SPINAL RESEARCH

2 - 3 September 2016

The 18th Spinal Research Network Meeting

ABSTRACTS

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

POSTER PRESENTATIONS

Poster session is scheduled from 6.25 pm at the end of the first day, immediately after the main meeting, on Friday, 2nd September. The posters are also available to view during the coffee and lunch breaks on Friday and Saturday.

SCIENTIFIC ORGANIZING COMMITTEE

Rescue of denervated muscle

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Post-mortem examination of injured human spinal cords typically shows severance of long nerve tracts, demyelination of axons, and maceration of gray matter.[1,2] Most studies focus on axon regeneration, axon sprouting, and remyelination to restore function after injury[3] but survival or replacement of spinal neurons is also crucial to retain spinal circuitry after injury, to provide sites for formation of new synapses, and for activity-based rehabilitation. Both physiological and morphological data from humans show that motoneuron death is common near the SCI epicenter.[4,5] Entire motor pools are destroyed in up to 30% of cases, resulting in complete muscle denervation. Not only would replacement motoneurons have to survive in a damaged spinal cord, they would also have to send axons long distance to reinnervate already atrophied muscles. Thus, transplantation of embryonic neurons into peripheral nerve near the denervated muscles was introduced for local reinnervation of muscles. In this situation, the distance axons have to grow to reach muscle, and the time for muscle atrophy, are both short.[6,7] The new motoneurons (ChAT-positive neurons) survive, regenerate axons, form functional neuromuscular junctions, and reduce muscle atrophy. The acute delivery of neurotrophic factors and/or activity improves this neuron survival, axon regeneration, and muscle reinnervation.[8,9] Electrical stimulation of the transplant elicits fatigue resistant muscle contractions of sufficient strength to move the ankle joint through its range.[7] Further, the function of the muscle is retained long-term. Remote placement of neurons is therefore an important model system for testing how to restore innervation to denervated muscles, to examine which mechanisms improve the function of the reinnervated muscles, to evaluate how to control these muscles, and to integrate their function into the movement of the entire limb.

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Optogenetic control of muscle function with stem cell-derived neural tissue

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The loss of motor neurons due to Spinal Cord Injury (SCI) or Motor Neuron Disease (MND) disconnects the CNS from skeletal muscle and leads to the impairment of vital motor function, such as breathing and locomotion. As there is no natural mechanisms for motor neuron regeneration in humans, such defects are usually irreversible, and currently, no established therapy exists that could reconstitute muscle function once motor neurons are lost. Motor neurons directly derived from pluripotent stem cells (Wichterle et al., 2002; Machado et al., 2014) could, in principle, be used to reconnect the CNS with muscle targets, but it is unclear how newly generated, embryonic-like neurons would integrate into lesioned adult spinal circuits. To circumvent this problem, we are developing an alternative approach that relies on stem cell-derived peripheral neural grafts which express optogenetic actuators like channelrhodopsin-2, to establish neuromuscular junctions with recipient muscle. Due to the photosensitivity of the graft, muscle contraction can then specifically be triggered by light flashes which are generated by an optoelectronic pacemaker device and transmitted to the graft via light sources such as LEDs. In a recent proof-of-principle study, we have shown that optogenetic motor neuron grafts can relay rhythmic contraction patterns from an artificial control system to skeletal muscle in vivo (Bryson et al., 2014). While such a neural prosthesis would not offer a cure for SCI or MND, the quality of life of patients could be dramatically improved by artificially driving respiration and avoiding the need for mechanical ventilation. If successful, our approach could also be applied to other key motor functions, for example swallowing.

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Evaluating the repair potential of neural stem cell transplants in spinal cord contusion injuries

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Transplantation of neural stem cells is a potential therapy for spinal cord injury and a recent report has suggested that they could be used to form a functional relay across an injury (Lu et al. 2012). In this study we have used combined behavioural, electrophysiological and anatomical approaches to investigate the repair potential of neural stem cells prepared from human embryonic spinal cord tissue (566RSC, NeuralStem Inc.) after transplantation into a contusion injury.

Cells were transplanted into contusions at the C6 level produced 3 weeks earlier using an Infinite Horizon impactor (175 kdynes). Most transplants were made into Sprague Dawley animals immunosuppressed from two days before transplantation to the end of the study. A few nude animals were transplanted for comparison. The transplanted cells were suspended in a buffer without additional reagents or neurotrophic support while control animals were injected with buffer only. Functional outcome was assessed weekly by behavioural testing for 8 weeks post transplantation and using terminal electrophysiology to look for changes in corticospinal and sensory pathways in spinal segments above and below the injury. Spinal cords at the injury site were sectioned and transplanted cells visualized using immunocytochemistry and confocal microscopy.

As reported previously for ischemic (Cizkova et al 2007) and lumbar compression injuries (Gorp et al 2013), transplanted cells filled the injury site and a proportion of the cells expressed the neuronal marker NeuN. The cells extended large numbers of axon-like processes for several mms above and below the injury site in grey matter, but especially in white matter, as described previously for cells transplanted into transection injuries within a matrix containing a cocktail of growth factors (Lu et al 2012). However, despite these anatomical observations, neither behavioural tests (grip strength and ladder walk) nor electrophysiological assessment of corticospinal-evoked and sensory-evoked cord dorsum potentials showed any difference between the control and transplanted animals. Retrograde tracing of the corticospinal tract showed very few labelled fibres extending into the transplanted injury and immunolabelling for neurofilament 200 also revealed relatively sparse numbers of axons within the transplant. This suggests a limited opportunity for host axons to connect with transplanted cells and this is one potential explanation for the absence of improved functional outcome in transplanted animals.

These experiments show that 566RSC NeuralStem cells i) will survive in a contusion injury with an appropriate immunosuppression regime, ii) can proliferate and differentiate into cells that express neuronal markers and extend axon-like processes for long distances in the host spinal cord, and iii) that these properties are not dependent on the provision of growth factors. However, to fully understand the repair potential of these cells, further experiments should investigate whether the transplanted cells have excitable properties, whether prolonged survival periods are necessary for full *in vivo* differentiation and whether neurotrophic support facilitates connectivity and integration into host spinal cord circuitry.

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Acute intermittent hypoxia: a potential adjuvant to spinal cord injury rehabilitation

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Spinal cord injury (SCI) leads to disrupted connections within and between the brain and spinal cord, causing life-long paralysis. However, most injuries are not complete, leaving at least some spared neural pathways to the motor neurons that initiate and coordinate movement. Consequently, neural plasticity contributes to spontaneous recovery of motor function following SCI. Although injury-induced plasticity in spared spinal synaptic pathways enables partial spontaneous recovery, the extent of this repair is slow and limited. Thus, there is an overwhelming need for new clinical strategies that enhance beneficial plasticity and subsequently improve motor function in persons with SCI.

Acute intermittent hypoxia (AIH) induces spinal plasticity, strengthening connections to motor neurons (Baker-Herman et al., 2003; Fuller et al., 2003). Considerable progress has been made towards an understanding of cellular mechanisms giving rise to AIH-induced respiratory plasticity (Mahamed and Mitchell, 2007). Repetitive exposure to AIH enhances the expression of plasticity-promoting proteins in respiratory motor nuclei (Satriotomo et al., 2007; Wilkerson and Mitchell, 2009) and elicits profound recovery of breathing capacity in spinally injured rats (Barr et al., 2007). Indeed, exciting results from collaborating laboratories demonstrate that AIH facilitates non-respiratory motor output in spinally injured rats and humans. Daily breathing exposures of AIH (5 min episodes, 5 min intervals, 7 consecutive days) completely restored lost forelimb function in a horizontal ladder-walking task in spinal-injured rats, and this effect lasted more than 3 weeks post-treatment (Lovett-Barr et al., 2012). With shorter hypoxic episodes (1.5 min, 1 min intervals, 15 episodes), a single-day exposure of AIH increased maximum ankle torque generation (Trumbower et al., 2012) while 5 consecutive days of AIH increased walking ability in persons with chronic, iSCI (Hayes et al., 2014). Although these findings are striking, much work needs to be done to determine the clinical feasibility of AIH as a plasticity-promoting therapy to elicit long-term enhancement of limb function (i.e., walking, hand opening, etc.) after spinal injury.

The purpose of this talk is to review translational studies aimed at uncovering possible mechanisms of AIH-induced motor plasticity and to assess the potential of AIH as an adjuvant to SCI rehabilitation.

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Shared control approaches in SCI: from assistive to rehabilitative technologies

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Brain-computer interfaces (BCI) are becoming an increasingly popular research area, with the ultimate aim of improving the quality of life of people with spinal cord injuries (SCI), as well as those with other pathologies that result in a degree of limb paralysis. However, current state-of-the-art non-invasive BCI systems are still somewhat limited in terms of the number of different mental commands that they can decode and/or the speed and accuracy at which a command decision can be made. For asynchronous, non-invasive approaches this is typically not more than 3 classes (different mental commands) and typically not faster than around 0.5-1Hz to achieve accuracies above 70% (Leeb et al., 2015). Several research groups around the world are working to improve BCIs in terms of signal processing and machine learning techniques, as well as hardware designs. However, other more traditional assistive technology interfaces also exhibit one or more of these same challenges, e.g. single-switch scanning interfaces are limited by the speed of the scan, whereas head arrays and sip-and-puff switches are limited by the number of discrete commands and false positive rates (Fehr et al., 2000). Therefore, we have been developing so-called shared control techniques (Mulder et al., 2015), whereby the assistive technology (e.g. a wheelchair) can be instrumented with sensors, such that it is able to interpret the user's imprecise commands in the current context. In this case, the assistive technology itself can offer practical assistance, whilst simultaneously reducing the user's workload (Carlson & Demiris, 2012). Moreover, the user's performance is rarely constant, so we have been developing techniques that can adapt the level of assistance that the shared control system provides to match each individual user's ever evolving needs, which has enabled people to use a BCI to drive a wheelchair in cluttered environments (Carlson & Millán, 2013). We anticipate that similar techniques could enhance SCI recovery, by more reliably integrating BCI into rehabilitation protocols and automatically adjusting the level of assistance to the capabilities of the patient. To this end, we have begun characterizing lower limb robotic exoskeletons, so that we can understand their impact on the user's gait (Barbareschi et al., 2015), physical interaction forces (Rathore et al., 2016) and brain signals, in terms of features present in electroencephalography (Zervudachi et al., 2016).

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Mechanisms of neuromodulation the recovery of function post paralysis

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Data from animal and human experiments will be presented which provide insights into the neuromodulatory mechanisms underlying significant levels of plasticity of multiple physiological systems and how this plasticity has been accompanied with significant levels of recovery of sensorimotor and autonomic functions. Fundamental mechanisms thought to underly previously unrecognized physiological responses in completely paralyzed individuals will be presented. But, further, these post-injury responses give reason to consider the importance and application of these responses in how movement is controlled in the uninjured state. The concept of neuromodulatory mechanisms of “enabling versus inducing” sensory-motor responses and the crucial role of activity-dependent supraspinal and spinal plasticity will be discussed. A brief presentation of how noninvasive transcutaneous stimulation techniques can be combined with the emerging exoskeletal technology will be presented. The significance of newly emerging data severely challenges the validity of several dogmatic assumptions about the potential to recover sensory-motor and autonomic function after “complete” paralysis. Thus, it is time for a new way of thinking of how paralysis can be treated in the acute and chronic post-injury states.

The Corticospinal Pathway following Spinal Cord Injury

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The corticospinal tract is an important target for motor recovery after spinal cord injury (SCI) in humans. Using noninvasive electrophysiological techniques we have demonstrated the presence of reorganization in corticospinal projections targeting spinal motor neurons of muscles located close and at a distance from the injury site in individuals with chronic anatomically incomplete cervical SCI. Our physiological findings indicate that corticospinal transmission in intrinsic hand muscles change in a task-dependent manner and to a different extent in individuals taking or not taking baclofen. Changes in corticospinal transmission present after SCI also extend to the preparatory phase of upcoming movements. We have used this physiological information to develop noninvasive protocols to strengthen transmission in residual corticospinal projections and spinal cord networks in humans with incomplete SCI. Moreover, we have novel data indicating cortical connections projecting to corticospinal neurons may represent a potential alternative target for enhancing motor recovery after SCI.

