MRI. I will then provide data derived from qMRI to highlight progressive volume and microstructural changes (myelin and iron content) over five years following acute spinal cord injury. Finally, I will show latest developments of high-resolution MRI sequences and optimized post-processing methods to assess the interaction of degenerative and reorganizational changes at the level of the spinal cord and brain, simultaneously.
Biomimetic 3D-Printed scaffold for spinal cord injury repair

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There exists a great unmet medical need to develop novel therapies that promote axonal regeneration after spinal cord injury. While bioengineered scaffolds have been reported to support axonal regeneration into spinal cord lesion sites, these technologies have been limited by foreign body responses at implantation sites, lack of linear axon guidance through the lesion, and limitations in scaling to human size injuries. We have recently developed an advanced 3D bioprinting technology to fabricate a scaffold mimicking the complex architecture of the spinal cord. The printed scaffold is simply and rapidly produced, reduces foreign body responses, and supports linear, aligned host axonal regeneration in the most challenging model of rat spinal cord injury, complete transection. We found that Neural Stem Cells loaded within the protected environment of scaffolds enables survival and filled the acute lesion site, while dissociated grafts into the acute lesion site had poor survival, did not fill the lesion hence cavities were evident. Host axons regenerated into stem cell loaded scaffolds and formed synapses on stem cells derived neurons. Those stem cell extended axons into the caudal intact spinal cord and form excitatory synapses on host ventral motoneurons, thus a neuronal relay was formed between rostral host - stem cells in scaffold – caudal host. Functional testing demonstrated partial functional recovery of animals that received NSCs in scaffolds, using the open field BBB score, along with detection of EMG signals in the hind limbs, that were abolished upon rostral re-transection. We conclude that biomimetic 3D printed scaffolds enable survival of NSCs to acute spinal cord lesion sites, likely by providing a neuroprotective environment, support linear regeneration of host axons through sites of severe SCI, and support regain of motor function.

References

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For better or worse: traumatic CNS injury and the inflammatory neural stem cell niche

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Therapeutic transplantation of human neural stem cells (hNSC) offers promise for neural repair in neurodegenerative disorders and central nervous system (CNS) injuries. However, functional integration of transplanted stem cells is heavily dependent on the interactions between donor cells and cellular and molecular cues in the host microenvironment. We have previously shown that while hNSC engraftment and survival are maintained, there is a striking effect of the acute versus sub-acute and chronic spinal cord injury (SCI) microenvironment on donor hNSC fate, migration, and functional integration (Cummings et al., 2005; Hooshmand et al., 2017; Nguyen et al., 2017; Salazar et al., 2010). HNSC transplantation into sub-acute (9dpi) and chronic (30-60dpi) SCI models results in extensive donor cell migration away from the injury epicenter, predominant oligodendrocytic and neuronal differentiation, with few cells exhibiting an astroglial fate, and robust recovery of locomotor function (Cummings et al., 2006; Cummings et al., 2005; Hooshmand et al., 2009; Nguyen et al., 2017; Salazar et al., 2010). Engraftment as oligodendrocytes is directly correlated with functional recovery, and selective ablation of human cells abolished recovery of function (Cummings et al., 2005). In contrast, hNSC transplantation into acute (0dpi) does not result in recovery of locomotor function and reveals striking hNSC migration towards the SCI epicenter, as well as predominant astrocytic (>90%) fate and clumping of these cells proximal to the epicenter (Hooshmand et al., 2017; Nguyen et al., 2017). Specific immunodepletion of polymorphonuclear neutrophils (PMN) inhibits donor hNSC astrogliogenesis after transplantation into the acute microenvironment, and rescues the capacity of donor hNSC to promote functional repair (Nguyen et al., 2017). In parallel, in vitro experiments using conditioned media (CM) from PMN and Macrophages (Mϕ) demonstrates that secreted factors derived from these two distinct immune populations drive hNSC migration and differentiation, identifying complement C1q and C3a as molecular mediators (Hooshmand et al., 2017). In addition, C1q in combination with C3a interacts with hNSC to control both migration and lineage selection (fate) in vitro, and in vivo after acute (0dpi) SCI transplantation (Hooshmand et al., 2017). A variety of data suggest the novel concept that C1q could have cellular functions as a ligand that can directly interact with a transmembrane receptor to mediate cell signaling. We therefore investigated the effects of blockade of C1q in the SCI microenvironment on NSC, and direct effects of C1q on NSC signaling in vitro, hypothesizing that C1q directly modulates NSC through direct interaction involving receptor mediated cell signaling transduction. Using an unbiased screening strategy, we identified a short list of transmembrane C1q signaling/receptor candidates in NSC, and identified specific functions for the first of these, CD44. Our results identify a novel element in the understanding of the neuro-immune interface and NSC biology, highlight the importance of host inflammatory microenvironmental cues in modulating NSC behavior, and identify a significant novel role for complement molecules in modulating NSC cell behavior in vitro and in vivo in the host microenvironment.

References

Supported by California Institute for Regenerative Medicine, Craig Nielsen Foundation
iPSCs-based regenerative medicine for spinal cord injury

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In our previous preclinical studies, when neural stem progenitor cells (NS/PCs)-derived from human induced pluripotent stem cells (hiPSCs) were transplanted into mouse or non-human primate spinal cord injury (SCI) models, long-term restoration of motor function was induced without tumorigenicity, by selecting suitable hiPSCs-lines1,2. However, NS/PCs derived from certain iPSC-lines gave rise to late-onset tumorigenicity after transplantation3. Here, to preclude these risks before clinical application, we developed molecular characterization of hiPSCs and hiPSC-derived NS/PCs together with transplantation to injured spinal cord of immune-deficient mice2. We investigated global methylation status of tumorigenic hiPSC-NS/PCs and found that aberrant hypermethylation of a tumor suppressor gene was induced along the passage. Based on these findings, we are establishing production and selection method of clinical grade NS/PCs stocks-derived from human iPSC stocks generated from HLA-homozygous super-donors by CiRA 4,5. As a fail safe system to prevent the hiPSC-NS/PCs, we found that pretreatment with γ-secretase inhibitor (GSI), which inhibits Notch signaling, decreases the proliferative capacity of transplanted hiPSC-NS/PCs, triggers neuronal commitment, and improves the safety of hiPSC-based approaches in regenerative medicine6. We submitted the detailed protocol of clinical research (Phase I–IIa) trials for treatments of sub-acute phase SCI using hiPSCs-derived NS/PCs to the Keio University Certified Special Committee for Regenerative Medicine in 2017 and approved by the Keio University Certified Special Committee for Regenerative Medicine on November 27 2018 and subsequently approved from the Ministry of Health, Labour and Welfare on February 18, 20195, 7. In this clinical trial, SCI patients with ASIA impairment scale A are the target subjects for our clinical study, and 2 × 10⁶ hiPSC-NS/PCs will be transplanted at 14–28 days after injury. The patients will be followed-up for one year, undergoing neurological and imaging evaluations and rehabilitation5, 7. In the future, validation of hiPSC-NS/PCs transplantation at the subacute phase will expand toward graft indication for chronic SCI with combination with GSI treatment6 and rehabilitation9.

References


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Repair and regeneration of the injured spinal cord using engineered neural stem cells

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There is a compelling rationale to consider the use of neural stem cells to facilitate repair and regeneration of the injured spinal cord. However, a number of obstacles exist which limit the clinical translation of neural stem cell technologies as a practical regenerative strategy. These include: the lack of autologous options, relatively poor survival and integration of transplanted neural stem cells and the non-permissive milieu of the injured cord including the presence of the glial scar. In this talk, I will present our recent work which seeks to address these issues. We have developed approaches to study human neural stem cells (NSCs) created through induced pluripotent stem cell or direct reprogramming in immunodeficient transgenic rodents and mice including the RNU rat. By engineering these human NSCs to express glial derived neurotrophic factor (GDNF), one can dramatically enhance NSC survival and integration into the injured cord. Work will also be presented demonstrating that expression of humanized enzymes to degrade the glial scar can have dramatic effects on repair, regeneration and plasticity.

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Technical development of a novel lumbar spinal cord injury rat model to identify the essential neuronal circuitry controlling bladder function as a target for future cell therapy approaches

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Loss in control of bladder function is crucial to most SCI patients, it leads to urinary tract infections and premature death, and it limits quality of life. The testing of therapeutic approaches is currently limited by the fact that animal models specifically targeting bladder dysfunctions after SCI do not exist. The aim of the present project is to develop such an animal model.

Obviously, the understanding of the neuronal circuitry controlling bladder function and its precise location is an essential prerequisite. Motoneurons innervating the striated muscles of the external urethral sphincter (EUS) controlling micturition exhibit a tonic discharge that increases during bladder filling1. This process is referred to as the guarding reflex or spinal reflex pathway and is activated by low-level afferent input from the bladder. Contractions of the EUS also induce afferent firing in the pudendal nerve that in turn activates spinal inhibitory mechanisms that suppress preganglionic neurons and interneurons within the micturition reflex pathway2. Thus; this bladder-to-EUS-to-bladder reflex pathway represents a two-fold negative feedback mechanism that promotes urinary continence. These efferent pathways to the EUS are carried in the pudendal nerve from motoneurons in the S3–S4 segments of the human spinal cord originating from cells in a circumscribed region of the lateral ventral horn, in a region called Onuf's nucleus and from various caudal lumbosacral segments in animals3. During micturition, the firing of sphincter motoneurons and the negative feedback are inhibited. In SCI patients, this inhibitory feedback is weaker or absent4.

We herein demonstrated the technical development of a lumbosacral spinal cord injury (SCI) model and investigated the functional and structural changes of the lower urinary tract (LUT) after injury in Lewis rats. Pilot experiments were conducted to identify the exact region of motoneurons at the lumbar reflex center (Onuf's nucleus) by means of retrograde double labelling at the lumbosacral spinal cord. The EUS was injected with 4% fast blue and 4% Fluorogold. Labelled motoneurons were found in the dorsomedial and ventrolateral portions of the L5-L6 spinal cord.

The primary objective of this study is to determine the impact of SCI in L5/L6 level on acute changes in bladder activity in two SCI groups; L5/L6 acute contusion and complete transection models. The extent of bladder dysfunction by using awake bladder assessment (Cytometry, Metabolic cage) as well as motor function by locomotion analysis (BBB locomotion score, Catwalk gait analysis) is assessed. The functional outcome after lumbosacral injury is compared to standard lesion height T9 (acquired from our previous studies). The second objective is to describe whether sparing of descending projections is related to sparing of bladder function. Finally, this animal model will provide an ideal situation for the testing of transplantation of identified neuronal populations serving for structural and functional regeneration of the lower motoneuron circuitry determining LUT function.

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Supported by funds of the Paracelsus Medical University Forschungsförderungsfonds (FFF) (R-19/03/119-AFR)
Neural cell transplantation is a promising therapeutic approach for spinal cord injury (SCI). Yet, despite the extensive research it still offers only limited recovery. One of the issues of cell transplantation is graft differentiation into undefined populations of cells, such as glial or oligodendrocyte progenitors, and a mix of excitatory and inhibitory neurons. This may dilute the beneficial effect that only appropriate cells can provide. Therefore, I am investigating if cells of defined neuronal fate can increase functional integration within the injured tissue. For example, spinal V3 interneurons are important in controlling the function of breathing, walking and swimming, and thus their enrichment could be key for functional recovery after the injury. V3 interneurons were derived using hESC differentiation protocols in vitro. They were separated from other unspecific progenitors by cloning and expressing a reporter on a cell surface, used for magnetic cell sorting. Human cell transplantation was tested in a hemisection spinal cord injury in rats. Cell grafting method was developed and tested in vivo, leading to cell survival for at least 2 weeks. Successful method for cell grafting can now be used to test a pure V3-graft for survival and integration. The ability of sorted V3 interneurons to innervate motor neurons connected to a muscle, and cause contractions, is also being tested in vitro. This should give a model for screening components to improve neural circuit formation and regeneration.

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Vagus nerve stimulation paired with rehabilitative training enhances motor recovery after bilateral spinal cord injury to cervical forelimb motor pools

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Closed-loop vagus nerve stimulation (VNS) paired with rehabilitative training has emerged as a strategy to enhance recovery after neurological injury. Previous studies demonstrate that brief bursts of closed-loop VNS paired with rehabilitative training substantially improve recovery of forelimb motor function in models of unilateral and bilateral contusive spinal cord injury (SCI) at spinal level C5/6. While these findings provide initial evidence of the utility of VNS for SCI, the injury model used in these studies spares the majority of alpha motor neurons originating in C7-T1 that innervate distal forelimb muscles. Because the clinical manifestation of SCI in many patients involves damage at these levels, it is important to define whether damage to the distal forelimb motor neuron pools limits VNS-dependent recovery. In this study, we assessed recovery of forelimb function in rats that received a bilateral incomplete contusive SCI at C7/8 and underwent extensive rehabilitative training with or without paired VNS. The study design, including planned sample size, assessments, and statistical comparisons, was preregistered prior to beginning data collection (https://osf.io/ysvgf/). VNS paired with rehabilitative training significantly improved recovery of volitional forelimb strength compared to equivalent rehabilitative training without VNS. Additionally, VNS-dependent enhancement of recovery generalized to two similar, but untrained, forelimb tasks. These findings indicate that damage to alpha motor neurons does not prevent VNS-dependent enhancement of recovery and provides additional evidence to support the evaluation of closed-loop VNS paired with rehabilitation in patients with incomplete cervical SCI.