Restoring the sense of touch in limb loss and spinal cord injury

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Loss of limb, spinal cord injury, stroke, cerebral palsy, Parkinson's disease, and other neurological impairments result in loss of sensation and of function for millions of people. Pharmacological or traditional surgical treatments cannot restore these losses. There are approximately 282,000 persons in the US with spinal cord injury[1] and 1.6 million with limb loss[2]. Loss of function is life-changing in spinal cord injury and significant in limb loss. In both, however, the *loss of sensation is devastating*. Somatosensation is the most significant connection to the world and others. Neural prostheses that connect to the nervous system are making significant advances in restoring sensory and motor function to these patients as demonstrated in several long-term clinical trials. Flat interface nerve electrodes (FINEs)[3] have been implanted on upper and lower extremity nerves of nearly 20 subjects with spinal cord injury or limb loss, demonstrating nearly a decade of clinically stable performance of this interface to the nervous system[4,5]. Following a decade of successful motor restoration, there are significant advances in restoring somatosensation. We have implanted FINEs with 8 or 16 stimulation points evenly distributed around the median, radial, and ulnar nerves of limb loss subjects. Over the past four years we have mapped the location, intensity, quality, and temporal stability of users' perceptions of electrical stimulation through the FINEs. We have connected sensors to their prostheses and mapped the tactile and hand position information directly to stimulation patterns applied to through the FINEs in extensive lab studies and in community usage. Greater than 90% of the individual contacts on the FINEs result in either a tactile, proprioceptive, or rarely, nociceptive sensation. These perceptions are distributed over the hand and are reported and being sensations directly on their hand, as thought it was not lost. The perception location and stimulation thresholds have remained stable for more than 4 years to date. We have shown that patterns of varying stimulation intensity encode the quality of tactile perception, resulting in a range of perceptions from paresthesia to vibration to motion to natural touch[6]. The subjects show reduction in long-term episodic phantom pain. With restored sensation, the users describe the prosthesis as their hand. Sensation improved fine control of the prosthesis and enables the user to perform tasks with visual and auditory occlusion that were not possible without sensation[7], sense motion, and have ability to discriminate texture. Sensation results in embodiment of the prosthesis, increased user confidence, and return to bimanual tasks. In the words of a subject, "I can feel my hand for the first time since the accident," and "feel my wife touch my hand." The systems developed for limb loss subjects are now being advanced toward spinal cord injury and offers an exciting future options for function and quality of life.

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Restoring functional movement in tetraplegia

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Millions of people suffer from diseases and injuries resulting in paralysis. Neuroprosthetic devices are being developed to restore lost function by decoding and re-routing neural signals around affected neural pathways. It has previously been shown that signals recorded in the brain can be decoded and linked to assistive technology and robotic devices. In non-human primates, these signals have also been used to activate chemically paralyzed arm muscles. More recently, it has been shown signals recorded within the brain can be linked in real-time to muscle activation to restore movement in a paralyzed human. Functional movement has been demonstrated for completing daily activities and neuroprosthetic research has opened many new possibilities for treating not only spinal cord injury, but potentially stroke and brain injury as well in the future.

Interfacing with the brain and spinal cord to restore upper-limb movement

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Neural interfaces, or Brain-Machine Interfaces, decode electrical activity directly from the nervous system to provide a new communication channel for the brain to interact with the environment. I will describe progress towards a neural interface that links cortical activity to spinal cord stimulation, providing an artificial motor pathway to restore movement to limbs paralysed by neurological injury. We find that a range of upper-limb movements can be elicited by microstimulation through intraspinal electrode arrays in the cervical enlargement (Zimmermann et al., J Neural Eng 2011) and that cortical control of spinal stimulation can restore simple volitional grasping in monkeys after temporary inactivation of primary motor cortex with muscimol (Zimmermann and Jackson, Front Neurosci 2014). Key technological barriers to clinical application include the long-term stability of cortical recording and spinal stimulation, as well as implementation within a low-power subcutaneous implant. I will describe several advances to address these challenges including the use of low-frequency local field potentials (Hall et al. Nature Communications 2014) and novel spinal cord stimulation techniques (Sharpe and Jackson, J Neural Eng 2014). Finally I will discuss neuroplastic changes induced by artificial motor pathways that suggest a role for neural interfaces in both the replacement and repair of the injured nervous system (Jackson and Zimmermann, Nat Rev Neurol 2013).

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Spinal cord stimulation: Tapping into neural circuits to modulate motor function after spinal cord injury

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Severe spinal cord injury (SCI) is a devastating condition, causing paralysis and impairment of vital body functions caudal to the lesion. One treatment approach besides the intense efforts undertaken to find neurobiological cure is to move the attention away from the pathology at the injury site itself, towards neural circuitry residing within the lumbar spinal cord, which is spared in the majority of incidents. The present talk will review the current knowledge of how this circuitry can be accessed through epidural lumbar spinal cord stimulation (SCS)[1–3] and harnessed for the treatment of motor dysfunction following SCI.

Previous work revealed that epidural SCS activates large-to-medium diameter sensory fibers within the posterior roots[3–6] that in turn trans-synaptically recruit spinal reflex circuits and plurisegmentally organized interneuronal networks controlling stereotyped multi-muscle activation patterns.[7,8] Indeed, it was demonstrated that in response to epidural SCS, the functionally isolated human lumbar spinal cord can generate motor output underlying stepping[3,8,12,13] and (full weight-bearing) standing.[9–11] Innovative approaches aiming at augmenting the outcome of locomotor training[10,14,15] and enabling some rudimentary translesional volitional motor control over otherwise paralyzed muscles by enhancing the central state of excitability[10,16] will be presented.

The role of SCS can go well beyond the immediate generation of motor output; when combined with complementary treatment modalities based on subclinical translesional motor control and proprioceptive feedback input as well as pharmacological interventions, it can become a major rehabilitation approach in SCI for augmenting and steering trans- and sublesional plasticity for lasting therapeutic benefits.

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Intramuscular neurotrophin-3 normalizes spinal reflexes and improves mobility after bilateral pyramidotomy injury in rats

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Brain and spinal injury reduce mobility and often impair sensorimotor processing in the spinal cord leading to spasticity (Adams et al. 2005). We established that complete transection of corticospinal pathways in the pyramids (Kathe et al. 2014) impairs locomotion and leads to signs of spasticity in rats. These include clonus (repeated muscle jerks), prolonged spasms and twitches (fast involuntary contractions, which are all symptoms associated with human spasticity after upper motor neuron lesions. The corticospinal tract lesioned rats also developed excessive monosynaptic and low threshold polysynaptic spinal reflexes.

Treatment of affected forelimb flexor muscles with an adeno-associated viral vector encoding Neurotrophin-3 at a clinically-feasible time-point after injury reduced spasticity including hyperreflexia. Neurotrophin-3 normalized the monosynaptic Hoffmann reflex to a hand muscle and polysynaptic spinal reflexes between afferents and efferents of treated muscles. Rats treated with Neurotrophin-3 also recovered more locomotor function. Furthermore, the balance of inhibitory and excitatory boutons in the spinal cord and the level of an ion transporter in motor neuron membranes required for normal reflexes (Boulenguez et al. 2010) were normalized. Our findings pave the way for Neurotrophin-3, which is safe and well-tolerated in humans (Parkman et al 2003, Sahenk et al 2007), as a therapy that treats the underlying causes of spasticity and not only its symptoms.

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Characterization of axon growth repellents in the developing spinal column

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During amniote development, the spinal column undergoes a segmentation process through the sequential formation of the mesodermal somites along the rostrocaudal axis. Somites differentiate subsequently into dermomyotome (giving rise later to skin and skeletal muscles) and sclerotome (giving rise to vertebral bone structures and cartilage). Sclerotomes divide at their antero-posterior intrasegmental boundary, and become polarized into an axon growth-permissive (anterior) region and an axon growth-repulsive (posterior) region. When outgrowing from the neural tube towards the periphery, motor and sensory axons respond to this binary system and follow a segmented pattern, ensuring that nerves develop without obstruction by the future cartilage and bones of the vertebral column (Kuan et al., 2004).

In this context, repellent molecules from posterior half-somites guide navigating axons by excluding them from “no-go” areas (Keynes et al., 1997). Among the candidate molecules, peanut lectin-binding glycoproteins, chondroitin sulphate proteoglycans, Eph/Ephrins and semaphorin 3A have been proposed as repellents acting on different receptor systems expressed by axon growth cones (Kuan et al., 2004; Bonanomi and Pfaff, 2010). Interestingly, similar repellent molecules are expressed in the adult central nervous system (CNS) by astrocytes. Following brain or spinal cord injury, these molecules are found to be upregulated in “reactive” astrocytes recruited at the lesion site, and to impede axon regeneration in this region (Silver and Miller, 2004).

I will present the results of a differential gene expression analysis of anterior and posterior half-sclerotomes, based on RNA-sequencing data. Several candidate genes are highlighted in this study and may play a role in the polarization and differentiation of the somite tissue, in the cell adhesion characteristics of half-sclerotome cells, and in the axon guidance properties of this system.

In addition, the growth cone collapse assay has been used to further characterize the axon growth-repulsive potential of a tissue or purified candidate proteins. Detergent extracts of rat grey matter and of a cultured line of human astrocytes have been shown to possess growth cone collapse-inducing activity. Furthermore, our experiments indicate that this CNS-derived activity has molecular properties similar to that in somites, so it is possible that this contact-repulsive system has been co-opted in the CNS to play an important role in regulating connectivity and plasticity.

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Modulation of the glial scar using GSK3β inhibition: a mechanistic study

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Astrocytes are the most abundant glial cells in the central nervous system (CNS) and constitute a major element of the mature glial scar the forms as a results of spinal cord injury (SCI). The glial scar is a physical and chemical barrier for axonal growth and is a key reason why axons do not regenerate. Our aim is to determine whether astrocytes can be regulated to form an astroglial scar that is more permissive to axon growth. To test the effect of GSK3β inhibition on astrocytes we used *in vitro*, *ex vivo* and *in vivo* models to study glia relevant to SCI.

In this study, in vitro cultures of astrocytes were used in a wound healing assay to test the effect of GSK3β inhibitors (Lithium chloride, AR-A014418 and Tideglusib) on wound healing. We developed a 'medium throughput' *ex vivo* slice models, using spinal cord and optic nerves from transgenic mice in which the astroglial promoter glial fibrillary acidic protein (GFAP) drives expression of enhanced green fluorescence protein (eGFP). Thoracic spinal cord slices (P10-15) or adult optic nerves from mice were maintained in culture for 3 to 7 days *in vitro* (DIV) and treated with a range of GSK3β inhibitors (lithium chloride, ARA014418, or Tideglusib. Inhibition of GSK3β significantly retarded wound closure in astrocyte cultures and induced morphological changes in astrocytes in the spinal cord and optic nerve, with the development of a polarised astrocyte phenotype. An equivalent effect of GSK3β inhibition was demonstrated in cultured optic nerves, with a profound effect on astrocyte morphology. To examine this astrocyte phenotype further, we performed a genome wide microarray analysis on the optic nerve following GSK3β inhibition compared to controls. Pathway analysis (IOA, Ingenuity Systems) indicated Axon Guidance Signalling as one of the major pathways significantly altered by GSK3β inhibition, with prominent effects on sema3, which is known to promote axon growth. Furthermore, using a three-way comparison of genomic data from cultured optic nerves with lithium chloride, AR-A014418 and Wnt agonist, we found lysyl oxidase (LOX) as the key regulated gene for the observed phenotypic changes in astrocytes.

The results support the possibility that GSK3β inhibition induces an environment permissive for axon growth and that the polarised astrocyte will provide a scaffold for axon growth and perhaps LOX may be a potential downstream target to be examined *in vivo* using a contusion model of spinal cord injury in adult rats.

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Rapid recovery of breathing after chronic cervical spinal cord injury

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Complete functional recovery of motor system activity after severe cervical spinal cord injury (SCI) has proved experimentally and clinically elusive. We provide evidence that a combination of extracellular proteoglycan matrix modification and conditioning may provide the key to the complete recovery of diaphragm activity following chronic cervical trauma in the rat. In our model, animals demonstrated complete ipsilateral hemidiaphragm paralysis lasting up-to 18 months following lateral C2 hemisection. There was no evidence of endogenous functional recovery. However, within two weeks, a single injection of chondroitinase ABC into the ipsilateral spinal cord at C4 restored robust bilateral diaphragm function. Further, conditioning with intermittent hypoxia training had a tendency to increase this recovered function. Indeed, we demonstrate that through regulation of serotonergic sprouting, our treatment strategy ensured rapid, patterned respiratory recovery in the chronically injured animal. This treatment was as effective when applied 1.5 years after trauma as when applied at 3 months and remarkably more efficacious than similar therapies applied acutely after SCI. Interestingly, the combination treatment strategy additionally lead to a small cohort of animals displaying structured, but abnormal, tonic activity in the ipsilateral hemidiaphragm caused by an excess of 5-HT sprouting. While transient, this may demonstrate the recapitulation of developmental processes occurring through treatment induced recovery in the adult and suggests considerable remodeling of spinal cord circuitry below the level of the lesion at chronic stages. These data illustrate that functional motor system recovery is possible following a near lifetime of paralysis through a universally applicable mechanism. Further, simple induction of plasticity via matrix modification may evoke recovery in certain motor systems that is more effective when applied chronically than in the initial weeks after SCI.

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Long-term activity monitoring and advanced assessments in spinal cord injury using wearable sensor technology

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Despite many years of research, functional recovery following an injury to the spinal cord is only partial. This is mostly because severed axons have restricted spontaneous regeneration and growth from remaining, intact fibres is limited. Pre-clinical data suggests that training, which enhances activity within damaged and intact fibres, leads to functional and anatomical recovery. Despite the lack of adequate clinical research into this issue, in humans rehabilitation paradigms in specialized spinal cord injury (SCI) centres focus on increasing the activity in spared fibres in the hope that this might activate remaining intact circuits or stimulate the formation of new ones. Outside of the clinical SCI setting, multiple activity tracking devices have recently become available. With these devices it is possible to track daily activity, usually in the form of step counts, as well as sleep patterns, eating and drinking behaviour, weight, calories burnt, distance walked etc. One of the key features of such devices is that the user can set themselves goals within each of the categories as well as use dedicated online portals to upload their data and compare themselves to others. Such devices could potentially be useful within the SCI community to help maintain health and to assist with the continuation of fitness training beyond the in-patient setting. However, existing commercial devices lack a number of SCI-specific features, such as wheeling instead of step counts, SCI-specific goals and suitable comparison data for people with SCI. In order to define these features research with such devices within the in-patient setting is essential. In recent years we have been investigating how to use inertial measurement units (IMUs) to provide meaningful, clinically relevant measures of arm movements and activity. We have used a novel IMU, ReSense, to measure arm movements during recovery from SCI in three rehabilitation hospitals in Switzerland. We have published several specific algorithms (Brogioli et al., 2016, Popp et al, 2016) that can analyse ReSense data to give details of the movement performed such as activity counts, kinetic and kinematic information and details of mobility and information about upper limb use. These algorithms allow us to investigate in detail how activity impacts recovery of function following human SCI.

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Axonal transport as a target for enhancing CNS regeneration

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Axons in the spinal cord fail to regenerate after injury due to a combination of intrinsic and extrinsic factors. Extrinsic factors preventing axonal regrowth have been well characterized, however the intrinsic factors that prevent robust regeneration are not completely understood. We have taken a cell biology approach to identify cellular mechanisms opposing successful regeneration, focusing on the axonal transport of growth promoting receptors. We have analysed axon traffic and transport *in vitro* and compared regenerative PNS neurons with non-regenerative CNS neurons, using GFP tagged integrins as an archetypal example of a molecule that can promote regeneration. We find that there are striking differences between the two neuronal types, such that PNS neurons allow dynamic transport of integrins throughout their axons, whilst CNS neurons selectively prevent integrin transport via a regulated trafficking mechanism. We find that this mechanism can be targeted to promote axon transport of integrins along with their associated growth machinery, and that this leads to a robust increase in intrinsic regenerative capacity.

Integrins are a family of transmembrane adhesion molecules. They control axon growth during development, and in adulthood they regulate dendritic function. They are also critical for axon regeneration in the peripheral nervous system (PNS). Adult CNS neurons do not regenerate their axons, and in these cells integrins are confined to dendrites. Why is this? We have found that integrins traffic into PNS axons via recycling endosomes, but that these are restricted from the axons of mature CNS neurons.

In PNS neurons (which can regenerate) integrins move into axons marked by the small GTPases Rab11 and ARF6. We find that integrins move bi-directionally in PNS axons, and that the direction of transport can be altered by manipulating the activation state of ARF6. Inactive ARF6 favours anterograde transport, whilst active ARF6 favours retrograde transport. In non-regenerative CNS axons, integrins are removed from axons by predominant dynein-dependent retrograde transport, regulated by the ARF6 GEFs EFA6 and ARNO. Rab11 and ARF6 collaborate to prevent integrins from localising to mature CNS axons. Recycling endosomes marked by Rab11 contain a large amount of machinery that is required for the dynamic regulation of cell membranes and the cytoskeleton – mechanisms that are required to establish a growth cone and drive axon growth. We have been targeting ARF6 in order to increase the axonal presence of Rab11 positive recycling endosomes and integrins, aiming to promote axon regeneration in the CNS. We have used in vitro laser axotomy to determine that ARF6 and Rab11 function to regulate axon regeneration after injury in vitro, and demonstrate that trafficking can be manipulated to increase the regenerative capacity of CNS axons.

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Transcriptome analysis identifies a developmental switch gene that limits regenerative ability in the adult CNS

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Injuries to the adult central nervous system (CNS) often result in permanent disabilities because neurons lose the ability to regenerate their axon during development. Both a non-permissive environment and reduced intrinsic growth ability account for the regeneration failure in the adult CNS. While several extracellular growth inhibitors expressed in the adult CNS have been characterized, the molecular signature underlying changes in neuronal intrinsic growth ability is largely unclear. Here, whole transcriptome sequencing and bioinformatics analysis followed by gain- and loss-of-function experiments identified *Cacna2d2*, the gene encoding the Alpha2delta2 subunit of voltage gated calcium channels, as a developmental switch that limits axon growth and regeneration. *Cacna2d2* gene deletion or silencing promoted axon growth in vitro. In vivo, Alpha2delta2 pharmacological blockade through Pregabalin administration enhanced axon regeneration in adult mice after spinal cord injury. As PGB is already an established treatment for a wide range of neurological disorders, our findings suggest that targeting Alpha2delta2 may be a novel treatment strategy to promote structural plasticity and regeneration following CNS trauma.

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Electrical stimulation to promote outgrowth of injured neurons

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The goal of this project was to investigate mechanisms and parameters that translate electrical stimulation (ES) to enhanced neurite outgrowth and possibly regeneration of injured neurons. Our research efforts began in the sensory system where the effects of stimulating sensory fibers were evaluated. Secondly we attempted to translate our findings to injured motor tracts, specifically the corticospinal tract (CST) following spinal cord injury (SCI). We found that a ES of sensory fibers over one hour increased the percentage of neurons with neurites >100um in vitro, with no change in the percentage of neurite bearing neurons, indicating that the effect on growth is due to enhanced elongation and not initiation. Longer duration stimulation (7h), as well as repeated stimulation for 7 days enhances growth comparable to the 1 hour ES. Growth effects of 1h ES of sensory fibers were also assessed in vivo in a model of SCI, together with cell transplantation of bone marrow stromal cells at 4 weeks post-injury. Animals with ES showed significantly increased axonal regeneration into the spinal graft compared to sham animals. To test the effect of ES on the lesioned CST, we stimulated the motor cortex over 30 min (either with 20 or 330 Hz) and found that axonal collaterals (i.e., axonal sprouts rostral to the lesion) were increased. Surprisingly, animals did not perform better, but worse in a reaching task, which might be linked to the unexpected finding of increased dieback at the lesion site.

To explore the molecular effects of ES, RNA sequencing was performed to investigate differential gene expression at 1 day and 7 days after sensory ES, collecting 30M SE reads/sample on a HiSeq2000. As expected condition lesion induces and represses an extensive number of genes compared to naïve animals. ES induced/reduced expression of a much lower number of genes relative to sham animals with smaller changes in gene expression. Several genes and pathways could be identified that are known to play a role in regeneration, suggesting that ES-mediated effects on axon regeneration are likely a summation of several activated pathways that overlap only partially with condition lesions.

We conclude that ES might become a viable approach to promote regeneration and plasticity of injured neurons after SCI, however various questions on mechanism and protocol have to be answered before translational approaches are attempted.

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Functional testing of candidate therapeutic genes in the injured corticospinal tract

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To successfully regenerate injured axons neurons must initiate complex transcriptional programs that orchestrate the production of diverse cellular materials. Peripheral neurons, and some central neurons in non-mammalian vertebrates like zebrafish, respond to injury by upregulating transcription factors that support axon growth. Mammalian CNS neurons often fail to do so, fundamentally limiting repair after injury. Thus a promising strategy to enhance regenerative ability in CNS neurons is to force the expression pro-regenerative transcription factors. Focusing on the corticospinal tract (CST), a clinically important cell type that historically has responded poorly to pro-regenerative treatments, we have tested viral overexpression of a variety of pro-regenerative factors. Two of these factors, KLF7[1] and Sox11[2] have demonstrated the ability to increase axon growth in models of partial cervical injury. Importantly, growth stimulation is also observed when transcription factors are delivered 8 weeks post-injury, suggesting efficacy in chronic injuries. Moreover, optogenetic stimulation of newly grown CST axons paired with electrophysiological monitoring of post-synaptic activity demonstrates effective synaptic integration[3]. To extend these findings we have tested the ability of genetically stimulated CST axons to regenerate into crush injuries that are more complete and severe. Neither KLF7 nor Sox11 promoted regeneration into these more challenging tissue environments, suggesting a continued role for environmental inhibitors. Accordingly, we next tested combined treatments of KLF7 or Sox11 with virally expressed chondroitinase, an enzyme that degrades growth-inhibitory CSPGs[4]. Although degradation of CSPGs was apparent and axons displayed reduced retraction from chondroitinase-treated injuries, axon regeneration into or beyond complete injury sites was not observed in any condition. Finally, because it is likely that cooperative activity by multiple transcription factors is needed for a full regenerative response, we have expanded single and combinatorial testing of transcription factors in injured CST neurons. These experiments identified KLF6 as an additional potent promoter of CST growth and current experiments are testing combined expression of KLF6 with other pro-regenerative TFs: cJun, Sox11, and Myc. Overall, these data demonstrate the ability of transcription-factor based interventions to enhance the intrinsic regenerative ability of injured corticospinal tract neurons, while also illustrating the continued challenges of achieving a full regenerative growth state.

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Realistic evaluation of current clinical status of cell therapeutics for spinal cord injury

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For decades the transplantation of cells to repair aspects of spinal cord injury (SCI) has been considered a leading therapeutic option. Now, in 2016, we have considerable data to indicate the impact of early clinical trials of cell therapy. It is clear that the effects of cell transplantation in complete acute thoracic injuries are not transformative. The impact in cervical injury and chronic injury is still under evaluation in Phase 1 and 2 studies. In addition, studies in ALS have helped to address the safety of multiple injections into the spinal cord. Although efficacy is not prominent, intraparenchymal injections into the spinal cord are apparently not especially risky. This assessment will require longer time periods for the highest risk cells, ES-derived and neural stem cells.

The mechanistic basis for possible repair, promotion of anatomical plasticity, remyelination, trophic support, and possible axonal regeneration remain valid but are difficult to specifically assess clinically. One leading problem in these studies is to verify cell survival. For allografted cells there is little data, other than in deceased ALS subjects, to inform the question of the need for, composition , and duration of immune suppression.