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DREADDs modulation of afferent excitability effects randomized ladder walk in female Sprague Dawley rats

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Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are chemogenetic tools that can selectively control the excitability of neuronal subpopulations upon activation by its ligand clozapine-N-oxide (CNO) [1]. DREADDs can thus be used to evaluate how modulation of sensory neurons influence locomotor functional outcomes with or without spinal cord injury. Our study evaluated DREADDs impact on randomized ladder walk, an assay of skilled walking, limb placement, and limb coordination [2]. Follow-the-leader (FTL) gait is a way of walking whereby moving legs are placed at the footprints made by the legs ahead of them, thereby significantly reducing the neural burden of foothold selection [3]. Thus, randomized ladder walk (i.e. random rung placement) was employed to eliminate FTL gait for analysis of subtle differences in motor function achieved via DREADDs activation. Encapsulated within an adenovirus (AAV2), excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs were injected into the right L2-L5 dorsal root ganglia (DRG). These DRG were chosen because their rostral segments are postulated to contain central pattern generation circuitry in rats. DREADDs depolarize (excitatory, hM3Dq) or hyperpolarize (inhibitory, hM4Di) neurons to increase or decrease their resting state excitability, respectively. Randomized ladder walk was recorded prior to DREADDs injection (baseline), as well as after DREADDs expression with and without administration of CNO (4 mg/kg). Using a 3-category scale (i.e. “hit”, “miss”, or “slip”), we scored the right hindlimb according to its placement accuracy (hit rung) and degree of error (missed rung or slipped off rung). For each animal, we then calculated percent of occurrence for each of the 3 categories relative to total steps (ranging from 56 to 68 steps per animal). We found a significant difference in percent hits for the excitatory group upon administration of CNO (excitatory DREADDs without CNO administration 0.988 +/- 0.016; CNO administered 0.945 +/- 0.024; mean +/- s.d., n=7; p=0.0002; 1-way ANOVA and Tukey post-hoc test on arcsine transformed data). We found a similar but nonsignificant (p=0.12) trend in the inhibitory DREADDs group. We further found a significant difference in percent misses for the excitatory DREADDs group upon administration of CNO (excitatory DREADDs without CNO administration 0 +/- 0; CNO administered 0.014 +/- 0.011; mean +/- s.d., n=7; p=0.002; 1-way ANOVA and Tukey post-hoc on arcsine transformed data). We found that for both types of DREADDs, there was no significant difference in baseline and DREADDs expression without CNO administration for all 3 categories (e.g. inhibitory DREADDs baseline percent hits 0.977 +/- 0.011; inhibitory DREADDs without CNO administration 0.962 +/- 0.033; mean +/- s.d., n=5; p=0.1 and p=0.75 for excitatory (n=7) and inhibitory (n=5) DREADDs groups, respectively; 1-way ANOVA on arcsine transformed data). These results indicate that activation of excitatory and inhibitory DREADDs by CNO (4 mg/kg) administration can modulate functional motor outcomes in randomized ladder walk.

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Improving grasp in spinal cord injury via a wearable electronic device

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In surveys of patients with spinal cord injury (SCI), restoration of upper limb function is selected as the most important target for therapy. The hand is usually assumed to be controlled mainly by motor cortical areas, which make corticospinal connections. However, recent research has revealed contributions to hand control from the reticulospinal tract originating in the brainstem\(^1,2\). It has also been demonstrated that reticular neurons respond powerfully to loud auditory clicks\(^3\), and that plastic changes in motor output can result from pairing clicks with electrical stimuli delivered to muscles\(^4\). Stimulus pairing was achieved using a novel wearable electronic device developed in the Baker group, which allows stimuli to be delivered for long periods while carrying out everyday activities.

This project seeks to exploit these advances to enhance grasp after SCI. Wearable device protocols are currently being improved and customised to target sub-cortical hand control, using studies in healthy human subjects. In order to assess the efficacy of these plasticity protocols, non-invasive measures of sub-cortical outflow first had to be optimised, to detect plastic changes following wearable device stimulation. RST inputs can be assessed using the \textit{startreact} paradigm, which measures the shortening of reaction time following a loud startling sound\(^5\). Furthermore, we are testing the effect of a loud acoustic sound on muscle responses evoked by transcranial magnetic stimulation (TMS) and transcranial electrical stimulation\(^6\), as well as a paired-pulse TMS protocol that targets late l-waves, which are proposed to be modulated by sub-cortical pathways\(^7\).

Our preliminary results indicate that changing the parameters of the device, such as the inter-stimulus interval, significantly affect our measurements. Future work will focus on finding the best possible parameters of the device and implementing several control groups to assess the device’s efficacy.

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Modelling spinal cord circuits using human organoids

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One of the hurdles impeding translatability of spinal cord injury (SCI) research is the reliance on rodent models. Due to rodent-human species differences in anatomy, physiology and gene expression, there is an unmet need for human specific platforms to further develop therapeutic strategies. Stem cell derived organoids are emerging as a new methodology to address these issues, creating human neural tissues with the broad cellular heterogeneity and structure as seen in vivo.

Our group previously described a novel cortical-specified organoid model which showed subcortical projecting axonal tracts originating from a population of corticospinal motor neurons. These tracts were capable of projecting to and innervating spinal explants from embryonic mice showing functional output. To further build upon this model we have developed and characterized a human spinal organoid interface aiming to mimic the diverse cellular heterogeneity seen in vivo. Immunohistochemical analysis of the organoids shows presence of a broad range of astrocytes, spinal interneurons and motor neurons found in the ventral motor column of the spinal cord. Future work will look at connectivity and circuit formation between cortical-specified and spinal-specified organoids to further develop an in vitro humanized model of the corticospinal tract. Utilizing single cell transcriptomics, we then aim to determine novel therapeutic targets important to circuit formation at the cortical-spinal interface relevant to repair in human SCI.

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Effectiveness of biomaterial-based combinational strategies for spinal cord repair – a systematic review and meta-analysis of preclinical literature

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Traumatic spinal cord injury (SCI) is a serious debilitating condition that affects millions of people worldwide and for which no effective treatments are available. Because of the complex pathophysiology and molecular changes that occur following SCI, combination therapy is more likely to be effective than monotherapy. One such combination therapy consists of a biomaterial to both bridge the injured site and deliver growth-promoting factors or transplanted cells or both. To summarise the evidence supporting this approach we performed a systematic review and meta-analysis of the effects of pre-clinical biomaterial-based combination strategies on locomotor and histological outcomes in in vivo models of SCI. We included 134 relevant publications and analysed separately the effects of biomaterials alone or biomaterials in combination with drug or cell therapies. Overall, treatment with biomaterials alone improved locomotor recovery by 7.7% (95% confidence interval [CI] 4.8 to 10.7) but biomaterial-based combination therapies improved locomotor recovery by 25% (95% CI, 20.0 to 30.0). Natural biomaterials were used more frequently than synthetic or mixed natural and synthetic biomaterials, and we did not detect an effect of the category of biomaterial used on observed recovery. However, efficacies were higher when poly (lactic-co-glycolic acid) (PLGA) based biomaterials were used but were lowest for natural collagen and fibrin biomaterials. Furthermore, we found that combination approaches were consistently more effective than monotherapy with drugs or cells or biomaterials. Our results suggest that future research on spinal cord repair might focus on combination therapies with an emphasis on synthetic polymers.

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AQP4 sub-cellular relocalization is a therapeutic target for functional recovery after traumatic CNS injury

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Spinal cord edema is a problematic and damaging secondary injury mechanism of spinal cord injury. All potential pharmacological interventions have failed in Phase III clinical trials. The astrocyte water channel, aquaporin-4 (AQP4), has been closely linked with the onset of CNS edema, but the mechanisms of its involvement have largely remained unknown. Blocking the AQP4 pore is not a feasible therapeutic approach. We present the first demonstration that targeting the mechanisms leading to sub-cellular relocalization and upregulation mechanism of a membrane channel protein is a viable therapeutic strategy. Acute inhibition of AQP4 expression and localization to the blood-spinal-cord barrier (BSCB) completely abolished CNS edema by 7 days post-injury in vivo. Ablation of both CNS edema and AQP4 BSCB translocation were accompanied by functional recovery 2 weeks post-injury compared to >6 weeks in controls. Our data provide a translational platform for the prophylactic treatment of CNS edema, setting the stage for eradication of risky symptom management strategies.

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Acute spinal cord injury: monitoring the lumbar cerebrospinal fluid provides limited information about the injury site

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Aim. To determine whether, after severe acute traumatic spinal cord injury (TSCI), monitoring from the lumbar cerebrospinal fluid (CSF) space provides comparable information to monitoring from the injury site.

Methods. In 13 patients with TSCI we simultaneously monitored lumbar cerebrospinal fluid pressure (CSFP) and intraspinal pressure (ISP) from the injury site for up to one week. Using CSFP or ISP, we computed the spinal cord perfusion pressure (SCPP), vascular pressure reactivity index (sPRx) and optimum SCPP (SCPP opt). We also assessed the effect of draining 10 mL lumbar CSF on ISP. Metabolites from the injury site, sampled by microdialysis, were compared with metabolites in the lumbar CSF.

Results. ISP and CSFP signals appeared different: ISP was pulsatile, but CSFP had low pulse pressure and was non-pulsatile 21% of the time. Overall, there was weak or no correlation between CSFP versus ISP (R = -0.11), SCPP (csf) versus SCPP (ISP) (r = 0.39) and sPRx (csf) versus sPRx (ISP) (R = 0.45). CSF drainage caused no significant change in ISP in 7/12 patients, a drop by <5 mmHg in 4/12 patients and a drop (by ~8 mmHg) in 1/12 patient. Periods of low ISP were associated with more pulsatile CSFP and stronger correlation between CSFP versus ISP. Patients with less cord oedema on MRI had stronger correlation between CSFP versus ISP. Metabolite levels in the lumbar CSF versus injury site did not correlate for lactate (R = 0.0007), pyruvate (R = -0.12) and lactate-to-pyruvate ratio (R = -0.05) with significant but weak correlations noted for glucose (R = 0.31), glutamate (R = 0.61) and glycerol (R = 0.56).

Conclusions. After severe TSCI, the cord swells against the dura thus obstructing CSF flow. As a result, monitoring from the lumbar CSF provides limited information about physiological and biochemical events at the injury site and lumbar CSF drainage does not effectively reduce ISP.

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Moderate training on forced exercise wheel following C2 SCI: locomotor and respiratory effects in mice

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Cervical spinal cord injury (SCI) results in permanent life-altering motor and respiratory deficits. Exercise training is the most frequently used strategy to improve the recovery of stepping in both animal models and clinical studies (1,2). The rat model of partial cervical spinal hemisection at the second cervical metameric (C2) segment is commonly used to study the effects of spinal injury on motor and respiratory functions (3,4). However, few studies have used the mouse model of cervical partial injury (5). In this model, mice recover partial locomotor ability 1 week following cervical hemisection, while the diaphragmatic activity remains impaired on the injured side. Here, we hypothesize that moderate exercise training may provide recovery of respiratory and motor functions following chronic C2 injury. We used the forced exercise wheel system for our training protocol (one 60 min session per day, 5 days a week during 6 weeks) to evaluate the running capacity of the animal through progressive maximal tests (Tmax) at different time points (Tmax1: before SCI; Tmax2: 7 days post-SCI; Tmax3: end of the 6 weeks of forced training). After Tmax2, the mice were distributed into 2 different groups: Sedentary (SED with naive, sham and C2 SCI) and 6 weeks trained mice (TR with naive, sham and C2 SCI). Trained animals were forced to run between 50-60% of their pre-surgical running capacity. To assess diaphragmatic EMG activity, both intact and injured sides were recorded during spontaneous and mild asphyxia breathing. As expected, our results demonstrated for both C2 SCI sedentary and trained groups a significant decrease in locomotor capacity (evaluated by comparison of Tmax1 vs Tmax2) at 7 days post-hemisection (C2 SCI TR: -60.4 ± 9.1 %; C2 SCI SED: -65.4 ± 6.3 %; p<0.05). Six weeks of moderate training on forced exercise wheel significantly increased the running capacity compared to the sedentary group (Tmax3: C2 SCI TR: +129.0 ± 21.4 %; C2 SCI SED: +14.2 ± 2.9 %; p<0.05). No difference in diaphragmatic activity was observed after 6 weeks of moderate exercise between all groups during spontaneous breathing (C2 SCI SED: -67.8 ± 6.1%; C2 SCI TR: -59.3 ± 11.6 %, injured side vs intact side) and mild asphyxia breathing (C2 SCI SED: +103.1 ± 28.5; C2 SCI TR: +85.6 ± 49.0 %, mild asphyxia vs spontaneous breathing). More experiments are required in order to determine the synergetic effects of moderate exercise in the locomotor functional recovery and the respiratory system, whether 6 weeks of moderate training could lead to a remodeling of the injured spinal cord cellular and molecular processes by creating a more favorable environment for axonal plasticity.

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Promoting axonal regeneration through enhancing anterograde transport of sub-cellular organelles


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The adult central nervous system (CNS) has highly limited intrinsic regenerative ability compared to other tissues. This is, in large part, due to the exclusion of growth machinery—integrins, growth factor receptors, organelles, etc.—from axons after development. We aim to promote axon growth and regeneration by enhancing the anterograde transport of these components.