It is possible to assess for subclinical changes, using electrophysiological and imaging methods, such as fMRI but clinical trial sponsors of multi-center studies are averse to adopting these methodologies due to their cost, need for specialized expertise, and lack of persuasive data that they add value to clinical determinations.

Although studies in incompletely injured subjects may indicate a greater efficacy, it is clear that we need to be solve some additional problems in order to meaningfully study cell transplantation for SCI. Advances in the delineation of possible specific targets for cell-mediated effects need attention, including a deeper understanding and quantification of myelin deficits in people after SCI. In cervical SCI, lower motor injury due to motor neuron loss, needs improved assessment methodologies as this injury can severely limit the impact of non-specific cellular therapies. Most current methods of cell delivery result in disruptive boluses of cells whose distribution and migration is uncontrolled. This is not optimal and relies on assuming that the cells will migrate and perform in a favorable way, despite the absence of normal tissue signals.

Thus, the next generation of cellular therapies for SCI requires solution of the above and other problems, if meaningful progress is to occur.

Secondary health conditions after spinal cord injury and standardization of information collected

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Based on our own research are secondary health conditions after spinal cord injury (SCI) illustrated. In brief causes of death – respiratory, cardiovascular, and suicide will be reported. Secondary health conditions including respiratory challenges and sleep disturbances, cardiovascular issues and heart rate variability (1-2), urinary tract problems related to long-term causes for renal deterioration (3-4) and possibilities for neuromodulation, constipation and the possible need for colostomy in severe instances (5), and sexual challenges as well pressure ulcers, osteoporosis, pain and spasticity (6), muscle changes, hand function (7), post-traumatic syringomyelia, and medicine requirements (8) after SCI will shortly be described.

A presentation of the development of the international initiatives for standardizing data collection within the SCI community for clinical as well research purposes will be given. This development started with the original Frankel classification continuing with the International Standards for Neurological Classification of SCI (9) to the International SCI Data Sets and the National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS), Common Data Element (CDE) Project for SCI (10-12).

The creation of the International SCI Data Sets started in 2002, and the International SCI Core Data Set, 19 International SCI Basic Data Sets, and four International SCI Extended Data Sets can all be downloaded free of charge from http://www.iscos.org.uk/international-sci-data-sets. Guidelines for uniform reporting of SCI data to facilitate comparison between studies are likewise provided.

The NIH NINDS CDE Project was initiated in 2006 with the aim of developing CDEs, data definitions, case report forms (CRFs), and guidelines relevant to clinical research in neurological diseases. The NIH NINDS CDE project specific to SCI began in 2012, and in all the NINDS CDEs for SCI clinical research and clinical trials there are 1150 data elements and measures with definitions, CRFs and guidelines, and all CDEs can be downloaded free of charge from http://www.commondataelements.ninds.nih.gov/SCI.aspx#tab=Data_Standards.

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The patient journey following SCI: prognosis, outcomes and preferences

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Spinal cord injury (SCI) is a devastating injury that results in impaired motor, sensory and autonomic function. As a result, patients experience limitations in physical function, obstacles when participating in daily activities and a reduction in their quality of life. While the number of new injuries is lower compared to health conditions such as stroke, the cost of the injury is substantial to both the person and society. To optimize patient outcome it is important to understand the patient's journey from the time of injury through to life in the community and consider the preferences of patients regarding their care.

Data from large SCI registries such as the Rick Hansen SCI Registry (RHSCIR) and the European Multicenter Study about SCI (EMSCI) has been used to assist clinicians prognosticate outcomes such as motor recovery and physical function for their patients. Motor recovery after injury is very heterogeneous and can be predicted using the neurological level and the severity of injury (ASIA Impairment Scale grade) obtained from the baseline neurological assessment. There is also evidence to suggest that motor recovery is enhanced in patients with an incomplete SCI who receive early surgery ($\leq$24hours). The development of clinical prediction rules such as the one developed by van Middendorp et al., enable clinicians to predict the probability a patient will be ambulatory at 1-year post injury using baseline motor and sensory data and the patient's age. Recent qualitative studies have examined the experiences and preferences of patients and their family members in receiving information on the SCI diagnosis and prognosis in acute care. The attitudes of clinicians as well as the type of information and the timing of when it is delivered after the injury were two major themes that emerged from this research. These examples from the acute care phase illustrate how clinical data and clinical prediction rules can assist clinicians with counseling their patients as well as the importance of considering the preferences of patients. Research has demonstrated that the needs and priorities of patients often change in each phase of care and over time. By personalizing care at each stage of a patient's journey and listening to their preferences, it will ensure patients achieve optimal outcomes following their injury.

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Corticospinal function in the control of gait following spinal cord injury

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Human gait is regulated by integration of descending motor commands, rhythm generating networks and sensory feedback signals in the spinal cord (1). The primary motor cortex contributes through the corticospinal tract to the activation of muscles during uncomplicated treadmill walking and is of major importance in modulating and adapting gait to changes in the environment as well as in the visual guidance of gait (1,2,3). This corticospinal contribution to gait may now be evaluated non-invasively by electrophysiological techniques in healthy individuals and in individuals with spinal cord injury. Transcranial magnetic stimulation (TMS) during gait elicits a direct monosynaptic excitation of ankle plantarflexors, which is largest at the time of push-off at the end of stance (4). Oscillations in EEG and EMG recorded from ankle plantarflexors also show the most pronounced coupling (corticomuscular coherence; CMC) in relation to push-off. Individuals with spinal cord injury show reduced activation of ankle plantarflexors by TMS with correlation to gait velocity. CMC and muscle activation by TMS recorded for ankle dorsiflexors are also reduced in individuals with spinal cord injury, but with a correlation to foot drop (5). All TMS and CMC measures show correlation to regional atrophy of the dorsolateral quadrant of the spinal cord where the corticospinal tract is located (6). These observations indicate that corticospinal transmission to ankle plantarflexors contributes to forward propulsion and gait velocity, whereas corticospinal transmission to ankle dorsiflexors is mainly important for toe lift at the end of swing.

Daily gait training for 11/2-3 month in adults with spinal cord injury or cerebral palsy have failed to demonstrate plastic changes in the corticospinal transmission to ankle muscle despite functional improvements. In contrast, corticospinal transmission to ankle muscles was strongly facilitated following 1 month of gait training in children with cerebral palsy below the age of 10 years (7). We suspect that this may reflect an age related difference in corticospinal plasticity and we speculate that different mechanisms may be responsible for functional improvements following training in adults and children. This may impact choice of intervention.

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Tracking trauma induced changes across the neuroaxis after acute SCI: insights from neuroimaging

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Recovery from spinal cord injury – and the attending neurodegenerative processes – can follow a complicated trajectory over several years following the initial insult. This is a prescient problem due to the ensuing diachisis (from Greek meaning "shocked throughout") that can affect the entire neuroaxis; encompassing the spinal cord and brain. Crucially, the cascade of neurodegenerative and compensatory (self-organized or re-organized) changes are further conflated by a circular causality between the patient's functional deficit and experience-dependent plasticity.

In the first part of my talk I will present findings from patients with acute spinal cord injury. By use of a longitudinal computational morphometry approaches, I show that trauma-induced ultra-structural and macroscopic measures of neurodegeneration (and reorganization) occur early and progress with a specific spatio-temporal pattern at the spinal and brain levels over two years. Myelin and iron sensitive quantitative MRI measures indicate that demyelination of corticospinal tract axons as well as iron accumulation accompany atrophy. Sub-acute changes in structural MRI measures at the spinal and brain levels predict two-year outcomes. Sample size calculations for cord area and corticospinal tract volume changes suggested that for randomised clinical trials, fewer than 30 patients per treatment arm would be required.

The second part of my talk is dedicated to pioneering data illustrating the feasibility to segment the grey and white matter at the level of the cervical and lumbar cord from high-resolution MRI data in chronic SCI. Importantly, both macroscopic as well as ultra-structural changes are evident above (i.e. cervical) and below the injury level (i.e. lumbar) within the spinal grey and white matter; thus providing novel insights into retrograde/anterograde degeneration as well as neuronal changes across the spinal axis in human SCI.

These observations illustrate the enduring neuroplastic processes induced by SCI and highlight a progressive (activity-dependent) diaschisis across the neuroaxis. Furthermore, these measurable changes are sufficiently large, systematic and have predictive validity to render them viable for scoring the effect of treatment.

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Patterns of bone loss after spinal cord injury

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Extensive muscle paralysis after a motor-complete spinal cord injury (SCI) is typically followed by significant bone loss in the long bones of the lower limbs. In chronic SCI, the weakened bones then become more susceptible to fracture from everyday activities such as transfers, or from falling out of a wheelchair. These fragility fractures are most common five years or more post-injury [Gifre *et al.* 2014], and typically occur around the knee (distal femur and proximal tibia) or above the ankle (distal tibia). Fractures often require surgical management and even long periods of bedrest in cases of severe complications such as pressures sores or osteomyelitis [Frotzler *et al.* 2015; Gifre *et al.* 2014]. Thus, avoiding fractures is a key rehabilitation goal in this patient population. Exercise and other intervention studies in chronic SCI illustrate that trying to reverse bone loss in the tibia and femur when the bones are already osteoporotic is challenging. A preventative strategy would be to intervene in the early phases of SCI, with the aim of attenuating the bone loss.

To achieve this, we need to identify and understand the factors that contribute to patterns of bone loss after SCI, which vary considerably between individuals [Coupaud *et al.* 2015]. We have performed longitudinal and cross-sectional studies using peripheral Quantitative Computed Tomography (pQCT) in patients with motor-complete SCI at the Queen Elizabeth National Spinal Injuries Unit (Glasgow, U.K.). The pQCT technique allows a much more detailed and quantitative picture of these patterns of bone loss after SCI than would be achievable with the clinical bone densitometry gold-standard method, dual-energy X-Ray absorptiometry (DXA). Using pQCT, we can quantify individual patients' rates of bone loss within months of their injury, identify those losing bone at the fastest rates, and target them for intervention [Coupaud *et al.* 2012]. Potential early rehabilitation interventions include whole body vibration and electrically-stimulated exercise. Early intervention in the patients most susceptible to rapid bone loss may prove to be an effective approach to the management of osteoporosis and prevention of fractures.

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Spontaneous recovery and opportunities for neuro-restoration of the upper limb after traumatic spinal cord injury

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As the demographic and profile of the traumatic spinal cord injury (SCI) population changes, the approaches to treat the altering presentation of this disease must also evolve. The field in the past 15 years has observed among other factors that: almost 2/3 of all SCI are cervical; the prevalence of incomplete injuries has increased to almost 60%; and the mean age of the population at time of injury is increasing (1-3). Declined upper limb (UL) (arm and hand) function is one of the most devastating consequences of cervical SCI (tetraplegia) and it has been shown to be the priority of recovery for this sub-group (4). Both subtle and significant improvements in UL function can lead to increased independence in daily activities, improving independence and quality of life (5).

Researchers in the rehabilitation field have developed a number of upper limb interventions that can enhance independence, function and restore neurological deficit. Over the last three decades, various interventions such as: functional electrical stimulation, exercise, practice and training coupled with peripheral nerve stimulation and activity based restorative therapy, have evolved in an attempt to improve UL function in individuals with SCI (4, 6, 7).

Despite the efforts and discovery of new concepts and interventions, widespread uptake and translation remain limited for a number of reasons: 1) Incidence of SCI is low, meaning that studies are often-underpowered, and multi-site studies are needed to conduct randomized controlled trials with even modest sample sizes; 2) development is funded, however uptake and translation methods are not well thought out or funded. As a result, methods found to be promising in initial studies do not always receive the follow-up work needed to refine and deploy them.

Thus, an algorithm to facilitate clinical decision making and progression for uptake from an already existing body of knowledge was defined on three principles: 1) Recovery profiles of the upper limb post SCI; 2) Scoping of current practices in UL rehabilitation; 3) Established neuro-musculoskeletal restorative approaches; and 4) Knowledge translation and implementation strategies needed for uptake.

This talk identifies the key components required for integrative knowledge translation in the evolving field for restorative upper limb strategies in SCI.