Previous work in the group has identified a number of potent promoters of CNS axonal regeneration in vitro. These molecules act upstream of integrin transport—by regulating small GTPases, and by linking integrin-containing Rab11 endosomes to kinesin for axonal transport. We are now applying this research to the injured spinal cord and diseased optic nerve, using animal models of CNS injury and glaucoma to investigate whether or not these molecules increase integrin transport in vivo, and how this translates to neuroprotection and repair. Studying axon transport in relation to CNS injury is helping to elucidate the role of key sub-cellular organelles in the regenerative process, leading to a better understanding of regenerative failure and the identification of new targets for CNS repair.

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Nimodipine prevents the development of spasticity after spinal cord injury

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Spasticity, one of the most frequent comorbidities of spinal cord injury (SCI), has been shown to disrupt motor recovery and quality of life (1). Despite major progress in neurorehabilitative and pharmacological approaches, no curative treatment for spasticity exists. Here, we show in a mouse model of chronic SCI that treatment with nimodipine – an FDA-approved L-type calcium channel blocker – starting in the acute phase of SCI completely prevents the development of spasticity measured as increased muscle tone and spontaneous spasms. The aberrant muscle activities are permanently blocked even after termination of the treatment. Constitutive and conditional silencing in neuronal subtypes of CaV1.3 channels shows that preventive effect of nimodipine on spasticity after SCI is mediated by the neuronal CaV 1.3 channels. This study identifies a potentially curative treatment protocol with a specific target for the prevention of spasticity after SCI (2,3).

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Inflammation and matrix remodelling after spinal cord injury: the role of TLR4

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Spinal cord injury (SCI) results in a cascade of secondary events including inflammation, extracellular matrix remodelling and scarring. Endogenous molecules present in the injury environment signal through toll-like receptor 4 (TLR4) to activate and amplify inflammatory responses. Therefore, the first aim of this project is to investigate whether inhibition of TLR4 can modulate the inflammatory and remodelling responses after SCI. The dose and delivery of the TLR4 inhibitor, TAK-242, was optimised first using bone-marrow derived macrophage (BMDM) culture, and subsequently in a rat model of T10 spinal contusion injury. Treatment of BMDMs with TAK-242 prior to LPS stimulation resulted in a dose-dependent reduction in inflammatory markers TNFα, IL-1β, IL-6 and iNOS. Pilot studies to optimise TAK-242 delivery in vivo revealed that twice daily intrathecal TAK-242 injections significantly reduced IL-6 and iNOS gene expression 24 hours post-injury and caused a significant reduction in the percentage of iNOS+ macrophages at 7 days post-injury. Following these optimisation studies, a long-term study was performed in which animals were treated with TAK-242 acutely after injury (2x daily, 7 days). Injured tissue was collected at 1- and 8-weeks post-injury and biochemically processed to separate cellular and extracellular tissue protein subfractions for proteomics. Bioinformatic analysis of this proteomics dataset is currently ongoing to identify changes in extracellular matrix proteins after injury and TLR4 inhibition. Any potential targets identified will be validated by Western blotting. Behavioural and histological assessments will also be performed to determine the effects of TLR4 inhibition on functional outcome, extent of injury pathology and changes in extracellular matrix expression.

References

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Chronic high frequency repetitive transcranial magnetic stimulation treatment following C2 SCI: respiratory and molecular effects

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High spinal cord injuries (SCI) induce respiratory deficits, often leading to death of patients. They disrupt the respiratory bulbospinal pathways innervating the phrenic motoneurons connected to the diaphragm, resulting in respiratory failure. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive device used in clinical practice to neuromodulate cortical and subcortical areas. We have previously established that TMS may be used to evaluate corticomotor and supraspinal pathways by recording specific motor evoked potentials of the diaphragm (MEPdia) in naive rats as well as in a preclinical rat model of neurological respiratory deficit1,2. We also showed that an acute high frequency rTMS protocol leads to a long lasting increase in diaphragm excitability in naive adult rats3. Based on these results, we tested a chronic rTMS protocol (9 trains of 100 biphasic pulses at 10 Hz, with 30 s intervals, 50% of maximum output, once a day for 7 days) on restrained, awake adult rats, starting 7 days after cervical SCI. We evaluated the global ventilation by plethysmography on awake rats, diaphragm activity by electromyography (EMG) recordings, and phrenic motoneurons excitability by recording MEPdia on anesthetized rats. Our chronic rTMS treatment did not have any effect on tidal volume (0.44 ± 0.05 vs 0.54 ± 0.02 ml/100g), respiratory rate (155 ± 6 vs 144 ± 11 BPM) and minute ventilation (69.09 ± 8.23 vs 78.32 ± 3.37 ml/min/100g) compared to sham treated animals. No variation in diaphragmatic EMG (treated (injured side): 0.106 ± 0.067 µV.s.s vs non-treated (injured side): 0.177 ± 0.177 µV.s.s) or in MEPdia (treated (injured side): 0.21 ± 0.16 mV vs non-treated (injured side): 0.21 ± 0.12 mV and treated (intact side): 0.31 ± 0.17 mV vs non-treated (intact side): 0.36 ± 0.15 mV) were observed after 7 days of rTMS treatment. We also looked at the reorganization of the perineuronal nets by studying expression of chondroitin sulfate proteoglycans (CSPGs) around the identified phrenic motoneurons pool in the spinal cord (C3-C6). These CSPGs were highly repulsive to neuronal outgrowth and were overexpressed following chronic SCI. Animals treated with rTMS presented a reduction in CSPGs production (8.28 ± 5.17% of occupied surface of the grey matter) compared to non-treated animals (21.25 ± 17.64% of occupied surface of the grey matter). Taken together, these results show that although chronic rTMS treatment did not elicit respiratory functional recovery at this post-lesional time point, profound modifications of the perineuronal nets following cervical SCI and rTMS treatment could be observed. Overall, our rTMS treatment protocol might change the cellular organization in the post-traumatic spinal cord and favor axonal sprouting. Additional experiments are needed with longer time points to evaluate whether this rTMS treatment protocol following cervical injury could lead to functional benefits.

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Recovery of proprioception in rats with dorsal root injury following human olfactory bulb cell transplantation

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The nerve fibers of an adult's CNS are unable to repair themselves after injury and so this leads to devastating long-term implications for the patient. Numerous attempts at treating spinal cord injury have been made and cell therapy is one of the most promising strategies. Olfactory ensheathing cells (OECs) are amongst the strongest candidates in this field. Our group has been carrying out studies on repairing of the CNS injuries by transplantation of OECs in experimental models. OECs are located in the nose and the brain and are able to aid nerve regeneration in the adult olfactory system. It has been shown that transplantation of OECs to the lesion site has the potential to treat spinal cord injury in both experimental rat models and clinical applications. The first clinical application by our collaborators showed encouraging outcome of axonal regeneration and functional recovery after transplantation of autologous peripheral nerve human OECs (hOECs) to the site of injury with simultaneous bridging of the spinal cord gap with autologous grafts. However, the clinical application did reveal a limitation of hOECs when treating injuries of large size and cavity. The yield of hOECs from the limited mass of biopsy tissue alone was not sufficient to bridge the severed connections. To overcome this limitation, we here present our latest work on the use of collagen as a substrate to fabricate 3D hOECs scaffolds to test its function in a rat spinal root injury model. This preliminary study is the first to transplant human olfactory bulb cells into a rat model of dorsal root injury. We have found:

1. Human olfactory bulbs can be harvested and cultured using similar protocols to rat olfactory bulbs;
2. half of hOEC transplanted rats recovered some degree of forelimb function compared with controls;
3. the study has shown that we can maximise the usage of limited cells by combining with a biomaterial;
4. the study has provided important information which is relevant for future clinical applications.

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The potential of perineuronal net modulation and daily rehabilitation to promote repair and recovery after acute spinal cord injury

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Given the complexities of spinal cord injury (SCI), it is imperative to understand the processes that impede its plasticity and regeneration. Specialised extracellular matrix (ECM) structures known as perineuronal nets (PNNs), play unique roles in this context. Indeed, removal of PNNs has been shown to open a window of plasticity⁴ to enhance neural regeneration and substantially restore functional deficits caused by SCI²³—effects enhanced in combinatorial therapy with rehabilitation⁴⁵. However, conventional methods of PNN removal are highly invasive, representing an obstacle hampering clinical application. Our lab thus utilizes a licensed oral drug termed perineuronal net inhibitor (PNNi), which is a novel strategy to remove PNNs in the context of spinal cord injury. A treatment paradigm of PNNi administration in conjunction with daily rehabilitation has been found to enhance repair and locomotor recovery in a rat model of acute SCI. PNNs, however, are important in stabilizing newly-formed neuronal connections⁶. Here, we sought to investigate if an extended duration of rehabilitation beyond that of PNNi administration would permit the reformation of PNNs and thereby confer even greater repair and recovery. Female adult Lister Hooded rats (n=10) received a moderate contusion injury at level T9, and were assigned two different treatment paradigms: one group received only PNNi (n=5), and the other received PNNi together with 15 weeks of rehabilitation (n=5). Locomotor recovery was evaluated using the open-field Basso, Beattie, and Bresnahan locomotor test (BBB), the horizontal ladder, as well as the von Frey test. Preliminary findings suggest that there may potentially be an improvement of locomotor function in rats receiving the combinatorial treatment. Future research will focus on elucidating the benefits of our combinatorial treatment in a chronic SCI rat model.

References

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Defining the molecular and cellular mechanisms of spinal cord regeneration

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Xenopus tadpoles have the amazing ability to repair and regenerate their tails upon amputation. Whilst some of the molecular and cellular mechanisms regulating tail regeneration have been characterised, little is understood about how the spinal cord itself can regenerate. Furthermore, the spinal cord is a complex tissue, comprising multiple cell types and how these different cell types respond to injury is unknown.

To start answering these questions, we have undertaken a genome-wide approach, by performing RNAseq on spinal cords isolated at 0, 1 and 3 days after amputation. By comparing the resulting datasets with those obtained on whole tail regeneration at the same time points, we were able to identify a number of genes specifically involved in spinal cord regeneration. In particular, we have identified the transcription factor Forkhead Box M1 (Foxm1) as being specifically expressed in the regenerating spinal cord tissue. Foxm1 is known to promote proliferation by promoting G1/S and G2/M transitions. Surprisingly, our data indicate that Foxm1 does not play a role in the proliferation rate of neuronal progenitors but in their fate after division. Comparing the fate of EdU-positive cells in wild type and foxm1-/- Xenopus tadpoles following amputation shows that Foxm1 is necessary for progenitors to differentiate into neurons in the regenerating spinal cord. We are currently investigating whether Foxm1 promotes neuronal differentiation in the regenerating spinal cord by regulating cell cycle dynamics.

In parallel, we have undertaken a single cell RNAseq approach to characterise the changes in gene expression of the different spinal cord cell types during regeneration. Differentiated neurons, Schwann-like cells and Oligodendrocytes show minimal changes in gene expression. However, neuronal progenitors display much larger changes in their transcriptomes. We are currently refining our analysis to establish how different subtypes of progenitors respond to injury.

References

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Respiratory training with intermittent hypercapnia to enhance plasticity following cervical spinal cord injury

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Cervical spinal cord injury (SCI) frequently leads to severe respiratory dysfunction due to damage of the spinal phrenic motor system which controls the diaphragm - the primary muscle of respiration. While some spontaneous functional plasticity occurs following cervical SCI, the extent is limited, and diaphragm paresis persists. The goal of this ongoing research is to test whether a novel activity-based therapy – daily acute intermittent exposures to hypercapnia – can enhance respiratory plasticity and diaphragm recovery after cervical SCI. We hypothesized that rehabilitation with this respiratory-specific activity-based therapy will stimulate anatomical and functional phrenic plasticity and improve diaphragm function following a moderate mid-cervical contusion injury in the adult rat. Anatomical plasticity following injury and treatment was investigated using transynaptic tracing and immunohistochemistry. Pseudorabies virus (PRV) was used to retrogradely and transneuronally trace the spinal phrenic circuitry ipsilateral to injury and assess integration of premotor spinal interneurons with phrenic motoneurons. Immunohistochemistry and western blot analysis were performed to assess changes in serotonin (5-HT), BDNF expression, and phrenic interneuron connectivity. Functional plasticity and respiratory recovery following dAIHc training was assessed with terminal diaphragm electromyography (dEMG). Hypercapnia trained animals showed a greater density of serotonergic axons within the spinal cord, and BDNF expression within the medulla and cervical spinal cord, when compared with untreated and air control animals. It was also found that 2 weeks of dAIHc training resulted in a greater recruitment of interneurons - specifically cholinergic interneurons - into ipsilateral phrenic circuitry when compared to untreated and air controls. Diaphragm EMG of the dAIHc trained animals resulted in a significant improvement of the ipsilateral and contralateral diaphragm inspiratory amplitude as well as response to respiratory challenge. These results therefore suggest that dAIHc is able to promote plasticity within the phrenic network following cervical SCI.

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A gut metabolite promotes axonal regeneration

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Spontaneous functional mammalian axonal regeneration after injury fails. High density and fast axon regeneration across distances are needed for efficient repair in humans. These are likely influenced by complex neuronal intrinsic and extrinsic metabolic and signalling mechanisms. Environmental factors such as exercise and diet have been shown to affect metabolism and signalling promoting health and repair processes in several diseases. Intermittent fasting (IF) has been shown to increase synaptic plasticity and neurogenesis that partially share molecular mechanisms with axonal regeneration. We recently discovered that IF promotes axonal regeneration after sciatic nerve crush in the mouse. Interestingly, we found that IF selectively modifies the gut metabolism leading to the specific increase in a gut bacteria-derived metabolite. Feeding mice with the metabolite increased sciatic axonal regeneration after injury phenocopying IF. Further, RNAseq transcriptomic analysis from dorsal root ganglion neurons suggests a role for the immune system in the regenerative phenotype. Currently, we are further investigating whether this metabolite affects axonal regeneration after spinal injury, which could offer a novel translational approach for nerve repair and functional recovery after nervous system injury.