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Functional electrical stimulation therapy for improving voluntary reaching and grasping function

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Neuroprostheses for grasping have been originally envisioned as tools that SCI patients will use daily to grasp and manipulate objects. In essence, up to 1999/2001 the neuroprostheses for grasping have been used as assistive devices only. Fifteen years ago we decided to try a new approach. We proposed to use the neuroprostheses as short-term therapeutic tools with the hope that use of these devices during early rehabilitation may potentially improve the voluntary upper limb function in individuals with cervical SCI. When we proposed that intervention we were uncertain if that therapeutic concept had any merit. Today, the use of the neuroprostheses for grasping as a therapeutic intervention is called functional electrical stimulation therapy (FEST). Already few commercial products have been developed with the objective to deliver FEST for upper limb. In this lecture I will provide a summary of what we learned about FEST for upper limb, and present a compelling case why this therapy should be used as a new best practice approach to improve voluntary upper limb function in the individuals with cervical SCI.

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Prediction, monitoring and appreciation of upper limb outcomes in human SCI

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Spinal cord injury (SCI) causes profound disability that affect mobility in fundamentally different ways, but which also present common challenges to patients, clinicians and society. Effective interventions to restore and preserve upper limb mobility and activity are essential for independent living, health maintenance, and quality-of-life. Current approaches to mobility assessment, however, are sporadic and time-consuming, based on subjective reports and low sensitivity tests, and thus limited in capturing clinical and non-clinical (real-world) conditions.

Clinically meaningful assessment must meet three main challenges: a) acquire functionally-relevant patterns of movement, mobility, and activity at different scales (real-world mobility, activities, and movement kinematics), b) assess patients across the care continuum (clinical and non-clinical settings), and c) account for considerable changes in movement, mobility, and activity as a function of both healthy and disease-modified aging.

The talk will address clinical and biomechanical matrixes to appreciate upper limb recovery while will be driven by the needs of frontline users (clinicians and patients) and researchers to provide support for clinical decision-making to improve therapies of specific motor impairments.

Upregulation of Astrocyte elevated gene (AEG-1) promotes axon regeneration after spinal cord injury

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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 we demonstrate by manipulating expression of protein molecule named astrocyte elevated gene-1 (AEG-1/ also known as MDTH, LYRIC1) that axon regeneration in the CNS is possible. Our microarray analysis of spinal cord injury (SCI) suggests that AEG-1 has a role in CNS axon regeneration. We found that AEG-1 was significantly up-regulated in regenerating pSN+DC lesions compared with intact control and non-regenerating DC lesion models. We also observed up-regulation of AEG-1 by up to 65 fold in regenerating SN injury model, correlating with axon regeneration. Semi-quantitative RT-PCR was used to confirm our microarray data along with immunohistochemistry and western blot to analyse AEG-1 protein levels. We found high levels of AEG-1 mRNA and protein in both regenerating SN and pSN+DC compared with non-regenerating DC or intact controls, with pSN+DC model showing the greatest levels of AEG-1 mRNA and protein. Moreover, knockdown of AEG-1 using short interfering RNA (siRNA) in 3 days cultured dorsal root ganglion neurons (DRGN) significantly suppressed DRGN neurite outgrowth suggesting that high levels of AEG-1 are required for axon regeneration. The mechanism by which AEG-1 promotes axonal regeneration is not yet known but we conclude that upregulation of AEG-1 plays a major role in axonal regeneration and could be harnessed to promote regeneration in the CNS of spinal injured patients.

EMG-triggered surface FES for arm reaching in tetraplegia

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A mid-cervical level spinal cord injury results in loss of wrist and elbow extension, while the muscles providing elbow flexion and shoulder abduction retain at least partial voluntary control. Surface functional electrical stimulation (FES) is typically used therapeutically to strengthen and retrain atrophied muscles, but if applied in a coordinated way, can be used to restore useful function[1].

In this case study, we used EMG triggered FES to restore some control of arm reaching in a 56- year old male with a C4 spinal cord injury. We recorded the activity of voluntary muscles with EMG and used these signals to control surface FES applied to paralyzed muscles, in order to achieve a functional arm movement. The FES control algorithm used was the following: when the deltoid exceeds a threshold and the biceps remains below a threshold, we assume that the FES user is trying to reach out, so the triceps and wrist extensors are triggered; when the biceps is above the threshold, we assume that the FES user is trying to flex their elbow (e.g. bring their hand towards their mouth), so the triceps and wrist extensors are turned off.

The patient was a 56-year old male, 19 months post injury (C4 Frankel A with some denervation at C8, but stimulatable C5,6 & 7). We used an Ottobock STIWELL surface stimulator and placed recording EMG electrodes on the biceps and anterior deltoid, and stimulating electrodes on the triceps and wrist extensors. With the algorithm described, the patient was able to successfully reach out, and bring his arm in at will.

In order to optimise this algorithm, we also implemented it in a computer simulation, using a musculoskeletal model of the upper limb[2]. At the start of the simulation, the deltoid activity is slowly increased while the biceps is off. When the deltoid reaches the threshold of 0.1 (10% of full activation), the triceps is stimulated and the elbow fully extends. Consequently, the biceps activity is slowly increased, and when it reaches a threshold of 5%, the triceps stimulation is turned off, and as a result the elbow flexes up to 100 degrees. When both deltoid and biceps are off, the triceps stimulation is also off.

Both the case study and our computer simulations show that the combination of EMG signals from voluntary muscles can be a useful control signal for functional, and not just therapeutic electrical stimulation. More detailed modelling will allow us to optimize the combination of EMG control signals, in order to maximize the benefit of FES for each individual.

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In vivo MRI tracking of MSCs labeled with Gadoteridol in a spinal cord injury experimental model

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Mesenchymal Stem Cells (MSCs) are multipotent stromal cells, able to deliver neurotrophic, anti- inflammatory and immunomodulatory molecules: for these reasons, their effects are exploited for the treatment of a multitude of pathologies, including neurodegenerative diseases and traumatic CNS lesions. In case of spinal cord injury (SCI), regrowth of severed axons is limited, but cell therapy can promote circuit repair, reorganization and axonal sprouting. Imaging-supported in vivo cell tracking provides a reliable method to assess the characteristics of cell grafts and to monitor the cell growth/movement after transplantation.

Here murine MSCs were labeled with the clinically approved MRI agent Gadoteridol through a procedure based on the hypo-osmotic shock (Hypo-MSCs), in order to be tracked in vivo in a murine model of SCI. Moreover in vitro and in vivo analysis have been performed to evaluate cell viability, proliferation and surface marker expression of MSCs.

Hypo-osmotic labeling did not alter the biological and functional profile of MSCs in vitro, but significantly increased the Gadoteridol cellular uptake, compared to iso-osmotic conditions. In vivo imaging on SCI mice revealed a substantial T1 contrast enhancement after transplantation of 300,000 labeled MSCs, enabling to circumscribe their spinal distribution and to follow their migratory dynamics for about 10 days. Histological validation of in vivo data corroborated the imaging results, highlighting the opportunity to perform a precise and reliable monitoring of the cell-based therapy. Finally, animals treated with labeled MSCs were monitored by behavioral tests and showed motor improvements, confirming the unaltered therapeutic efficacy of Hypo-MSCs.

Our results suggest that the presented cell labeling procedure is endowed with high efficacy and clinical translatability, and could possibly be beneficial for stem cell-based medical protocols required in tissue repair.

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SCIO - The Spinal Cord Injury Ontology, a prerequisite for automated data extraction from publications of preclinical research in spinal cord injury

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Background: Injury of the adult central nervous system of mammals results in lasting deficits like permanent motor and sensory impairments due to lack of profound neuronal regeneration. Preclinical research in the field of central nervous system trauma is advancing at a fast pace and yields over 8,000 new publications per year growing at an exponential rate, accumulating in a total number of approximately 150,000 PubMed-listed publications today. This large amount of unstructured textual information in scientific publications is not accessible to the individual researcher in a structured and searchable way. Moreover, manual information extraction of relevant experimental data, even from the relatively small field of preclinical research (compared to e.g. stroke) of spinal cord injury (SCI) with only approximately 40,000 publications is error prone. Textual descriptions of preclinical experiments are not standardized today and thus contain very heterogeneous vocabulary. Due to the exponential rise in publication numbers, such manually curated data would be outdated in a very short period of time. Therefore, an automated information extraction system is needed to extract relevant experimental data from scientific publications on SCI. For this purpose, an ontology for preclinical experiments on SCI (SCIO) was developed.
Methods: Descriptions and concepts related to experimental research on SCI in animal models were manually extracted by domain experts from 143 peer-reviewed publications. The typical process pipeline of the published scientific experiments was modeled and concept superclasses as well as their relations were designed. Subclasses and terms were defined for each superclass. Terms were aligned to appropriate terms in other related ontologies or vocabularies (e.g. MeSH terms). The building of the ontology was accompanied by a textual annotation to cover a substantial amount of actually used concepts.
Results: The SCIO was developed which consists of all relevant concepts for formalizing preclinical SCI experiments in animal models. The specific quality of the SCIO is a relational structure as opposed to a purely taxonomic representation of concepts as in the Neurological Disease Ontology (ND) ontology. Besides the representation of types of investigation methods, animal strains or experimental lesion types, an important feature of SCIO is the process modelling of the scientific experiment. Each single outcome of an experiment which is formalized as a “change” or “no change” of a certain parameter (e.g. locomotor function) between two experimental conditions (e.g. treated versus untreated) is linked inside SCIO to its specific experimental groups containing all information about the experimental setup and investigation methods. Thus, SCIO reflects the specific domain knowledge on how to read and process information from scientific publications on SCI. Moreover, a data granularity was selected which suits the domain needs for objective level-of-evidence estimation on therapeutic effects. SCIO can, therefore, be used to build an extraction model for automated information extraction from scientific publications and to design data processing pathways for enhanced meta-analyses on published data. The ontology will be made freely available.

Regulatable Chondroitinase ABC gene therapy as a treatment for spinal cord injury

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Following spinal cord injury the extracellular matrix undergoes significant remodeling. Scar formation is associated with upregulation of molecules known to be inhibitory to neural plasticity and recovery of function, including chondroitin sulphate proteoglycans (CSPGs). Enzymatic removal of CSPG glycosaminoglycan chains by the bacterial protein Chondroitinase ABC (ChABC) renders the matrix more permissive to recovery, however this is curtailed by rapidly diminishing enzyme activity. We have previously demonstrated that gene therapy using a modified ChABC gene compatible with expression and secretion by mammalian host cells confers sustained and long-term delivery of ChABC to the injured spinal cord following a single administration. This treatment resulted in dramatic reduction in pathology and significant improvements in functional recovery following clinically relevant spinal contusion injury at both thoracic and cervical levels in adult rats. We now use novel immune-evasive vectors to enable regulatable gene therapy to exert greater control over ChABC expression, where ability to switch off delivery of ChABC greatly improves safety of the treatment. Using this system, doxycycline administration results in high expression of the ChABC gene and extensive functional enzymatic removal of inhibitory components present in the extracellular matrix. We also show this is accompanied by pro-reparative changes in inflammatory markers. We are currently utilising this system to manipulate timing and duration of ChABC delivery to adult rats which have received a clinically-relevant contusion injury to the cervical spinal cord and are investigating its efficacy in promoting functional recovery. This represents both an experimental tool to optimise and control ChABC delivery, to understand the role of timing in ChABC treatment, and a step towards clinical feasibility of ChABC gene therapy.

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Identification of intrinsic axon growth modulators in intact CNS neurons after injury

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Neurons in the adult central nervous system (CNS) are unable to regenerate after spinal cord injury (SCI) due to an inhibitory environment and a decreased intrinsic growth capacity. Modulating environmental inhibitors and their neuronal receptors such as Nogo Receptor 1 (NgR1) results in increased regeneration and sprouting of intact neurons spared by injury, which correlates with enhanced functional recovery. Cell intrinsic factors also increase regeneration and sprouting, but side effects and limited functional improvements suggest these factors do not activate endogenous sprouting mechanisms. We sought to identify the mechanisms underlying spontaneous sprouting of intact neurons after incomplete SCI. We completed a unilateral corticospinal tract (CST) lesion (pyramidotomy, PyX) in transgenic wild type (n=6) and NgR1 knockout mice (*ngr1-/-*, n=6) expressing GFP under the µ-crystallin (*crym*) promoter (*crym*- GFP) for intrinsic corticospinal tract (CST) labeling. Two weeks post-lesion, mice received infusion of the retrograde tracer fast blue (FB) into the denervated spinal cord to label sprouting CST neurons. Two weeks later, we used laser capture microdissection to isolate CST neurons in a quiescent (GFP+FB-) or active (GFP+FB+) growth state. With enhanced sprouting in *ngr1-/-* mice, an abundance of FB+ sprouting neurons allowed us to complete RNAseq and conduct a transcriptomic analysis. 1174 genes were significantly differentially expressed (SDE) between sprouting and quiescent neurons, with lysophosphatidic acid (LPA) receptor 1 (*lpar1*) the most downregulated gene in sprouting neurons. Lpar1 interactors, including a negative regulator of Lpar1, lipid phosphate phosphatase related protein 1 (*lppr1*), were also SDE in sprouting neurons, suggesting a role for the LPA pathway in regulating intrinsic CNS axon growth. Overexpressing Lppr1 in cortical neurons *in vitro* resulted in an increase in neurite outgrowth and an increase in growth in an *in vitro* injury model. Next we sought to determine if modulating the LPA pathway *in vivo* would enhance functional sprouting. Adult wild type mice received PyX or sham lesion and either cortical infusion of AAV-Lppr1 (n=21), oral treatment with an Lpar1 antagonist AM095 (n=15), or vehicle control (n=19). Lppr1-expressing and AM095-treated mice had significantly enhanced sprouting of CST neurons into the denervated ventral horn and AM095-treated mice recovered greater fore and hind limb function in a grid walking task. With these data, we have demonstrated that bidirectional modulation of the LPA pathway is beneficial for axon growth with therapeutic potential for restoring function after SCI.

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Unexpected extracellular matrix remodelling after spinal contusion injury in rats

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One of the central pathological characteristics of spinal cord injury (SCI) in mammals is the aggressive and maladaptive remodelling of the spinal extracellular matrix (ECM). Excluding the large chondroitin-sulphate proteoglycans (CSPGs) the composition of the ECM after SCI has not been studied extensively. Recently, we performed high-throughput proteomics analysis targeting the mature matrix that accumulates in the injury epicentre following spinal contusion in adult rats (**Didangelos et al, 2016**). Our work identified a unique set of matrix proteins, many shown for the first time in the spinal cord and with unknown function in the tissue. One interesting feature revealed in our study was the high relative abundance of type I collagen, the prototypical component of post-injury fibrosis in mammalian organs. The spinal matrix also contained numerous collagen-associated glycoproteins and minor collagens suggesting an organized fibrotic response following contusive SCI in rats. Here, we describe for the first time the unexpected accumulation of type I collagen in mature lesions following contusive SCI in rats and we characterize the expression of the unknown collagen-associated proteoglycan asporin.

Transcript levels of asporin and type I collagen were significantly increased 4 days after contusive SCI and were further increased 14 days after injury. mRNA returned to baseline 10 weeks post SCI. Moreover, primary rat meningeal fibroblasts and cortical astrocytes expressed and secreted asporin and type I collagen in culture. Next, itype I collagen and asporin were visualized for the first time in mature SCI lesions. Type I collagen formed a dense and disorganised meshwork that occupied the entire lesion core. Type I collagen delimited and bordered the lesion cavities. Strong staining was also seen in the pia and attached perispinal blood vessels. The collagen pattern in the lesion epicentre was distinctively complex combining disorganised bundles of fibers, normally seen in fibrotic organs, together with ring-like structures, characteristic of blood vessels. Type I collagen did not costain with asporin. The proteoglycan was clearly enriched in the dura and was upregulated in injured spinal cords with a dispersed puncta-like pattern. In the remodelled and hypertrophic dura, asporin appeared to form diffuse fibers running parallel to type I collagen-stained structures. In contrast to collagen, the glycoprotein was not able to accumulate beyond the meningeal layers inside the lesion core. The identification of type I collagen in mature rat spinal contusion lesions is interesting given that contusion injuries are not associated with classic fibrosis. To understand the origin of type I collagen we costained chronic rat lesions (12 weeks) with type I collagen and CD34, an endothelial marker of capillaries, neovessels and circulating endothelial progenitor cells that enhance angiogenesis during wound healing. In the lesion core, CD34 colocalized with type I collagen and showed a disorgansed capillary-like appearance indicative of vascular remodelling taking place in the injury site. Costaining of CD34 with CD45 revealed a clear distinction between the capillary staining of CD34 within the lesion core and the cellular staining of CD45 in macrophages decorating cavities, most of which were CD34-negative.

In conclusion, ECM remodelling and composition in the spinal cord after contusive SCI is much more complex then previously thought and unexpectedly type I collagen is highly abundant in contusive lesions. Type I collagen accumulation is likely the result of aberrant and disorganised neoangiogenesis taking place after SCI rather than the product of exogenous cell types such as macrophages, fibroblasts or fibrocytes. Our work highlights that future therapeutic approaches to SCI need to examine options to stabilize and organize the vascular supply to spinal lesions in order to reduce pathology and improve neuronal repair.

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ChinMotion: preserved sensorimotor pathways rapidly enable 3D computer interaction after tetraplegia

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Individuals with severe paralysis require hands-free interfaces to control assistive devices that can improve their quality of life. Current interfaces lack effective kinaesthetic feedback, preventing fast and accurate multidimensional prosthetic control. We developed an open-source interface (ChinMotion[1]) that noninvasively translates chin, lip and tongue motion into 3D control commands. After brief calibration, ChinMotion rapidly enabled individuals with high tetraplegia and age-matched uninjured controls with fast and accurate point-and-click function, considerably surpassing the performance of existing interfaces. Furthermore, within the same session, all participants were able to use ChinMotion to autonomously control a virtual robotic arm and successfully complete a 3D reach-and-hold task. This is the first demonstration to our knowledge of precise and intuitive 3D computer interaction by individuals with high tetraplegia. These results have significant implications in advancing affordable mainstream wearable technology to restore independent function for the majority of individuals with severe paralysis.

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A combinatorial approach to axon regeneration: Epac activation and biomaterials

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Spinal cord injury leads to major motor and sensory functional disability below the level of injury. The post injury environment is non-permissive for axonal regeneration due to extrinsic factors (e.g. the presence of glial scar, cystic cavities and axon growth-inhibitory molecules) and intrinsic factors (e.g. the decline of intracellular cAMP). Therefore, the development of a single strategy for spinal repair seems inadequate and a combinatorial approach is likely to be more effective. Increasing intracellular cAMP by pharmacological or genetic means partially restores the capacity for axon regeneration after spinal injury. Recently, Epac, a guanine nucleotide exchange factor for cAMP, has been implicated in neurite outgrowth and axon regeneration[1,2]. Two isoforms of Epac (Epac 1 and 2) have been identified with different expression patterns during neural development. The use of biomaterial scaffolds to support axon regeneration has achieved some success in peripheral nerve injury repair. A novel silk-based biomaterial, Spidrex®, has been shown to promote axon regeneration *in vivo* in a rat model of sciatic nerve injury[3] by providing contact guidance. Here, we used *in vitro* preparations to study the potential of combining Epac activation and Spidrex® to enhance axon regeneration.

Dissociated primary cortical and dorsal root ganglion neurons were cultured from postnatal Wistar Rats. Specific Epac 1 and 2 agonists and a general Epac antagonist were applied to cultures for 24 hours respectively. In addition, Epac inactivation was studied using siRNA knockdown. The growth of neurons in the presence of Spidrex® was examined by seeding cells on to Spidrex® silk fibres aligned in parallel on polylysine-coated glass coverslips. HCA-Vision and Neuron J software were used to quantify total neurite length, maximal neurite length and morphological classifications. Time-lapse live imaging was used to assess the dynamic interaction between cells and Spidrex®.

The results reported herein demonstrate that Epac 1 and Epac 2 agonists at 2 □M concentration significantly increased neurite outgrowth, while the Epac antagonist seemed to decrease neurite outgrowth. siRNA knockdown of Epac 1 and Epac 2 also significantly decreased neurite outgrowth. Moreover, Spidrex® fibres were capable of supporting excellent outgrowth of dorsal root ganglion and cortical neurons *in vitro.* Finally the application of FRET SE imaging in live neuronal growth cones whilst elongating upon Spidrex® silk fibres was established, providing a method to observe Epac protein dynamics at the neuron-Spidrex® interface. These preliminary results demonstrate the potential of combining Epac and Spidrex® for spinal repair and future studies will further investigate this approach using *in vitro* preparations with axon growth-inhibitory molecules and *in vitro* and *in vivo* spinal cord injury models.

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Overexpression of the fibroblast growth factor receptor 1 (FGFR1) in a model of spinal cord injury in rats

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Spinal cord injury is a severe condition that affects many people and results in high health care costs. Therefore, it is essential to find new targets for treatment. The fibroblast growth factor receptor 1 (FGFR1) signalling pathway has a history of being explored for spinal cord injury treatment. Several groups have examined the effect of high availability of different FGFR1 ligands at the injury site and reported corticospinal tract (CST) regeneration as well as improved motor functions. In this study, we investigated overexpression of the FGFR1 in rat corticospinal neurons *in vivo* after injury (unilateral pyramidotomy) and in cerebellar granule neurons (CGNs) *in vitro*. We show that overexpression of FGFR1 using AAV1 intracortical injections did not increase sprouting of the treated corticospinal tract and did not improve dexterity or walking in a rat model of spinal cord injury. Furthermore, we show that overexpression of FGFR1 *in vitro* resulted in decreased neurite outgrowth compared to control. Thus, our results suggest that the FGFR1 is not a suitable therapeutic target after spinal cord injury.

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Environmental enrichment induces a lasting increase in axon regeneration potential via activity-dependent epigenetic modifications

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Injury to the adult mammalian nervous system leads to permanent deficits in sensory and motor function. This is partly due to the inability of neurons to initiate an effective molecular regenerative response, resulting in failed axon regeneration. Sensory neurons in the dorsal root ganglia (DRG) are vital for physiological and post-injury sensorimotor function as they receive and convey sensory information from the environment to motor circuits in the spinal cord and brain. Here we show that exposing mice to environmental enrichment (EE) prior to an injury induces a long-lasting increase in the regenerative potential of DRG neurons. Specifically, EE stimulated an increase in sensory axon regeneration by enhancing neuronal activity and calcium signalling, leading to increased axonal transport and CBP-dependent histone acetylation, which were required to reprogram the DRG neurons for regeneration. Moreover, prior exposure to EE augmented sensory axon regeneration and functional improvements after spinal cord injury, which was further enhanced when combined with a conditioning injury and that was mimicked by pharmacological enhancement of CBP activity. Overall this work portrays EE as a physiological means of priming sensory neurons for functionally relevant axon regeneration after injury, which is elicited through increased neuronal activity leading to enduring epigenetic reprogramming.

Restoring upper limb function using neurophysiological rehabilitation

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The most common form of traumatic spinal cord injury observed in the clinical setting involves a contusive type injury occurring at the cervical level. Patients surveys have identified that improvements in upper limb function is a top priority for individuals that have suffered an injury such as this[1,2]. We have therefore carried out initial studies to develop and optimise a rehabilitation paradigm combining behavioural and electrophysiological techniques to repetitively activate key neural circuitry controlling upper limb function, with the aim of restoring useful function. Here we present data obtained using one such neurophysiological rehabilitation paradigm in a clinically relevant model of cervical contusion injury in rats. Adult rats were implanted with epidural bipolar electrodes over the forelimb motor cortex with an external connection fixed to the skull and one week later received a contusion injury of moderate severity (225 kdyne) at spinal level C5/6. Animals in the rehabilitation group began rehabilitation two weeks post-injury, this involved daily four hour sessions of sub-threshold, cortical stimulation (in awake, freely moving rats) followed by a one hour session of intensive physical rehabilitation targeted primarily at skilled forelimb function. All animals were functionally assessed using a variety of behavioural techniques on a weekly basis as well as undergoing terminal electrophysiological assessments at the end of the study. We find that, compared to animals undergoing no rehabilitation, this combinatorial rehabilitation paradigm leads to significantly improved function in various aspects of forelimb function when assessed using behavioural techniques. Additionally, this repetitive activation of forelimb neural circuitry results in enhanced activity in numerous forelimb muscles as well as the radial nerve following stimulation of the forelimb motor cortex. These functional improvements were associated with significant increases in the colocalisation of synaptic and motorneuron markers (VGlut2 and ChAT), suggesting enhanced plasticity as a result of rehabilitation. These initial findings are greatly promising, but now must be investigated further to determine the mechanisms underlying the observed improvements. More extensive studies must also now be carried out to further refine and optimise our neurophysiological rehabilitation paradigm.