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Intramuscular AAV-Neurotrophin 3 as a therapy for forelimb deficits following spinal cord injury

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Injury to the cervical spinal cord causes profound and lasting sensorimotor deficits in upper limb function. The impaired function caused by disrupted transmission through the sensory and motor pathways are further complicated by the formation of abnormally excitable reflexes chronically. This hyperreflexia occurs in the majority of patients and disrupts any voluntary function remaining in the affected limbs. By measuring the H reflex, a proprioceptive reflex circuit with both mono and polysynaptic components, it is possible to detect hyperreflexia in both patients and preclinical injury models. Mechanisms causing hyperreflexia include maladaptive plasticity in the circuitry below the spinal cord lesion and alterations in several ion channels in motor neurons.

A promising therapy to restore sensorimotor function after spinal cord injury is neurotrophin 3 (NT3). Previous studies from our lab have shown NT3 to improve locomotion, skilled forelimb function and hyperreflexia following brain and brainstem injury. Here we use a gene therapy approach to assess its efficacy at restoring sensorimotor deficits following a clinically relevant model of spinal cord injury. Moderate, 225KDyn, bilateral contusion to the mid cervical region was performed in 20 rats. 24 hours after injury several muscles in the forelimb were injected with Adeno associated virus encoding hNT3 (AAV-NT3). Studies from our lab show that the dose of AAV-hNT3 results in significantly elevated NT3 levels in DRG, serum and muscle. A pilot study showed that this injury causes extensive cavitation pathology, sustained hyperreflexia, and forelimb deficits. Using H reflex testing and fine motor behavioral tasks, including pellet reaching and horizontal ladder, we are assessing functional forelimb recovery following AAV-hNT3. Future histological analysis will identify if any maladaptive plasticity occurring in the proprioceptive circuitry can be normalized with elevated NT3 levels.

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The contribution of extracellular vesicles to the development of the acute phase response following spinal cord injury

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Inflammation following spinal cord injury (SCI) persists long after the primary insult, and its presence in the cord leads to further cell death in tissue that was initially spared after injury. It has been shown that this progressive destruction of CNS tissue is dependent on a systemic inflammatory response known as the acute phase response (APR), which, when suppressed, affords some neuroprotection. The communication pathways between the spinal cord and the periphery remain poorly understood though the production of extracellular vesicles (EVs), membrane encapsulated nanoparticles, by the damaged cord have been proposed as the missing link. It was the aim of this study to characterise the APR during the acute phase of SCI, and to determine whether this response is influenced by EV signalling. A contusion model of SCI in female C57BL/6 mice was employed. Animals received a 70kD SCI, or sham surgery as a control, at T9. Blood, spleen, liver and spinal cord were collected at 2, 6, 12 and 24 hours post-surgery, and were analysed for markers of central and peripheral inflammation. SCI induced an APR as evidenced by a significant increase in neutrophil numbers in the blood, spleen and liver, which peaked at 2 hours post-injury and decreased through time. However, neutrophil infiltration of the spinal cord peaked at 24 hours, suggesting a ‘therapeutic window’ for intervention. The peripheral response was accompanied by elevated hepatic expression of pro-inflammatory molecules, and the magnitude of the response was dependent on injury severity. IL-1β and CXCL10 expression increased with injury severity, whilst CXCL1 and SAA-2 expression decreased with injury severity. EVs were isolated from platelet-free plasma by ultracentrifugation, and concentration was determined by nanoparticle tracking analysis (NTA). SCI induced a significant increase in circulating EVs at 2 hours post-injury, correlating with peak neutrophil mobilisation, which returned to baseline by 6 hours. To determine their biodistribution, SCI plasma EVs were dual labelled with a synthetic miRNA and membrane dye, and injected into naïve mice. EVs were found predominantly in the lung, spleen and liver, organs associated with the APR. Ongoing experiments will determine whether EV signalling is causative of the APR, might act as a potential biomarker of injury severity, or provide a therapeutic delivery tool.

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Suppresion of Fibroblast Growth Factor Receptor-5 (FGFR-5) has no effect on axon regeneration after SCI

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Spinal cord injury (SCI) is one of the major types of mammalian central nervous system (CNS) injuries and any injury to the CNS can cause permanent functional disability due to the inability of CNS axons to regenerate. In this study, regenerating SN, pSN+DC and non-regenerating DC injury models had been applied to investigate the relative axon regenerating genes after spinal SCI. Using microarray analysis around 350 genes were upregulated in all injury models and suggested manipulating expression of these molecule that axon regeneration in the CNS is possible. We found that Fibroblast growth factor receptor-5 (FGFR-5) was significantly up-regulated in non-regenerating DC lesions compared with intact control and regenerating SN and pSN+DC lesion models. We also observed up-regulation of FGFR-5 by up to 5.1fold in non-regenerating DC injury model. Western blot was used to confirm our microarray data along with immunohistochemistry to analyse FGFR-5 protein levels in which suggested that suppression of this molecules would promotes the regenerating of the axons. However, we found levels of FGFR-5 proteins is elevated in non-regenerating DC model which is correlated with our microarrays data but the localisation of the protein using immunohistochemistry was found out of the neurons and accumulated in dots. Moreover, knockdown of FGFR-5 using short interfering RNA (siRNA) in 3 days cultured dorsal root ganglion neurons (DRGN) showed no effect on DRGN neurite outgrowth suggesting that suppression of this gene has no role in axon regeneration and seems to regulate vascular reparation.
Modern health care allows people with spinal cord injuries (SCIs) to live longer and healthier lives. The incredible improvements in the last century have perhaps overshadowed the simple fact that people with SCI continue to have a decreased lifespan, potentially due to ongoing health challenges. SCI changes the physiology of tissue and organ functions and we hypothesize that it accelerates the aging process. We know of no studies investigating age related changes following SCI. We therefore wanted to separate normal aging from SCI induced changes in aging. We aimed to identify age-dependent changes at the cellular and physiological level known to relate to cellular aging following SCI that could compromise health and longevity in rodents.

We conducted T9/T10 moderate contusion SCIs or sham-operated controls on rats. One group was perfused early after SCI (aging control; 8 weeks of age) while the SCI and sham operated groups were allowed to age and perfused at 1 year later to understand the long-term health associated with a moderate thoracic SCI.

SCI resulted in long term mortality (~40%) that was not observed in control rats. Despite this, we found no differences between aged rats with and without SCI on a battery of tests including: detailed blood cell counts and chemistry assessments, telomere lengths, bladder elasticity, or bone mineral density. Our physiological investigations of common age-related changes did not reveal a mechanism underlying mortality in rats aging with SCI. Further work is required to determine whether SCI-related mortality is related primarily to complications, premature physiological aging, or a combination of both.
Neuregulin-1 and spontaneous repair after spinal cord injury

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The pathophysiology of spinal cord injury (SCI) is immensely complex. The primary trauma, resulting in axonal injury and rapid death of neurons and glial cells, is followed by secondary injury progression that comprises uncontrolled inflammation, axonal demyelination and degeneration, extracellular matrix remodelling, glial scar formation and cavitation, all of which contribute to the lack of repair and permanent disability. However, despite the severe pathological and neurological deficits associated with SCI, some degree of spontaneous functional recovery is observed in almost all cases, both in humans and experimental animals. Although this recovery is limited, it nevertheless reflects an innate capacity for repair. Accordingly, at the cellular level, there is a capacity for the activation of a number of intrinsic repair mechanisms after SCI, including neurogenesis, neuroplasticity and remyelination of spinal axons. It is crucial to better understand the cellular and molecular mechanisms underlying endogenous repair events after SCI, which are suboptimal, and determine how they contribute to restoration of function. This may provide a route to modify and exploit these processes in order to improve functional outcome after SCI. We have recently discovered that different isoforms of the neuregulin-1 (Nrg1) growth factor family may drive different components of spontaneous regenerative processes and functional repair after SCI. Using conditional knockout and fate mapping transgenic approaches, we have shown that while the cysteine rich domain (CRD)-containing Nrg1 type III isoform is responsible for spontaneous functional remyelination of dorsal column axons after SCI, the immunoglobulin (Ig)-containing isoforms of Nrg1 are dispensable for this process¹. Furthermore, the differentiation of centrally derived precursor cells into functional remyelinating cells depends on signalling via the Nrg1 canonical receptor ErbB tyrosine kinase². Here we investigate the role of Ig Nrg1 isoforms in mediating spontaneous repair after spinal contusion injury. The Ig isoform of Nrg1, which is almost exclusively expressed around muscle spindles, sensory neurons and afferents that feed into the spinal cord, appears to mediate the communication between proprioceptive afferents and spinal cord that provides the sensory feedback from muscles to the injured spinal cord that is necessary for spontaneous locomotor recovery³,⁴. Our data suggests that Nrg1 mediates multiple aspects of SCI pathophysiology and that specific Nrg1 isoforms mediate distinct aspects of repair. A greater understanding of how Nrg1 modulates SCI pathophysiology could aid in the development of target-specific disease-modifying treatment strategies.

References

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Direct macrophage to ependymo-radial glial progenitor cell signalling via Tnf-a promotes regenerative neurogenesis in the zebrafish spinal cord

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Zebrafish, in contrast to mammals, regenerate spinal neurons after injury. Immune system activation promotes regeneration in the larval spinal cord, but the mechanistic basis for this is unclear (Ohnmacht et al., 2016, Development 143: 1464-74). We have previously shown that the pro-inflammatory cytokine Tnf-a is mainly produced by blood-derived macrophages in the injury site (Tsarouchas et al., 2018, Nat Commun. 9, 4670). Using pharmacological and gRNA-mediated interference with Tnf-a signalling, we show that up-regulation of hdac1, a known epigenetic regulator of neurogenesis, is impaired in progenitor cells. Moreover, proliferation of progenitor cells and regeneration of motor neurons is strongly reduced in the absence of Tnf-a signalling. Recombinant human Tnf-a stimulates expression of hdac1 and the motor neuron marker mnx1 in isolated progenitors. Conversely, genetically inhibiting hdac1 function only in progenitor cells reduces regenerative neurogenesis in vivo. These results suggest direct signalling from blood-derived macrophages to progenitor cells to effect essential gene regulation for regenerative neurogenesis. We are now analysing Tnf-a induced gene expression changes in spinal progenitor cells in unbiased approaches, including single cell RNA seq. Our data reveal a signalling axis from immune response to regenerative neurogenesis in a vertebrate spinal cord, providing targets for future interventions in non-regenerating mammals.

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Sensory-motor interneurons funnel sensory inputs into abnormal patterns of muscle activity after spinal cord injury

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Spinal cord injury (SCI) results in devastating sensory/motor impairments whose origin has been related to the permanent alteration of the spinal circuits caudal to the injury site\(^1\). A poor repertoire of functional movements characterized the motor impairment typical of the chronic phase of SCI with the simultaneous appearing of involuntary and abnormal muscle contractions, collectively called spasticity. To date, several cellular mechanisms have been linked to the appearance of spasticity after SCI but no spinal circuit has been identified to generate this aberrant motor activity.

Here we used mouse genetics, optogenetics and electrophysiology to show that V2a (Chx10\(^+\)) interneurons together with sensory afferents, motor neurons and muscles form a close-loop feedforward excitatory system in the spinal cord that drives abnormal muscle activity after SCI. Indeed, V2a interneurons receive segmental afferent inputs and project onto motor neurons located in the same segment. Simultaneously, V2a also project to more caudal motor pools that do not receive the same sensory inputs. Lastly, the V2a-induced motor activity of more caudal segments, in turns, activates the sensory afferent that re-excite the spinal network. In this way optical activation of V2a interneurons can generate multi-joints movements during motor behaviors.

However, activation of V2a interneurons by sensory inputs trigger sustained activity of the spinal network after SCI and funnel this activity into abnormal and involuntary patterns of motor neuron activation, leading to the appearance of spasticity and muscle spasms after SCI.

In conclusion, here we have identified an excitatory interneuron at the interface of proprioceptive afferent and motor output that alters sensory inputs processing and integrate it into an abnormal muscle activity after SCI.

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\(^1\)Nielsen et al., 2007

This work is supported by the Novo Nordisk Foundation and NINDS.
Temporally regulating chondroitinase gene delivery following spinal cord injury

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Chondroitinase ABC is a promising preclinical therapy that promotes neuroplasticity and functional recovery after spinal cord injury by degrading extracellular matrix inhibitors. Efficient delivery of chondroitinase ABC to the injured mammalian spinal cord can be achieved by viral vector transgene delivery. This approach dramatically modulates injury pathology and restores sensorimotor functions. The ability to exert control over chondroitinase gene expression has further optimised this system and enables the timing of delivery to be manipulated. In a comparison between short-term and long-term treatment over 8 weeks, only the group receiving sustained treatment recovered skilled reaching and grasping function. We now further investigate the therapeutic time window of chondroitinase gene therapy by switching on the transgene with a 1 week delay following injury, and at a chronic 4 week timepoint and investigate reaching and grasping function whilst using chemogenetics to silence corticospinal projections during this behaviour.

References

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Enriched conditioning: intrinsic redox signalling regulates neuronal regenerative potential after spinal cord injury


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Overcoming the limited axonal regenerative ability that impairs functional recovery following a central nervous system injury remains a challenge. Here we report the discovery of a novel regenerative paradigm that we call enriched conditioning. Enriched conditioning consists in the combination of exposing mice to environmental enrichment (EE) followed by a conditioning sciatic nerve axotomy, both preformed prior to a spinal cord injury. Enriched conditioning significantly increases the regenerative ability of dorsal root ganglia (DRG) sensory neurons compared to EE or conditioning lesion alone, propelling axon growth well-beyond the spinal injury site. Gene expression analysis of DRG after enriched conditioning showed a striking upregulation of NADPH oxidase 2 (NOX2) pathway with upregulation of all the components of NOX2 complex. Mechanistically, we found that PKC-dependent STAT3 phosphorylation induces binding of the transcription factor STAT3 on hyper-acetylated NOX2 promoter regions. This triggers the expression of the NOX2 complex and enhances redox signalling. Moreover, NADPH oxidase 2 conditional deletion or overexpression respectively blocked or phenocopied enriched conditioning-dependent axon regeneration after spinal injury. Together, these studies provide a paradigm that enhances the regenerative ability of sensory neurons beyond what was previously recognized, offering an optimized regenerative model for mechanistic and therapeutic discoveries.
Stationary indoor FES Cycling with virtual racing to encourage voluntary effort and promote recovery: a pilot study with incomplete-lesion patients

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Introduction In 2000 we reported how a man with an incomplete lesion at T11/12 recovered some motor function after using FES with a recumbent mobile tricycle to improve his fitness [1]. He became less disabled and permanently able to walk better. We hypothesized that it was the combination of voluntary effort with the stimulation that promoted neuroplastic related recovery. If this effect is not uncommon, then to make it a practical and useful therapy, an indoor stationary cycling machine with a virtual reality representation of over-ground cycling might provide motivation for voluntary effort. A key requirement was that the person’s effort should be separate from the effect of the stimulation. To achieve this, the iCycle has been developed [2] and we have completed a clinical study at the Royal National Orthopaedic Hospital [3].

Method Eleven participants (C1-T12) with incomplete SCI (5 sub-acute; 6 chronic) were recruited and completed 12-sessions of iCycle training over four weeks. Function was assessed using the bilateral ISNC-SCI motor score and other outcome measures including mechanical power, before and after training, and after a further four weeks.

Results Two of the 6 participants with chronic injuries, and 4 of the 5 participants with sub-acute injuries, showed improvements in ISNC-SCI motor score >8 points (scale: 0-50). Average (SD) improvements were 4.7 (5.5) points for participants with a chronic SCI, and 7.4 (7.4) points for those with sub-acute SCI. The two chronic participants who changed the most were both in their late 50s and had been injured 13 months and 6½ years respectively before the therapy.

Discussion Improved ISNC-SCI motor scores in chronic participants may be attributable to neuroplastic changes due to the iCycle training. Yaşar et al. [4] trained 10 people with incomplete SCI (AIS C or D) with FES-cycling only: participants were instructed not to make any voluntary effort. As in our study, they trained for one hour, 3 times per week, but for 3 months, and outcome measures were made at the end of the therapy and again three months later. Average motor scores improved by 1.7 after the therapy and 4.7 at follow up. These average improvements are nearly equal to ours but took three times longer; suggesting that voluntary effort coincident with the stimulation may accelerate recovery by a factor of three.

We found no correlation between increase in power output and improved motor score at follow-up. This suggests that the neurological recovery had not, after only four weeks of training, led to strong fatigue-resistant motor units [5] so perhaps longer iCycle training will be necessary for functional recovery.

References

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Facilitating SCI research, translation and transparency: going public with the Open Data Commons (ODC)

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Data shared in publications about preclinical research, represent only a fraction of the data produced. For example, surgical data or data that do not fit the ‘story’ of a manuscript, are often not included in published papers. Further, experiments with so-called “negative” outcomes will likely not be published in a journal at all. Thus, a large amount of data will not be accessible. These ‘dark data’ are estimated to make up 85% of all data collected, which creates various problems including publication bias. Furthermore, the inability to discover and access all data impedes research and translational efforts on various levels. For example, the lack of transparency does not allow funders to access the results of the studies they supported, journals are unable to deliver the entire picture of the experiments, and researchers repeat studies that have been performed by others, incorporating incorrect protocols and flawed scientific premises without ever knowing it. This causes a significant waste of time and resources and also triggers significant ethical concerns regarding animal use and human subjects who receive experimental therapies. Another issue is that the current publication model does not facilitate replications and independent validations. Lastly, collecting a large body of data would allow a new form of meta-analysis based on raw data, thereby facilitating new discoveries and translation.

To tackle these challenges, we have developed an Open Data Commons for preclinical research in SCI (ODC-SCI). With support from the Craig Nielsen foundation (to A.F.), a functional data platform has been built that already houses 145 data sets from 43 laboratories (http://odc-sci.org) and allows data sets to be associated with a citable DOI (digital object identifier), similar to published papers. Together with our steering committee representing the SCI research community we have recently submitted a multi-funder proposal to transition the ODC-SCI into the larger community and into a self-sustaining community governed database under the FAIR data stewardship principles. This will make SCI data Findable, Accessible, Interoperable and Reusable. This 5-year proposal has been recently approved for funding (to K.F., administrative PI) by Wings for Life and the Craig Neilson Foundation, (with ISRT possibly joining the effort in the future). The proposal has 3 separate components (AIM 1-3) focusing on outreach and communication with the community, establishing a community governed curation process for uploading data/metadata, and to finalize tools for selection of cohorts, within and across available datasets, creation of these aggregated datasets and tools for analyzing them. Over the next years we hope to engage all SCI researchers in this effort, as user, member of the curation board, and/or activist for a new and more effective way to publish experimental data.

This effort is supported by the Craig Neilsen Foundation, Wings for Life and the International Spinal Research Trust
Semaphorin 7A controls the proper targeting of serotonergic fibers in the spinal cord

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Serotonin (5-HT) is a monoamine neurotransmitter synthetized in various populations of brainstem neurons\(^1\). In the spinal cord, descending serotonergic projections regulate postural muscle tone, locomotion and rhythm and coordination of movements via the Central Pattern Generator (CPG)\(^2\). The molecular signals that control the precise patterning and targeting of serotonergic inputs onto CPG networks in healthy or after injury are still unknown. Semaphorin 7A (Sema7A) belongs to Semaphorins family involved in guiding growing axons and controlling plasticity of synaptic connections\(^3\). We hypothesize that Sema7A signaling could be an important molecular actor that instructs the patterning of 5-HT inputs to spinal CPG networks.

Here, we show that Sema7A controls the wiring of descending 5-HT axons in the spinal cord. Our results reveal that mistargetting of 5-HT fibers in the spinal cord is compensated in Sema7A deficient mice so that their gross locomotion proceeds accurately. We also demonstrate that when the system is challenged with a spinal lesion, the time course of post-injury 5-HT expression is significantly altered in Sema7A deficient mice with specific ectopic targeting of 5-HT fibers in the lumbar grey. Compensatory mechanisms at play in unlesioned Sema7A deficient mice are lost and injured Sema7A deficient mice exhibit a worsening of their post-injury locomotor abilities. These disregulation of spinal network modulation by 5-HT are accompanied by modifications in the rewiring of supraspinal input. Our findings identify Sema7A as a critical determinant of serotonergic circuit formation.

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Molecular and cellular responses of immune cells following therapeutic targeting of the extracellular matrix after spinal cord injury

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Traumatic Spinal cord injury (SCI) is a devastating neurological condition that causes irreversible axonal damage and neuronal death, resulting in permanent disability for which currently there are no effective treatments. One of the most promising experimental therapies for SCI is the enzyme chondroitinase-ABC (ChABC). ChABC targets inhibitory chondroitin sulphate proteoglycans (CSPGs) in the extracellular matrix (ECM) which accumulate at the injury site, impeding functional recovery¹. The beneficial effects of ChABC after SCI have been demonstrated by multiple labs and in different injury models, thus providing strong validation for ChABC therapy as a promising therapeutic for clinical development¹,².

In addition to the initial mechanical injury, a wide range of secondary cellular and molecular events occur after SCI, constituting the secondary injury, which leads to further tissue damage and consequentially, functional impairments³. One major contributor to this pathological cascade is the inflammatory response which is aggressive and unresolved. The factors that impede the clearance of immune cells after the injury have not been fully characterized and several lines of evidence imply a role for altered extracellular matrix (ECM) in SCI inflammation⁴,⁵. However, the mechanisms that underlie immunomodulatory effects of ECM after SCI are not yet understood. To evaluate whether ECM alteration modulates the inflammation after SCI we performed a large-scale digestion of inhibitory scar matrix by ChABC enzyme delivered via lentiviral vector (LV-ChABC) after thoracic SCI. By flow cytometry, we assessed the number of immune cells within the injury epicentre. Our results show that ECM digestion by LV-ChABC promotes inflammatory resolution after SCI revealed by accelerated clearance of neutrophils and reduced accumulation of macrophages and microglial cells compared with control animals (LV-GFP). Furthermore, we evaluated changes in cytokine and chemokine expression after SCI showing a reduction of inflammatory cytokines in animals after CSPGs digestion.

These data suggest that ECM alteration after SCI impedes the inflammatory resolution, contributing to tissue damage and functional impairments. Understanding the cellular and molecular mechanisms underlying ECM-mediated modulation of the inflammatory response may lead to more effective therapies to treat SCI.

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Magnetic field stimulation and iron oxide nanoparticles facilitates functional and morphological recovery in spinal cord injury

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Electromagnetic field (EMF) stimulation improves the microenvironment for the nerve regeneration by stimulating neurotrophic factor release, attenuating oxidative stress and apoptosis after injury ¹,². Iron oxide nanoparticles (IONPs) in combination with an external electromagnetic field (EMF) have the ability to facilitate axon regeneration \textit{in vitro} ³,⁴. The present study was designed to unravel the \textit{in vivo} potential of EMF and IONPs to facilitate morphological and functional recovery in rats with either complete or contusion spinal cord injury. In male albino Wistar rats, the spinal cord was completely transected at T11 vertebra or contusion injury was produced. The injured rats were exposed to EMF after 24h of surgery daily for five weeks (50 Hz, 17.96 μT for two hours). In a separate group of injured rats, iron oxide nanoparticles (25μg/mL) were implanted at the site of injury and the rats subsequently exposed to EMF. No cytotoxic effects of IONPs were observed. In both complete and contusion injury rats, we observed a significant improvement in locomotor behavior as well as significant reduction in lesion volume and scar formation following IONPs implantation and EMF exposure. Immunohistological analysis of spinal cord tissue for GAP-43 in these rats showed a significant (P<0.001) increase in the expression and confocal images showed abundant sprouting from mature neurons and axons near the site of lesion ⁵,⁶. In complete SCI rats, we could not record motor evoked potentials, but in contusion SCI rats significant improvement was observed after the intervention. These novel findings highlight the therapeutic potential of IONPs along with EMF in SCI.

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The role of proprioceptive afferents in activity-dependent, spatiotemporal epidural stimulation of the cervical spinal cord

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Contusion of the cervical segments is the most common form of spinal cord injury (SCI). Patients surveys have identified improvements in upper limb function as a top priority for individuals that have suffered this type of injury, but no clinical solution is available for improving the recovery of skilled arm movements. Electrical neuromodulation therapies of the spinal cord have enhanced lower limb function in numerous preclinical models, from rodents to primates, as well as a number of clinical case studies. Despite this success, and the high priority of improved upper limb function for the SCI patient community, efforts to translate this promising technique to the cervical spinal cord and upper limbs have so far been limited. Given the complex patterns of muscle activation required for the execution of skilled arm movements, adaptation of epidural electrical stimulation (EES) to the cervical spinal cord necessitates a thorough understanding of the functional specificity that can be achieved using this technique, as well as the neuronal circuitry that underlies its effect. Here we present functional and anatomical data indicating that lateralised epidural stimulation of the rodent cervical spinal cord effectively targets specific upper limb motor pools, dependent on the rostrocaudal location of the stimulation site. Based on these findings, we have developed and assessed the efficacy of a stimulation paradigm in which cervical EES is delivered in a spatially selective manner and is temporally patterned in accordance with the real time activity of selected upper limb muscles. Furthermore, to gain a greater understanding of key neuronal circuitry we have utilised targeted pharmacological and genetic manipulations, allowing us to demonstrate the pivotal role of proprioceptive feedback circuits in the generation and modulation of motor responses during cervical EES. Taken together, these results establish a conceptual framework for the design and optimisation of targeted cervical implants to facilitate upper limb movements after spinal cord injury.
Development of crosswalks to aggregate international spinal cord injury functional data

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Introduction
In the field of spinal cord injury (SCI), there are multiple, independent databases containing information on neurological and functional recovery, but data cannot be pooled or compared due to differences in how function is measured. For example, the two largest databases, the United States based Spinal Cord Model Systems (Model Systems) and the European Multicenter Study about Spinal Cord Injury (EMSCI) use slightly different functional outcome assessments. A crosswalk (where scores from two separate assessments are linked or converted to a common metric) is needed to allow comparisons. Thus, the primary objective was to create a crosswalk between the Functional Independence Measure (FIM) and the Spinal Cord Independence Measure III (SCIM III) for items reflecting voluntary motor function.