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Perineuronal nets (PNNs) in the spinal cord show different molecular composition in comparison to the brain

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Perineuronal nets (PNNs) are dense extracellular matrix structures surrounding neuronal subpopulations throughout the central nervous system and are involved in the control of plasticity during development and after spinal cord injury (SCI)(1). PNNs are mainly composed of chondroitin sulphate proteoglycans (CSPGs) bound to a hyaluronan backbone. While the molecular composition of PNNs from various brain regions have been studied, much of the composition and associated neuronal populations in the spinal cord is yet unknown. A clear understanding of the molecular composition of PNNs will facilitate the manipulation of PNNs in promoting plasticity after SCI. Immunostaining for choline acetyltransferase (ChAT) with the "PNN marker" *Wisteria floribunda* agglutinin lectin (WFA) and for CSPGs, including aggrecan, was used to characterise the molecular heterogeneity of PNNs in neurones. In the cerebral cortex, WFA co-localises with 97% of CSPG-positive neurons (2). However in the spinal cord, WFA and aggrecan show less co-localisation (~50%), but they denote distinct sub-populations of ChAT-positive motoneurones. A similar pattern was shown with other CSPGs, including versican, phosphacan and brevican. In the ventral horn, distinct populations of ChAT-positive neurones are surrounded by CSPG- positive but WFA-negative PNNs. This indicates a difference in both the PNN composition and the targeted neuronal populations between the spinal cord and cortex. The molecular heterogeneity of PNNs displayed in spinal motoneurones may indicate a functional role. Insights into the role of PNNs in the spinal cord could aid functional recovery studies post-injury.

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Systematic analysis of the epigenetic events driving axonal regeneration

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Insight into the molecular events that drive axonal regeneration after spinal cord injury has revealed that the interplay and cross talk of multiple injury-induced signaling cascades, is key to activating long- term regenerative gene reprogramming. Given that after sciatic nerve injury, transcriptional control is regulated by specific epigenetic signatures and that these signatures drive long-term changes in gene expression, we hypothesized that a number of upstream injury-induced signals converge on the epigenome to determine whether axonal regeneration occurs or fails. Recently, it has been established that histone acetylation occurs on selected regenerative gene promoters during axonal regeneration after peripheral but not central injuries[1]. To explore both permissive or inhibitory histone marks that are related to gene transcription under regenerative vs non-regenerative conditions, we performed a systematic investigation, employing and integrating RNA- and ChIP-Seq profiling from the dorsal root ganglia after sciatic nerve vs dorsal column lesions.

Here we show for the first time that H3K27ac functions as a major transcriptional switch exclusively after sciatic nerve injury. Using protein-protein interaction predictions we unveil a core H3K27ac-dependent transcriptional network that drives the expression of a multitude of genes that enable axonal regeneration to occur. Central to this transcriptional core, we identify CITED2, an interacting transactivator and adaptor protein that recruits and binds H3K27ac-dependent transcription factors to the histone acetyl-transferases CBP and p300. We find that CITED2 may function as a convergence point for upstream injury-induced signalling and that it may be vital for regeneration through its ability to direct the composition of regenerative transcriptional complexes. Taking the above into account, we propose that the loss or “silencing” of CITED2 after spinal cord injury is a major contributing factor underlying central regenerative failure.

To recapitulate the H3K27ac-dependent regenerative programme after central spinal cord injury, we strived to find small pharmacological molecules that could target and drive the expression of CITED2. Using literature-mining tools we identify LBH589, a novel FDA-approved broad-spectrum HDAC inhibitor that has the potential to induce a 25-fold increase in CITED2 mRNA[2]. We find that LBH589 treatment enhances axonal outgrowth in dorsal root ganglion neurons and that these effects on outgrowth depend on CITED2 to drive the H3K27ac-dependent regenerative programme. Ultimately this work aims to prove that LBH589 can promote *in vivo* regeneration and functional recovery after central spinal cord injuries.

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Delayed intramuscular Neurotrophin-3 normalises abnormal spinal reflexes, reduces spasms and improves mobility after corticospinal tract injury

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CNS injury often causes spasticity and disability. We now show that bilateral transection of the corticospinal (CST) tracts in the pyramids causes flexor spasms in the forelimbs, hindlimbs and tail that are easily quantified from movies of freely moving rats in the open field. Fortnightly H-reflex testing confirmed that naïve rats show rate-dependent depression of a monosynaptic reflex to a flexor forelimb muscle whereas rats with bilateral pyramidotomy exhibit less rate-dependent depression (i.e., hyper-reflexia). Walking on a horizontal ladder with irregularly spaced rungs was impaired. Grip strength was slightly reduced. Polysynaptic reflexes between antagonist muscles were increased after CST injury, which may cause co-contraction. Injection of AAV1 encoding human prepro neurotrophin-3 (NT-3) unilaterally into forelimb flexor muscles reduced all these signs of spasticity (relative to AAV1-GFP). Intramuscular NT-3 progressively reduced flexor spasms in the open field and normalized proprioceptive monosynaptic H-reflexes to a flexor forelimb muscle. This is consistent with expression of TrkC receptors in proprioceptive muscle afferents. NT-3 also normalized polysynaptic reflexes to muscles supplied by the ulnar nerve but only those involving afferents from injected muscles (e.g., synergist flexor muscles but not antagonist muscles). NT-3 normalised the pattern of excitatory synapse-like boutons from primary afferents upon motor neurons. NT-3 also normalized the pattern of VGAT+ boutons on VGluT1+ afferents, indicating that pre-synaptic inhibition may have been restored. NT3 also normalized the level of the KCC2 ion transporter in motor neuron membranes. Finally, NT-3 was transported in afferents from injected muscles to the DRG. RNAseq of cervical DRG identified mRNAs and miRNAs whose levels were dysregulated by CST injury but were normalized by NT-3 treatment. These findings are exciting because (1) we administered NT-3 in a clinically relevant time frame and by a straightforward route. (2) Recombinant NT-3 is safe and well-tolerated in five Phase I and II clinical trials (unlike NGF). (3) The world's first gene therapy (Glybera) involves i.m. injection of an AAV1 encoding a different transgene, which paves the way for NT-3 as a therapy for CNS injury.

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AMPK as regenerative inhibitory signaling after nerve and spinal injury

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While axonal regeneration and partial functional recovery in the injured peripheral nervous system (PNS) occur, axonal regeneration fails in the central nervous system (CNS) such as after a spinal cord injury (SCI), strongly contributing to unsuccessful functional recovery. Lack of regeneration in the spinal cord can be partially enhanced by an injury to the peripheral branch (conditioning lesion) or by overexpression in DRG neurons of selected regeneration-associated genes. We hypothesize that key retrograde signaling following peripheral but not central axonal injury regulates pathways that control the regenerative phenotype. Therefore, we believe that the combined investigation of protein as well as gene expression changes in the "DRG-axonal signaling unit" after central versus peripheral nerve injury is critical to identify crucial regenerative pathways. We performed combined RNAseq from DRG and proteomics from sciatic axoplasm in mice following an equidistant sciatic or spinal cord axotomy to investigate differential molecular responses in the "DRG-axonal signaling unit". Integrated bioinformatics analysis of the RNAseq and proteomics data followed by axonal injury experimental approaches identified key regulatory metabolic mechanisms involving AMPK signaling in the control axonal regeneration. Conditional deletion of AMPK alpha1 but not alpha2 promotes significant axonal regeneration of sensory ascending DRG axons across an injured spinal cord. Both AMPK upstream and downstream pathways as well as the impact of AMPK deletion on neurological recovery are currently being investigated.

Electrical stimulation and targeted rehabilitation to restore upper limb function after spinal cord injury

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Spinal cord injuries can have a severe impact on a patient's life and restoring upper limb and hand function is one of the highest priorities for tetraplegic patients. The aim of this study is to develop a targeted neurorehabilitation programme designed to maximize and restore useful upper limb, hand and digit function. To achieve this, behaviorally trained Lister Hooded rats were implanted bilaterally in their forelimbs with electromyography (EMG) electrodes plus an epidural stimulation electrode over the dominant side of their motor cortex (based on left or right handedness during tasks). Pilot experiments were conducted to ensure successful cortical stimulation and recordings of EMG signals after electrode implantation. These showed that EMG signals can be successfully recorded and the implantation process does not affect the animals' performance in behavioral tasks. Furthermore, the cortical stimulation successfully produced motor evoked potentials. We then assessed the potential for using targeted neurorehabilitation to improve upper limb function in spinal contused rats, with the aim of determining the optimal combinatorial paradigm. Clinically relevant spinal contusion injuries were performed (using an Infinite Horizon impactor; 225kD at level C5- C6) and different combinations of the following treatments were applied: behavioral rehabilitation (intensive training on skilled forelimb tasks), neurophysiological rehabilitation (repeated electrical activation of pathways important in forelimb function) and intraspinal injections of lentiviral Chondroitinase ABC (to enhance neuroplasticity within the spinal cord). Rehabilitation and treatment were specifically focused on elbow extension, pronation, and digit dexterity. In an initial study, animals were assessed for two weeks post injury but no significant differences between treatment groups were observed at this early time period. However, longer term studies are currently underway, with animals undergoing a targeted neurorehabilitation paradigm over a period of 10 weeks post injury. We aim to determine the extent of recovery over a chronic time course of targeted neurorehabilitation and to establish whether different treatment combinations will reveal different levels of recovery. This work should provide essential information on the temporal effects of these treatments, and identify key time points during recovery and rehabilitation, and ultimately may lead to the fine tuning of treatment paradigms for achieving optimal recovery of upper limb function after spinal cord injury.

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Developing ultra-high resolution 3D synchrotron radiation tomography for imaging the contused rat spinal cord

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Classical histology requires mechanical sectioning and collection of tissue followed by staining or immunohistochemistry. Tissue can be inaccurately represented by sections lost or damaged during mechanical processing, while tissue staining can be frustrated by contaminated and non-specific reagents. Mechanical disruption issues can be circumvented with 3D imaging and sectioning *in silico* by various techniques, but we were keen to develop a non-destructive methodology to image *ex vivo* tissues already destined for tissue processing to maximize information gained from valuable samples. To this end, we developed an x-ray computed tomography methodology to image paraffin embedded *ex vivo* spinal cords from adult rats including naïve uninjured tissue or following moderate severity contusion injury at the cervical level using the IH impactor device.

X-ray computed tomography traditionally relies on detecting differential absorption of x-rays passing through an object. Dense features like bone or tumours are more opaque to x-rays and appear darker than surrounding tissue. Subtler differences like those found within soft tissues are not detected by this method as they do not block the passage of x-rays to differing degrees. However, x-rays can be attenuated or 'slowed' as they pass through a material and by exploiting this feature tissue contrast can be drastically enhanced and soft tissue features visualized by phase contrast imaging.

Using the tomography beamline i13-2 at the Diamond Light Synchrotron facility, we have developed a protocol which can highlight white and gray matter within the spinal cord along with the extent and size of tissue damage and injury spread after spinal cord contusion injury. Scans through a 30 mm length of spinal cord take ~40 minutes and the combination of an effective pixel size of ~1.6 µm with phase- contrast and an *ex vivo* stain mean the internal spinal cord vasculature can be discerned at the capillary level along with large motor neurons and axonal projections in the white matter. Notably, following imaging the paraffin embedded spinal tissue remains viable for subsequent histological processing meaning additional animal experiments do not need to be performed for 3D information, with the associated time, cost and ethical benefits.