Design/Method
Common person equating, in which the instruments are administered to the same group of individuals, was used to create and validate the crosswalk. The Swiss Network of Spinal Cord Injury database (Swiss) (n = 663) was used to develop the crosswalks. The Rick Hansen Spinal Cord Injury Registry (n = 557) and data from the United States based SCIM III reliability study (n=390) were used as validation databases. Only FIM and SCIM III Items reflecting voluntary functional movement were considered, as they reflect a similar construct. Three conceptually different crosswalk methods were used. 1) Expert panel evaluation where experts in the field establish equivalency for similar items and scores from FIM and SCIM III, developing a third common scale. Individual FIM and SCIM III administrations were then re-coded to the common scale; 2) Equipercentile equivalency in which a crosswalk was developed based on aligning total score distributions and rank ordering both FIM and SCIM total scores; 3) Rasch analysis based on co-calibrated item difficulties was used to co-calibrate the items from both instruments to develop the crosswalk.

Results
The expert panel evaluation method resulted in a correlation of 0.911 (p<0.01) between the expert panel recalculated FIM and SCIM III scores. The equipercentile method produced a correlation of 0.901 (p<0.01) between the original SCIM III scores and the equipercentile FIM to SCIM III converted scores. The correlation between the original FIM scores and the equipercentile SCIM III to FIM converted scores was 0.918 (p<0.01). Rasch analyses resulted in a correlation of 0.917 (p<0.01) for FIM to Rasch FIM scores, while the correlation for SCIM III to Rasch SCIM III scores was 0.897 (p<0.01).

Conclusion
We demonstrated three viable methods to create a FIM/SCIM III crosswalk such that data collected on items reflecting functional movement with one measure can be converted to the other measure. This will allow comparisons of functional recovery across multiple databases reflecting different systems of care and rehabilitation approaches. The optimal crosswalk table will be presented.

These methods can be used when linking clinical or pre-clinical outcome measures.

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Plasticity in bulbospinal connections to thoracic alpha and gamma motoneurones following a chronic lateral spinal cord lesion

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Previous evidence from electrophysiological experiments in anaesthetised cats with a chronic lateral thoracic spinal cord lesion indicated an expansion of the functional projections of expiratory bulbospinal neurones (EBSNs) in the segment above the lesion (Ford et al. 2000). Here we investigate connections that may be involved in those projections, in particular the connections made by the same EBSNs to motoneurones, estimated via cross-correlations between their discharges. Motoneurones were assigned as α or γ according to efferent spike amplitudes and the connections at 2 weeks and 16 weeks post-lesion were compared with those in unoperated animals. The connections to α motoneurones of the internal intercostal nerve were found to be no different from controls, consistent with intracellular data (Ford et al. 2016). However, a significant increase in the number of EBSNs showing connections to γ motoneurones was found, at both 2 weeks and 16 weeks (11/28 and 18/49 EBSN/nerve pairs respectively, compared to 4/33 in controls). Furthermore, at 16 weeks a significant increase was also found in the connections to γ motoneurones of the external intercostal nerve (8/24, compared to 1/16). These motoneurones are usually activated in inspiration and are therefore, with respect to the EBSNs, antagonists for respiratory movements. The validity of the results may be limited by a number of caveats, including whether different populations of γ motoneurones might have been active in the control and lesion groups. The results are likely to be most secure for the external intercostal nerve, followed by the internal intercostal nerve at 16 weeks and least secure for the same nerve at 2 weeks. Overall, the results support the view that the expanded projections include more connections being made to gamma motoneurones.

References

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Twenty years of Spinal Research PhD studentships

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PhD studentships have a vital role in recruiting and supporting a new generation of scientists in spinal cord injury research. Over two decades Spinal Research has funded 49 students to study at the UK’s leading neuroscience research laboratories at a cost approaching £5 million pounds. Since 1998 forty students have completed their studies, with thirty-seven gaining a PhD (92.5%), nine studentships are currently in progress. Over half have continued with a career in academia with more than 20% completing a medical degree. Currently approaching 20% are using their knowledge on spinal cord injury in research related occupations.

The rate of completion of PhDs varies by institution, discipline and Funding Body. The reported UK/EU benchmark is 72% completion within seven years and 82% at twenty-five years. Two (Lakatos and Warren) now run their own SCI labs and two (King and Dunning) have their own research departments. The Lakatos lab was awarded a studentship in 2018 completing the cycle.

The high completion and retention rates of ISRT funded projects are a credit to the assessment process, the students, the host scientists and their institutions.

If you are interested in our awards, visit our website (www.spinal-research.org) and sign up for notifications about all our forthcoming call for proposals.
Low sulfated heparins target multiple proteins for CNS repair

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The ineffective repair that follows spinal cord injury leads to permanent disabilities for which effective treatments are limited. Previously, we demonstrated that heparins modified by selective desulphation (mHeps) reduce features of astrogliosis1, 3. mHeps are a class of glycomolecules with structural similarities to resident heparan sulfates (HS) that comprise repeating disaccharide units with variable sulphation patterns. HS are key modulators of cell signalling by both sequestering ligands (including chemokine/cytokines) in the ECM and acting as cofactors in the formation of ligand-receptor complexes. We have now assessed whether mHeps affect repair in two in vitro CNS injury models; a mechanical injury model in which a cell-free region is created with a scalpel blade in myelinating CNS cultures and have also used them in an antibody mediated demyelination injury model. We have demonstrated that the degree and positions of the sulphate moieties on mHeps are crucial for their biological effects. Specifically, monosulphated compounds at C2 and N positions have the greatest effect on promoting neurite outgrowth and (re)myelination after injury, whereas, highly sulphated heparin isoforms had detrimental effects. mHeps had no effect on the natural process of myelination, suggesting that any beneficial/detrimental effects were due to their interactions with factors secreted as a result of the injury process. Comparison of the secreted factors from the various MCs illustrated differences in the profile of chemokines/cytokines released. To identify factors that interact with our lead compound that was the most effective with regard to repair, low sulphated mHep7 (mHep7), we carried out TMT-LC/MS analysis on affinity purified conditioned media. Numerous factors were identified including amyloid beta A4. Further investigation established that amyloid beta peptide (1-42) could inhibit myelination which was overcome with co-treatment of mHep7. We therefore propose that desulphated mHeps may be novel therapeutics for CNS repair.

References

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Planaria (a type of flatworm) share many neuronal proteins with humans, but remarkably can very successfully regenerate their nervous system after injury, making them a useful model with which to study neuronal regeneration. Recent studies e.g.¹ have suggested that classical neurotransmitter proteins have other roles during neuronal regeneration outside of their functions in neural synaptic transmission. We examined the requirement for classical neurotransmitters in planarian neural regeneration. Pharmacological disruption of the serotonin and glutamate pathways in planaria (Dugesia japonica and Schmidtea mediterranea) caused abnormal locomotion and movement, as expected, and additionally showed varied effects on head/CNS or eye regeneration in transected or eye-ablated planaria. Planaria are a useful invertebrate model that may provide insights into how human neuroregeneration may be encouraged.

References
The endothelial cells transcriptome after spinal cord injury
A tale of two opposite models: zebrafish vs mouse

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After a spinal cord injury (SCI), loss of neurons promotes the proliferation of neural stem cells that reside in the ependymal region of the spinal cord, in both mammals and in zebrafish. Nevertheless, while in zebrafish these ependymal stem cells are able to replace all lost neuronal types and regenerate the spinal cord and motor function, the mammalian counterpart forms a glial scar. Endothelial cells (ECs) are no longer seen as mere coating of vessels, as they are essential in the regeneration of various organs via production of microenvironment modifiers. Recently, it was shown that acute and chronic injuries modulate differently the EC transcriptome, which in turn controls whether the organ regenerates or forms a fibrotic scar. This raises the appealing possibility that modulating the microenvironment of mammalian spinal cord, towards a zebrafish-like signature, could help to stimulate the regenerative potential in mammals along with the suppression of the scar.

We performed FACS sorting and RNA-seq to obtain the specific gene expression profile of spinal cord ECs, at 0 days post-injury (dpi), 3 and 7 dpi, that would be required to promote and support a regenerative response after an injury by comparing zebrafish vs mouse. Our data shows that after injury the majority of genes differentially expressed in both animal models are cytoskeleton and extracellular matrix (ECM)-related and have been described to influence axonal growth and do have distinct patterns in terms of progression in both models. Fluorescent in situ hybridization (FISH) and qPCR data will validate these pools of genes and future functional in vivo studies will be performed in both models.

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Epigenomic signatures underlie differential regenerative potential of Dorsal Root Ganglia after peripheral versus central spinal axonal injury

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Upon injury, opposite to peripheral axons, axons in the central nervous system fail to regenerate. The dorsal root ganglia (DRG) neurons represent a model to investigate the molecular mechanisms of this differential ability: a cell body extends one regenerative competent axon in the peripheral nerves and one regenerative incompetent in the spinal cord. A sciatic nerve lesion elicits regenerative gene expression changes radically distinct from those that follow a spinal cord lesion. Recent evidence suggest that epigenetic modifications can underlie this differential response [1-3], however a systematic study of epigenetic state upon regenerative vs non-regenerative injury is still missing, and could suggest specific therapeutic strategies.

To systematically characterize gene expression changes and chromatin state upon regenerative vs non-regenerative axonal injury, we have performed RNAseq, ChIPseq for active (H3K27ac and H3K9ac) and repressive (H3K27me3) histone modifications, and ATACseq for chromatin accessibility, from sciatic DRG in response to a sciatic nerve axotomy (SNA) vs a dorsal column axotomy (DCA). The integrated genome wide analysis showed that: 1) upon SNA, but not after DCA, chromatin is in a more accessible, transcription competent, state; 2) upon SNA, but not after DCA, promoters and enhancers are more enriched for H3K9ac and H3K27ac; 3) SNA leads to a more robust transcriptional response than DCA, with upregulated genes involved in regulation of transcription and regenerative signaling cascades; 4) DNA footprint analysis and machine learning identified repertoires of transcriptional regulators and chromatin remodelers uniquely associated with regenerative or non-regenerative injury respectively; 5) injury-dependent genes are enriched in binding sites to CTCF, a chromatin folding organizer involved in correct development of neural networks in the brain [4, 5]. Neuronal CTCF null mice failed to respond to conditioning-dependent increase in DRG outgrowth and showed reduced axonal regeneration after sciatic nerve injury. These results suggest that proper chromatin state is required for axonal regeneration and lead to the intriguing hypothesis that a poorly dynamic chromatin state might contribute to the low level of axonal regeneration after injury in the central nervous system.

References
Priorities of spinal cord injured patients

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Patients of the Queen Elizabeth National Spinal Injuries Unit were invited to complete a survey ranking the importance of the following functions: (a) arm/hand function (b) upper body/trunk strength and balance (c) bladder/bowel function, elimination of dysreflexia (d) sexual function (e) elimination of chronic pain (f) normal sensation and (g) walking movement. Anderson (2004) demonstrated that the most important function for tetraplegics was arm/hand function and the most important function for paraplegics was sexual function. The results of this survey showed that in an older population in Scotland the top priority for tetraplegics was arm/hand function however the top priority for paraplegics was walking movement whilst sexual function was the least important function in both groups.

References


Supported by Queen Elizabeth National Spinal Injury Unit
Dynlrb1 is essential for dynein mediated transport, axon growth and neuronal survival

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The cytoplasmic dynein motor complex transports essential signals and organelles from the cell periphery to perinuclear region, hence is critical for the survival and function of highly polarized cells such as neurons. Deficits in dynein functions have been implicated in neuronal injury responses and in the pathogenesis of neurodegenerative diseases1. In previous work, we identified a role for the dynein complex in regulating axon growth rates2,3. Dynein Light Chain Roadblock-Type 1 (DYNLRB1) is thought to be an accessory subunit required for specific cargos4,5. However, here we show that DYNLRB1 is essential for general dynein-mediated transport, axon growth and sensory neuron survival. Homozygous Dynlrb1 null mice are not viable and die during early embryonic development. Furthermore, heterozygous or knockdown animals display reduced neuronal growth, and selective depletion of Dynlrb1 in proprioceptive neurons compromises their survival. Conditional depletion of Dynlrb1 in sensory neurons causes deficits in several signaling pathways, including β-catenin subcellular localization, and a severe impairment in the axonal transport of both lysosomes and retrograde signaling endosomes. Hence, DYNLRB1 is an essential component of the dynein complex.

References
Dynamic electrical stimulation: a novel tool to neuromodulate spinal networks pre and post lesion

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Recovery of volitional motor control after injury is facilitated by epidural electrical stimulation of the spinal cord. We defined a novel neuromodulation method from hindlimb EMG signal during stepping and named it Dynamic Stimulation (DS). DS was dorsally applied to four lumbosacral segments using a high-density epidural array, counting 18 independent platinum-based micro-electrodes, used for both delivering DS and acquiring cord dorsum potentials. Finite element analysis visualized, at the interface array/spinal cord, the temporal and spatial features of DS neuromodulation, which uniquely affect the entire lumbosacral network, particularly the most rostral and caudal segments. In fully anesthetized adult rats, DS temporarily increased spinal cord excitability and strongly potentiated spinal-ly induced motor output compared to tonic stimulation. Furthermore, DS facilitated subthreshold cortical input to recruit muscle contractions and, based on spinally evoked EMG responses, allowed a greater recovery of motor output shortly after severe spinal cord contusion or complete transection. Experiments on chronically injured animals also confirmed increased spinally-induced motor responses from lower limb muscles in behaving rats at rest or during treadmill quadruped ambulation. In summary, DS uniquely amplifies both spinal and cortico-spinal input to spinal locomotor networks, which in turn increases the potential to regain significant levels of functional recovery after a spinal lesion.

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Docosahexaenoic acid reduces microglia phagocytic activity and induces neuroprotection in mouse and rat models of spinal cord contusion injury

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Microglia are activated after spinal cord injury (SCI) but the mechanisms involved in their phagocytic post-injury response and the link to neuroprotection remains incompletely characterized. The omega-3 fatty acid docosahexaenoic acid (DHA) has significant neuroprotective effects after hemisection and compression SCI (1,2) and has been shown to affect microglia in these injury models. Using experimental models of rat and mouse contusion SCI, we show that DHA (500 nmol/kg) administered acutely post-injury confers neuroprotection and enhances locomotor recovery, and also exerts a complex modulation of the microglial response to injury. In rats, at 7 days after SCI, the level of phagocytosed myelin within Iba1-positive cells was significantly lower after DHA treatment, and this occurred in parallel with an increase in intracellular miR124 expression. In primary microglia cultures from adult rats, the phagocytic response to myelin was also reduced by DHA and this was associated with an increase in miR124, but not miR155. A similar response was observed in a microglia cell line (BV2) treated with DHA, and the effect was blocked by a miR124 inhibitor. Furthermore, the phagocytic response of BV2 cells to stressed neurons was also reduced in the presence of DHA. In peripheral monocyte-derived macrophages, the expression of the M1, but not the M0 or M2 phenotype, was reduced by DHA. These findings show that DHA induces neuroprotection in contusion injury, and this adds further support to the neuroprotective potential of this fatty acid in SCI. Furthermore, the improved outcome in this type of injury is accompanied by a reduction in the phagocytic response of microglia, and this is linked to a modulation of miR124.

Reference

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Combining neural transplantation with intermittent hypoxia after cervical spinal cord injury

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There is a growing interest in the use of neural progenitor cells (NPCs) to repair the injured spinal cord. Despite extensive preclinical research, it remains unclear as to how donor cells develop, differentiate, and integrate with host injured circuitry, and if integration can be enhanced and/or guided using non-invasive means such as activity-based therapy. With a focus on the phrenic circuit and respiratory dysfunction after cervical spinal cord injury (SCI), the present work tests the hypothesis that pairing cellular transplantation with a clinical rehabilitation strategy (daily acute intermittent hypoxia, dAIH) will enhance neuroplasticity and promote donor-host connectivity. NPCs isolated from GFP rats and cultured for 3 days prior to cryopreservation yielded neuronal and glial restricted progenitor cells. While the phenotype of these progenitors is being determined in ongoing work, excitatory, inhibitory and modulatory neuronal precursors are known to be present. These cells were then transplanted into a clinically-relevant cervical (C3/4) contusion injury in adult Sprague Dawley rats, one week after injury. Animals received 4 weeks of dAIH (10 x 5minute exposures to 10% oxygen intermittent with normoxia, 5 days a week), beginning one-week post-transplantation. Donor cells survive, differentiate, and integrate with the host spinal cord as assessed with transynaptic pseudorabies virus tracing (PRV) and immunohistochemistry. Respiratory training resulted in significantly enhanced donor-host connectivity to ipsi- and contralateral-to-injury phrenic circuit, compared to untrained transplant recipients. At least a subset of these newly integrated donor spinal interneurons are cholinergic. Preliminary data suggests the underlying mechanism for directing donor-cell outgrowth towards phrenic inter- and motoneurons is in part mediated via BDNF expression within the cervical spinal cord. Transplant recipients, with and without dAIH training, showed greater muscle (diaphragm) recovery than vehicle-controls, as measured by terminal electromyography. Interestingly, transplant and dAIH training recipients demonstrated a greater ability to respond to hypoxic but not hypercapnic respiratory challenge. Ongoing experiments are focused on assessing host-to-donor axonal integration (e.g. descending serotonergic and host afferents into site of transplant), and assessing whether respiratory training is selective in the subsets of donor neuron phenotypes and targets. These experiments suggest that rehabilitative strategies such as dAIH may be an effective way for enhancing donor cell outgrowth and connectivity.

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Speakers and Panelists

Speakers – Friday 6th September

Simone Di Giovanni holds a Chair in Restorative Neuroscience at Imperial College, where his research group investigates the molecular signalling and transcriptional mechanisms that control axonal sprouting and regeneration. Simone also holds an honorary post within the NHS as a consultant in Neurology. Previously, since 2006, he worked at the University of Tuebingen, Germany as a Research Group Leader, where he was also a consultant clinician in Stroke and General Neurology. Simone did his post-doctoral training in Neuroscience studying gene expression regulation after spinal cord injury at Georgetown University, Washington DC, 2001-2004 where he became research Instructor (2004-2006). He studied Medicine at La Sapienza University and did his Neurology training at Catholic University, Rome, Italy.

Frank Bradke after studying at the Freie Universität Berlin and University College London, Bradke carried out research at the European Molecular Biology Laboratory (EMBL) in Heidelberg as part of his doctoral thesis. As a postdoctoral researcher, he moved to the University of California in San Francisco and Stanford University in 2000. In 2003, he was appointed a group leader at the Max Planck Institute of Neurobiology in Martinsried. In 2011, he was awarded the IRP Schellenberg Prize, one of the most prestigious awards in the field of regeneration research. In the same year he became full professor at the University of Bonn, and was appointed head of the Axon Growth and Regeneration research group at the DZNE. Bradke is an elected member of the Leopoldina (the German National Academy of Sciences), the Academia Europaea, and the European Molecular Biology Organization (EMBO). In 2016, he was awarded the Leibniz Prize, which is the most important research award in Germany. In 2018, he received the Roger de Spoelberch Prize.

James Fawcett qualified in Medicine in Oxford and London. After a period in clinical practice he studied for a PhD under Michael Gaze at NIMR Mill Hill. 5 years at the Salk Institute followed, after which he moved to Cambridge University, first in Physiology then as Chairman of the Brain Repair Centre. His main interest is the restoration of CNS function lost through spinal cord injury, neurodegenerative disease and ageing. He has focused on activation of axon regeneration and plasticity through manipulation of the extracellular matrix and integrins. He also works on interfacing electronics with the damaged nervous system and on the design of protocols for clinical trials in spinal cord injury.

Tammy Szu-Yu Ho received her Ph.D. from Baylor College of Medicine in Houston supervised by Prof. Matthew Rasband. She was investigating sodium channel clustering at the axon initial segment and nodes of Ranvier. In 2016, she joined the laboratory of Prof. Clifford Woolf at Boston Children’s Hospital and Harvard Medical School as a post-doctoral research fellow. Her work is aimed to identify molecular targets for promoting axon regrowth by performing drug screening using human stem cell derived neurons.

Philippa M. Warren received her Ph.D. in Physiology, Development and Neuroscience from Cambridge University under Prof's James Fawcett and Roger Keynes. During a Postdoctoral Fellowship at Case Western Reserve University (under the mentorship of Dr. Warren Allain and Prof. Jerry Silver) she was trained in respiratory physiology, addressing the recovery of respiratory function after high cervical and chronic spinal injury. Philippa developed these skills as a Fellow at the University of Leeds receiving additional training in muscle physiology and dynamics. She has recently established her own laboratory at King’s College London, where she continues to investigate the mechanisms of respiratory motor recovery and plasticity following acute and chronic spinal injury.
Andras Lakatos having obtained his undergraduate medical degree in Budapest, András carried out his PhD studies in glial cell biology in the Beatson Institute and at Cambridge, being supported by Spinal Research. This was followed by two years of postdoctoral research in neuroregeneration at the University of Cambridge. He then completed basic specialty training in general medicine and higher specialist training in neurology at the Cambridge University Hospitals. During his clinical training he held a Clinical Lectureship in medicine and later a Walker Fellowship in neurosciences between 2010-2013, helping to kick-start his independent research group. He was then awarded the MRC Clinician Scientist Fellowship in 2017. He is leading a regenerative neurobiology laboratory at the Department of Clinical Neurosciences, University of Cambridge and is affiliated to the WT-MRC Cambridge Stem Cell Institute. He also practices as Academic Consultant in clinical neurology in Addenbrooke’s Hospital.

Jessica Kwok was trained as a glyco-/neuro-scientist. Her research focuses on modulating the extracellular matrix environment in the central nervous system to enhance plasticity and regeneration after injury. Dr Kwok received her Ph.D. at the University of Hong Kong investigating the role of chondroitin sulphate proteoglycans (CSPGs) in neural development. She then moved to the University of Cambridge joining the laboratory of Prof. James Fawcett. There, she elucidated the hierarchical assembly of perineuronal nets and how this extracellular matrix assembly regulates neuroplasticity. Dr Kwok established her lab in 2015 at the University of Leeds, combining her knowledge in CSPGs and neuroplasticity in designing new tools for enhancing functional recovery after spinal cord injury.

Dana McTigue received her PhD in Physiology in 1995 from the Ohio State University where she studied autonomic control of gastric function. She then did a postdoctoral fellowship at OSU where she studied the response of adult progenitor cells to spinal cord injury in rodents. She then was hired as an Assistant Professor in the Department of Neuroscience at OSU in 2003, where she rose through the ranks to Full Professor in 2015, at which time she also become the Vice Chair for Research for the department. The focus of Dr. McTigue’s laboratory is two-fold. One area is continuing to examine the role of adult progenitor cells in cellular repair and remyelination after spinal cord injury. The 2nd theme of research is systemic pathology after SCI, with particular emphasis on liver inflammation and metabolic disease, which is exhibited by a substantial portion of SCI individuals. Ongoing work suggests that a negative feedback cycle forms after injury in that SCI causes liver inflammation which in turn exacerbates intraspinal and systemic pathology.

Luming Zhou obtained his Ph.D at Hertie Institute for Clinical Brain Research (HIH), University of Tuebingen in Germany supervised by Prof. Simone Di Giovanni. He has been investigating the epigenetic mechanisms controlling axonal regeneration in dorsal root ganglion (DRG) neurons following spinal cord and sciatic nerve injury. He is currently a Research Associate in Prof. Simone Di Giovanni's laboratory at Imperial College London where he investigates age-dependent mechanisms that underpin regenerative decline.

Karim Fouad (PhD) is a Professor in the Faculty of Rehabilitation Medicine at the University of Alberta in Edmonton, Canada. He began his career in Germany working in the field of locomotor control and then ventured to Canada for a postdoc in the laboratory of Dr. Pearson. A second post-doctoral position in Switzerland with Drs. Schwab and Dietz allowed him to enter the field of spinal cord injury. In 2001 he was offered a position at the University of Alberta, where he enjoys the refreshing winters and the evenings in front of his fireplace. His pioneering research on understanding and promoting injury induced neuro-plasticity using pharmacological approaches and rehabilitative training has been published in prestigious journals including J. Neurosci, Brain and Nature Medicine.
Jan Schwab MD PhD is a physician-scientist and board-certified Neurologist specialized on spinal cord injury (SCI). His main interest is focused on deciphering the underlying pathomechanisms of the maladaptive immune response after SCI. In addition, he has an interest to develop and apply tools to improve prediction of animal models for clinical trials, reduce inherent bias and increase experimental value in SCI research. After internships at Tel Aviv and Cornell University he received his MD from the Eberhard-Karls University of Tuebingen followed by a PhD from the Max Planck Research School. He completed Neurological residency at the Charité - School of Medicine (Berlin). Since 2015 he serves as director of the Spinal Cord Injury Division of the Department of Neurology, since 2016 as Program Director of the Central Ohio SCI Model System and since 2018 as medical director of the Belford Center for Spinal Cord Injury.

Elisabeth Serger studied Biosciences and Molecular Biosciences with a Major in Neuroscience at the University of Heidelberg in Germany. In 2016 she started her PhD with Professor Simone Di Giovanni in the Department of Brain Sciences at Imperial College London. Her work focuses on the link between fasting induced changes in the microbiome and axonal regeneration.

Grégoire Courtine was trained in Mathematics, Physics, and Neurosciences. After a Postdoc in Los Angeles (UCLA), he established his own laboratory at the University of Zurich in 2008. He was appointed Associate Professor at the Center for Neuroprosthetics at EPFL in 2012. He is also affiliated to the department of Neurosurgery at the University Hospital Lausanne (CHUV). In 2014, he launched the startup GTX Medical that aims to translate the medical and technological advances gained over the past 15 years into a medical treatment to treat paraplegia.

Speakers – Saturday 7th September

Marina Martinez has had a long-standing interest in spinal cord injury research with a focus on the mechanisms supporting recovery of walk. She completed her PhD degree in Neuroscience at the University of Aix-Marseille, France. She next worked as a postdoctoral fellow in Dr. Serge Rossignol’s laboratory at the University of Montreal, Canada. Since 2016, she is an Assistant Professor in Neuroscience at the University of Montreal and a regular researcher at the Sacred Heart Hospital of Montreal. Her lab uses animal models and a systems neuroscience approach to test strategies aimed at guiding recovery. Her work is funded by the main federal and provincial agencies, in addition to foundations, and by a salary award.