With a now established methodology for ultra-high resolution 3D synchrotron radiation tomography imaging of the adult rat spinal cord, future work will include analysis of the physical development of the contusion model of spinal cord injury over acute and chronic time-points, as well as imaging other nervous system tissue such as dorsal root ganglia, spinal nerves and peripheral nerves.

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The potential of novel silk-based biomaterial in combination with growth promoting cues to promote central nervous system axonal regeneration

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INTRODUCTION: The mammalian central nervous system (CNS) is poor at spontaneous repair following spinal cord injury (SCI) and there is currently no cure available. An ideal treatment would be a tissue engineered scaffold to support neurites growth in combination with a drug to stimulate their regeneration and guidance cue to direct neurites growth across the lesion. A silk-based biomaterial called Spidrex® has been shown to support excellent axonal regeneration in a peripheral nerve injury model[1]. The omega-3 polyunsaturated docosahexaenoic acid (DHA) is essential for neurodevelopment and has been shown to promote neurite outgrowth of rat DRG neurons[2]. However, the potential of Spidrex® or DHA or a combination of both for central nervous system (CNS) axonal regeneration has not yet been investigated. **METHODS:** Tensile tests were conducted by mounting the specimens on cardboard frames and using a Zwick testing machine. For in vitro degradation tests Spidrex® fibres were kept a 70 °C for 0, 20, 40 and 60 days and tensile properties tested in the same way. Dissociated CNS neurons were cultured from *Xenopus Laevis* embryos and from cerebral cortex of postnatal (P1) Wistar Rats. Neurons were seeded on to Spidrex® silk fibres aligned in parallel. DHA at 0.8, 4, 8, and 32 µM was added to cortical neuronal cultures for 48h. The optimal concentrations of DHA were then applied to cortical neurons seeded on silk fibres for 48h, during which the interaction of neurites with silk fibres was observed with time-lapse microscopy followed by immunocytochemistry. The host immune response was tested by exposing microglia cells, isolated from the cortex of postnatal (P3-6) Wistar rats, to Spidrex® for 48h or LPS for 24h and by *in vivo* implantation up to 5 months. Expression of iNOS inflammatory marker and levels of nitrite release were determined using immunocytochemistry and Griess assays respectively.

RESULTS: Tensile tests results showed that Spidrex® fibres bundle has a tensile elasticity as low as fresh rat spinal cord. Moreover, Spidrex® silk can degrade *in vitro* as showed by a significant decrease in mechanical strength.We showed that Spidrex® silk fibres support excellent outgrowth of CNS neurons. Particularly, there was a significant proportion of Xenopus and rat cortical neurons engaging with the silk. We demonstrated that one of the key features of Spidrex® is the presence of numerous repeated sequences of arginine-glycine-aspartic acid (RGD) facilitating cell attachment to the material by integrins. Omega-3 DHA promoted neurite outgrowth of rat cortical neurons in a concentration-dependent manner. Furthermore, rat cortical neurons with 32 µM DHA in combination with Spidrex® silk fibres significantly increased total neurite length/neuron when compared to either the biomaterial or DHA alone. We showed minimal microglial activation, *in vitro* and *in vivo*, with levels of iNOS and nitrite release similar to controls and significantly lower when compared to LPS treated cells.

CONCLUSIONS: Spidrex® silk supports neurite outgrowth of CNS neurons, this is further enhanced with the combination of the omega-3 DHA fatty acid. Future work will explore the potential of applying electric field to guide and further enhance neurite growth along the biomaterial as well as testing this combinatorial strategy in the presence of a growth inhibitory molecule.

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EEG predictors and markers of central neuropathic pain in sub-acute spinal cord injury

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Central Neuropathic Pain (CNP) affects approximately 40% of patients with Spinal Cord Injury (SCI).[1] CNP is related to changes in cortical activity, in particular in the area of the primary motor cortex, controlling movements. Compared to patients with no CNP, SCI patients with long-standing CNP have the overactive cortex while they imagine to move either painful or non-painful limbs.[2,3] Most notably these patients have strong electroencephalographic (EEG) activity in the theta band, otherwise not seen in SCI patients with no pain.[2] While relation between EEG and CNP has been confirmed, it is still not known whether changes in the cortical activity are a cause or a consequence of CNP.

Thirty sub-acute SCI patients (24 M, 6 F; age: 45 ±15.2), within 6 months post-injury, free of nociceptive pain, level of injury C4-T12, incomplete or complete, participated in this study. They were asked to imagine tapping with both legs while their brain activity was recorded with 48 channel EEG device (usbamp. Guger technologies, Austria). They performed 60 repetition of imagined tapping, every time they saw a cue on a computer screen. EEG analysis was performed on signal averaged over all 60 repeated trials. EEG artifacts were removed prior to further analysis and EEG was re-referenced to a common average reference. Time frequency analysis (event-related spectral perturbation) was performed to define EEG responses to imagined movements.

Prior to EEG analysis patients were divided in three groups. Group I (11 patients) had early CNP symptoms at the time of EEG recording and most of them received pharmacological treatments. Group 2 (9 patients) had no pain at the time of EEG recording but developed pain within 6 months following EEG recording. Group 3 (10 patients) did not have pain at the time of EEG recording and did not develop pain within next 6 months.

Group 2 that developed pain within 6 months after EEG recording had the strongest EEG activity in the alpha (8-12 Hz) and in the beta band (16-24 Hz), On the contrary, group 1 with CNP at the time of EEG recording, had weakest alpha and beta band activity but had strongest theta band activity not present in the other two groups. Differences in EEG activity between groups were most evident in the centro-parietal areas of the cortex.

We hypothesize that pain related changes in EEG activity have two phases. The first phase, stronger alpha and beta band activity precedes the physical symptoms of pain. These EEG markers could be used to identify patients at the high risk of developing CNP and for creating pharmacological and non- pharmacological preventive treatments. Second phase, shifting of EEG activity towards lower, theta band, occurs within few months following the physical symptoms of pain. These theta band markers of CNP are similar to EEG markers seen in SCI patients with long-standing CNP. [2]

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Degron peptide mediated inhibition of PTEN as a non-genetic approach for mTOR activation after SCI

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Robust CNS axonal regeneration in the optic nerve has been observed following deletion of the genes encoding PTEN and SOCS3, two endogenous inhibitors of intrinsic neuronal growth capacity. However, translation to humans requires alternate approaches that do not depend on transgenic gene deletion. To promote regeneration and recovery following SCI, we have here designed cell-membrane permeable, TAT-coupled peptides carrying protein-binding domains to either PTEN or SOCS3, coupled to a proteasomal degradation peptide (coined degron peptides) targeting PTEN or SOC3 for degradation. This TAT-peptide approach could be clinically translatable and has been used with promising results in a clinical trial on stroke patients with 92 subjects, evaluating neuroprotection using a PSD-95 interacting peptide termed NA-1. A phase 3 trial has recently been initiated for stroke in Canada (Frontier Trial).

Our western blot data revealed successful degradation of PTEN by our peptides in vitro using cultures of cortical neurons. This degradation was inhibited by the proteasome inhibitor MG132. In vitro, PTEN targeting peptides as well as Tat-SOCS3 degrons increased neurite length of dissociated cortical neurons and DRG neurons analysed 24-72 hours after plating. Injection of PTEN-degron into the sensorimotor cortex of mice produced a decrease in PTEN and an increase in phospho-S6 immuno-reactivity; the latter indicates increased mTOR pathway activity. Similarly, the Tat-SOSC3 degron reduced SOC3 immunostaining in the cortex and an increased the pSTAT3 staining. This provided the rationale to apply the most effective of our PTEN-Degrons (based on our in vitro studies) for 14 days into the sensorimotor cortex of mice that underwent a crush of the dorsal column. This was followed by injecting to different axonal tracers (dextranes amines) into the left and right cortex (4x 0.4ul) at week 6 and perfusion at week 8 post injury. The analysis of this study is still ongoing and will be presented.

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Transplantation of excitatory neural precursor cells to promote respiratory recovery after cervical spinal cord injury

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The majority of spinal cord injuries occur at the cervical level resulting in persistent, life-threatening respiratory deficits. This can be attributed in large part to the direct compromise of the phrenic motor circuitry that controls the diaphragm – the primary respiratory muscle. Despite this devastating outcome, experimental and clinical studies have demonstrated an intrinsic capacity of the injured spinal cord to exhibit limited spontaneous functional recovery. A key contributor to this plasticity are spinal interneurons, which undergo axonal sprouting, promoting the formation of novel functional neuronal relays. With a primary focus on these interneurons, the present study tests whether transplantation of spinal neural precursor cells enriched with excitatory interneurons can contribute to anatomical repair and improve phrenic motor recovery. Previous work has shown that therapeutic efficacy may be greater if transplanted interneuronal precursors are derived from the ventral spinal cord (White et al. 2010*). Additional studies by our research team have revealed that ventrally derived Chx10-positive (putative-V2a) spinal interneurons become synaptically integrated with the phrenic motor system weeks following high cervical injury. Building upon these results, the goal of the present work was to assess the therapeutic benefit of transplanting Chx10 interneuronal precursor cell into the injured phrenic motor circuit.

Neuronal and glial restricted progenitor cells derived from developing spinal cord tissues were enriched with stem cell derived Chx10-driven excitatory interneurons and transplanted into a cervical (C3-4) spinal cord contusion injury in adult rodents. Anatomical connectivity of grafted cells was assessed using a retrograde, transynaptic tracing technique while the functional contribution of grafted cells was analyzed with terminal bilateral diaphragm electromyograms. Anatomical analysis revealed donor cell survival, differentiation and integration with the injured host phrenic circuitry. Functional diaphragm electromyography demonstrated altered patterns of activity one month following treatment in transplant recipients. These ongoing studies not only test the efficacy of a promising therapeutic strategy, but also offer insight into the neuronal phenotypes that can be effective for neural transplantation into the injured nervous system.

* White et al. (2010). *Experimental Neurology*, 225(1):231

Neuregulin-1 signalling controls an endogenous repair mechanism after spinal cord injury

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The injured spinal cord maintains some capacity for spontaneous repair, although this is suboptimal. Understanding the cellular and molecular mechanisms underlying endogenous repair may provide a route to exploit and enhance these processes in order to improve functional outcome after spinal cord injury (SCI). We have identified neuregulin-1 (Nrg1) to be essential for Schwann cell-mediated spontaneous remyelination of injured spinal axons within the dorsal columns and to be a significant contributor to spontaneous locomotor recovery. We found that Nrg1 ablation in adult mice leads to complete failure of Schwann cell-mediated remyelination after contusive SCI. The type III isoform appears to be critical for this process, while other Nrg1 isoforms regulate different repair mechanisms. Importantly, we found that conditional Nrg1 ablation leads to chronic demyelination and conduction failure in dorsal column axons and worse functional outcome in mice with clinically relevant spinal contusion injuries. Finally, although some remyelinating Schwann cells are likely to be infiltrating the injured spinal cord from the periphery, we have evidence that at least a large proportion of centrally remyelinating Schwann cells are derived from precursor cells present in the spinal cord and that Nrg1 serves as a molecular switch that influences the differentiation fate of centrally derived precursor cells. Through a genetic fate mapping approach that assesses both the infiltrating and the de novo-produced central Schwann cell lineages, we show direct evidence that ErbB receptor activation on oligodendrocyte precursor cells is required for their transformation into remyelinating Schwann cells after SCI. Moreover, we found that specific ablation of ErbB receptors on these central precursor cells in contused mice not only prevents a large part of Schwann cell-mediated remyelination, but also worsens spontaneous locomotor recovery, further highlighting the significance of this spontaneous repair response. Our data provide novel mechanistic insight into endogenous regenerative processes after SCI. These findings could lead to the design and development of combinations of effective and safe target-specific therapies for improving spontaneous repair and functional recovery after SCI.

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