Claudia Angeli is an Assistant Professor in the Department of Neurological Surgery, University of Louisville and Senior Researcher at Frazier Rehab Institute in Louisville, KY. Her research background and interests are focused in understanding mechanisms of control of human locomotion following neurologic injury. Dr Angeli received her B.S. in Health and Human Performance at East Carolina University, Ph.D. in Kinesiology with a concentration in Mechanical Engineering from Michigan State University. She holds faculty appointments in the departments of Neurological Surgery, Mechanical Engineering and Physical Medicine and Rehabilitation at the University of Louisville and the department of Physical Therapy at Bellarmine University.
Marc Bolliger was trained in Sports & Movement Science. He completed his Ph.D. at the Humboldt University Berlin, investigating brain metabolism after extensive dehydration in endurance athletes. He then moved to Zurich for a postdoc position at the University of Zurich and ETH Zurich. During this postdoc, he investigated the influence of spinal cord injury on plasticity of human spinal locomotor circuitry and he was involved in the development of new technologies to enhance the therapy outcome after SCI. In 2010, he became the head of the research lab at the Spinal Cord Injury Center at Balgrist University Hospital.

Armin Curt is a Professor for Paraplegiologie and Medical Director at the Balgrist University Hospital Zurich, Switzerland. After receiving the Medical Degree at the University of Cologne, Germany, and full training in neurology and clinical neurophysiology he started his specialization in Spinal Cord Injury care and rehabilitation at the University of Zurich in Switzerland. From 2005 – 2008 he was an Associate Professor in Neurology and SCI Research at the University of British Columbia Vancouver, Canada. In 2013 he gave the Sir Ludwig Guttmann Lecture. He is holder of numerous national and international grants and Principal Investigator of the Horizon 2020 project “NISCI – Antibodies against Nogo-A to enhance regeneration and functional recovery after SCI”. His research interests are translational research in human SCI, neuro-rehabilitation, clinical neurophysiology and neuro-imaging in human SCI.

Joost Verhaagen was trained in molecular biology, obtained a Ph.D. from the University of Utrecht, received post-doctoral training at the Roche Institute of Molecular Biology (Nutley, NJ, USA) and was a visiting scientist in the Laboratory of Neurobiology and Behavior (The Rockefeller University, NY, USA). He founded and heads the Laboratory for Neuroregeneration at the Netherlands Institute for Neuroscience (NIN) and is strategic professor in the Molecular Biology of Neuroregeneration at the Vrije Universiteit Amsterdam. Verhaagen is an expert in axon regeneration and in gene therapy. His laboratory studies structural and functional plasticity in the injured and intact peripheral and central nervous system with a focus on the role of transcription factors, chemorepulsive and neurotrophic proteins. His research is relevant to advance our understanding of how neurons survive injury and form and maintain new functional connections. He published over 225 papers and edited a number of books, including the Handbook of Clinical Neurology on Spinal Cord Injury (2012) and a volumen of Neuromethods on Gene Therapy for Neurological Disease (2015).

Michael Sofroniew received an M.D. from Ludwigs-Maximillians University in Munich and a Ph.D. from University of Oxford. After a surgical internship at Johns-Hopkins University he pursued a full time research career as a faculty member at University of Cambridge (1986-2000), where he was a founding member of the Cambridge Centre for Brain Repair. He moved to UCLA in 2000, where he is currently a Distinguished Professor in the Department of Neurobiology. Work in his laboratory is directed at understanding the cell biology of injury and repair in the adult central nervous system (CNS).

Patrick Freund has obtained a PhD (2008) and MD (2015) in Biology and Medicine, respectively. In 2016, he was awarded the “venia legendi” in Paraplegiologie at the University of Zurich. In 2018 he was awarded the SNSF Eccellenza Professorial Fellowships. He holds affiliation with the Wellcome trust Centre for Neuroimaging and the Brain Repair and Rehabilitation Department at UCL Institute of Neurology, as well as with the Department of Neurophysics at the Max-Planck-Institute in Leipzig. Since 2013, he leads the Neuroimaging group at the Spinal Cord Injury Center Balgrist. His main research focus is set on the multimodel assessment of functional and structural changes throughout the central nervous system induced by focal injuries to the brain or spinal cord.
Yakov Koffler was trained in Biomedical Engineering, Biophysics, and Neurosciences. He completed his Ph.D at the Technion, Israel under the supervision of Prof. Shulamit Levenberg, studying the contribution of blood vessels and angiogenesis to formation of functional engineered vascularized skeletal muscle tissue. He then moved to the University of California San Diego (UCSD), USA where he developed the technology to 3D-print spinal cord scaffolds under the supervision of Prof. Mark Tuszynski. He is now an Asst. Proj. Scientist and work on scaling up and testing the technology in preclinical models of Spinal cord injury and Peripheral nerve injury.

Aileen Anderson received her B.S. in Bioengineering from the University of Illinois Urbana, and Ph.D. in Neurobiology from the University of California Irvine (UCI). After a post-doctoral work at Harvard, she returned to UCI as a faculty member, where she is currently Director of the UCI Sue & Bill Gross Stem Cell Research Center, and a Professor at the University of California Irvine in the departments of Physical Medicine, Neurological Surgery, and Anatomy & Neurobiology. Research in her laboratory focuses on neural stem cell (NSC) populations and spinal cord injury mechanisms, investigating intrinsic and extrinsic factors defining the migration and differentiation potential of NSC, non-traditional roles for the innate inflammatory system in the pathophysiology of spinal cord injury and control of NSC fate and migration, and potential for implanted biomaterial scaffolds to provide an environment supporting robust axonal regeneration.

Hideyuki Okano received M.D. in Physiology from Keio University in 1983. After he obtained Ph.D. degree from Keio University in 1988, he held post doctoral position at Johns Hopkins University Medical School. He has appointed full professors at Tsukuba University School of Medicine in 1994, Osaka University School of Medicine in 1997, and returned to Keio University Medical School in 2001 as a full professor of Physiology. Since 2007, he has been a Dean of Keio University Graduate School of Medicine or a Dean of Keio University School of Medicine. He has been conducting basic research in the field of regenerative medicine including, neural stem cells and iPS cells, spinal cord injury, developmental genetics and RNA binding proteins. He has awarded numbers of awards and honors including the Medal with Purple Ribbon in 2009 and the first prize of the 51st Erwin von Bälz Prize in 2014. He aims to establish and provide patients-specific iPS cells and genetically modified non-human primate models for neuroscience research and to explore the pathogenic mechanisms of neurological/psychiatric disorders. Currently, he is the leader of Brain Mapping Project in Japan (Brain/MINDS).

Michael Fehlings is the Vice Chair Research for the Department of Surgery at the University of Toronto and Head of the Spinal Program at Toronto Western Hospital. He combines an active clinical practice in complex spinal surgery with a translationally oriented research program focused on discovering novel treatments to improve functional outcomes following spinal cord injury (SCI). He has published over 850 peer-reviewed articles (h-index 86) chiefly in the area of central nervous system injury and complex spinal surgery. His work examining the use of regenerative approaches, including neural stem cells, to repair the injured nervous system has led to numerous international awards and has helped lead the field toward clinical translation in this area. In 2017, he led the international, multi-disciplinary initiative to create Clinical Practice Guidelines for the management of degenerative cervical myelopathy and traumatic SCI. Most recently, Dr. Fehlings’ work demonstrating that midcervical excitatory interneurons are essential for the maintenance of breathing in non-traumatic cervical SCI and critical for promoting respiratory recovery after traumatic SCI was published in Nature. Dr. Fehlings has received numerous prestigious awards including the Gold Medal in Surgery from the Royal College of Physicians and Surgeons (1996), the Olivecrona Award from the Karolinska Institute (2009) and the Reeve-Irvine Research Medal in Spinal Cord Injury (2012).
**Friday Panelists**

Murray Blackmore is an Associate Professor in the department of Biomedical Sciences at Marquette University. He received his undergraduate degree from Stanford University and graduate degree in Neuroscience from the University of Minnesota, followed by postdoctoral training at the Miami Project to Cure Paralysis. Since 2011 Dr. Blackmore has led a research lab at Marquette University devoted to developing new approaches to address spinal cord injury. His lab uses genetic and epigenetic profiling, high content screening, and gene therapy vectors in animal models of spinal injury, with the ultimate goal of discovering new genetic interventions to boost the regenerative ability of cells in the nervous system.

James Fawcett qualified in Medicine in Oxford and London. After a period in clinical practice he studied for a PhD under Michael Gaze at NIMR Mill Hill. 5 years at the Salk Institute followed, after which he moved to Cambridge University, first in Physiology then as Chairman of the Brain Repair Centre. His main interest is the restoration of CNS function lost through spinal cord injury, neurodegenerative disease and ageing. He has focused on activation of axon regeneration and plasticity through manipulation of the extracellular matrix and integrins. He also works on interfacing electronics with the damaged nervous system and on the design of protocols for clinical trials in spinal cord injury.

Stephen McMahon is Sherrington Professor of Physiology at King’s College London, and Director of the London Pain Consortium. He is a neuroscientist who trained with Patrick Wall in the 1980s. He is principally interested in somatosensory systems and actively engaged in work ranging from molecular biology to electrophysiology to human psychophysical studies. He has published more than 250 original research articles, many highly rated (H-index >80) and is co-editor of the Textbook of Pain. His work has been published in leading scientific journals including, Nature, Nature Medicine, Science, Nature Neuroscience, Cell, Neuron and Brain.

Phillip Popovich completed his PhD training in physiology and spinal cord injury (SCI) at Ohio State University (OSU) where he is currently Professor and Chair of Department of Neuroscience, Director of OSU’s Center for Brain and Spinal Cord Repair and Executive Director of the Belford Center for Spinal Cord Injury. As a post-doctoral fellow, also at OSU, he was awarded a Sandoz Research Fellowship that supported his formal training in immunology and CNS autoimmune disease. His research program is focused on understanding how SCI disrupts communication between the nervous and immune systems leading to a state of chronic immune dysfunction, including immune suppression and “metainflammation”.

Joost Verhaagen obtained a Ph.D. from the University of Utrecht, received post-doctoral training at the Roche Institute of Molecular Biology. He founded and heads the Laboratory for Neuoregeneration at the Netherlands Institute for Neuroscience (NIN) and is strategic professor in the Molecular Biology of Neuoregeneration at the Vrije Universiteit Amsterdam. Verhaagen is an expert in axon regeneration and in gene therapy. He published over 225 papers and edited a number of books, including the Handbook of Clinical Neurology on Spinal Cord Injury (2012) and a volume of Neuromethods on Gene Therapy for Neurological Disease (2015).
**Saturday Panelists**

**Claudia Angeli** is an Assistant Professor in the Department of Neurological Surgery, University of Louisville and Senior Researcher at Frazier Rehab Institute in Louisville, KY. Her research background and interests are focused on understanding mechanisms of control of human locomotion following neurologic injury. Dr Angeli received her B.S. in Health and Human Performance at East Carolina University, Ph.D. in Kinesiology with a concentration in Mechanical Engineering from Michigan State University. She holds faculty appointments in the departments of Neurological Surgery, Mechanical Engineering and Physical Medicine and Rehabilitation at the University of Louisville and the department of Physical Therapy at Bellarmine University.

**Armin Blesch** received his PhD at the University of Wurzburg, Germany in Genetics and completed his postdoctoral training at the University of California, San Diego. While in San Diego, he moved up the faculty ranks to Assoc. Prof. before joining the Spinal Cord Injury Center in Heidelberg, Germany to establish the Laboratory for Neuroregeneration. In 2015 he was appointed as Professor in the Department of Neurosurgery at Indiana University School of Medicine. His research is focused on axonal regeneration in the spinal cord, mechanisms of pain after spinal cord injury and the role of stem cells in functional recovery.

**Armin Curt** is a Professor for Paraplegiology and Medical Director at the Balgrist University Hospital Zurich, Switzerland. After receiving the Medical Degree at the University of Cologne, Germany, and full training in neurology and clinical neurophysiology he started his specialization in Spinal Cord Injury care and rehabilitation at the University of Zurich in Switzerland. From 2005 – 2008 he was an Associate Professor in Neurology and SCI Research at the University of British Columbia Vancouver, Canada. In 2013 he gave the Sir Ludwig Guttmann Lecture. He is holder of numerous national and international grants and Principal Investigator of the Horizon 2020 project “NISCI – Antibodies against Nogo-A to enhance regeneration and functional recovery after SCI”. His research interests are translational research in human SCI, neuro-rehabilitation, clinical neurophysiology and neuro-imaging in human SCI.

**Linda Jones** was trained in kinesiology, physical therapy and advanced neurological physical therapy. She transitioned from clinical practice to research, conducting two cell based trials in spinal cord injury, one of which was the first embryonic stem derived trial in the United States. She subsequently joined the Craig H. Neilsen Foundation, managing the translational research portfolio, as well as initiating and overseeing special projects. She is currently completing a PhD at the University of Colorado, in clinical science. Her interests lie in clinical trial design, outcome measures and bridging gaps between pre-clinical and clinical research.

**Wolfram Tetzlaff** obtained his MD degree in Germany and his PhD in Calgary followed by faculty appointments at the University of Calgary, Ottawa and British Columbia, where he holds the John and Penny Ryan BC Leadership Chair in Spinal Cord Injury Research. He serves as the Director of ICORD, the International Collaboration on Repair Discoveries and leads a research program focusing on spinal cord injury modeling in rodents and experimental strategies for neuroprotection and neural repair after spinal cord injury (SCI). In particular, his group found that diets affect the cascades of secondary damage after spinal cord injury and can improve outcomes; and that a skin-derived progenitors when differentiated into Schwann cells can be used for neural repair in the chronically injured rodent spinal cord. More recently, he focused on the role of endogenous oligodendrocytes progenitor cells in spontaneous repair after SCI. Dr. Tetzlaff’s work is funded by the Canadian Institutes for Health Research, Wings for Life, Spinal Research and the MS Society of Canada.
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