4 - 5 September 2015

The 17th Spinal Research Network Meeting

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

POSTER PRESENTATIONS
Poster session is scheduled from 7pm at the end of the first day, immediately after the main meeting, on Friday, 4th September. The posters are also available to view during the coffee and lunch breaks on Friday and Saturday.

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Neurogenic immune deficiency after spinal cord injury: mechanisms of action and therapeutic opportunities

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Most who suffer a traumatic spinal cord injury (SCI) above spinal level T5 develop autonomic dysreflexia (AD), a pathological condition characterized by severe episodic paroxysmal hypertension. Left untreated, AD can cause pulmonary embolism, stroke or even death. Data from our lab indicate that maladaptive plasticity in the spinal cord circuitry responsible for causing AD also causes chronic immune suppression. In SCI mice, the onset and frequency of AD correlates with the magnitude of immune suppression. We predicted that as large segments of spinal cord lose supraspinal input, the periodic activation of viscera-sympathetic reflexes (e.g., due to bladder/bowel filling) will cause uncontrolled activation of sympathetic motor neurons with heightened release of catecholamines and glucocorticoids (GCs) into blood and lymphoid tissues. Data indicate that GC and catecholamine-dependent signaling synergize to elicit apoptosis in leukocytes and that remaining immune cells are functionally impaired. Retrograde trans-synaptic labeling from the spleen of SCI mice reveals the formation of new and complex intraspinal circuitry, presumably due to ongoing plasticity and synaptogenesis between primary sensory afferents, interneurons and sympathetic preganglionic neurons. Moreover, the receptive field for activating this new circuitry expands beyond the thoracic spinal segment that controls secondary lymphoid tissues in naïve/uninjured mice. Thus, after SCI, an uncontrolled “supercharged” autonomic circuit develops that recapitulates convulsive neuropathology (“autonomic spinal epilepsy”) and causes immune suppression. New preliminary data indicate that this aberrant circuitry can be “silenced” and immune cell ablation reversed by injecting inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) into the spinal cord. Neurogenic immune ablation may explain why people with high-level SCI are more susceptible to infection – a leading cause of morbidity and mortality in this patient population. Overcoming this deficit will reduce mortality, significantly improve quality of life and also recovery of neurological function after SCI.

References:


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There is a more profound increase in the recruitment of potentially damaging populations of leukocytes, and neutrophils in particular, to the spinal cord than to the brain after equivalent injuries. The microinjection of cytokines, such as IL-1 or CXCL1, into the spinal cord results in the recruitment of large numbers of neutrophils, which damage the blood-spinal cord barrier and cause axonal injury. In the absence of circulating neutrophils, the injection of these cytokines has very little impact on spinal cord integrity. Factors, such as peripheral injury or infection, that give rise to an increase in the number of circulating neutrophils will increase the number of neutrophils recruited to the brain after an injury. However, the pattern is more complicated for spinal cord injury. Here, in a counterintuitive manner, the injection of bacterial cell wall products (endotoxin) into the periphery can reduce the number of neutrophils recruited to the cord and reduce damage. It is clear that the orientation of the response to peripheral challenges, be it a pro- or anti-inflammatory effect, appears to be dependent on the nature and timing of events. A better understanding of the interactive pathways from the injured cord to immune cell populations in distant organs is required in order to take advantage of the potential benefits of immune-modulatory therapy after SCI.
Spinal cord injury (SCI) acutely triggers dramatic inflammatory changes throughout the body. These inflammatory changes include robust activation of the innate immune complement system, along with the mobilisation and recruitment of peripheral immune cells to the damaged spinal cord. These early SCI-induced inflammatory events are thought to contribute to secondary pathology and thus represent targets for therapeutic intervention. The first part of this talk will discuss acute peripheral immune activation following SCI, focusing on the effects of complement components C3a and C5a. In particular, the roles of C3a and C5a in immune cell mobilisation and recruitment will be highlighted (Brennan et al., 2015; unpublished observations). The second part of this talk will follow on from our previous findings that surgical removal of the spleen dramatically reduces macrophage presence at the lesion site during the sub-acute phase of SCI. This phenotype ultimately coincided with improved behavioural and histological outcomes (Blomster et al., 2013). The mechanism(s) via which the spleen influences macrophage presence at the site of SCI have remained largely unclear. I will discuss some of our most recent work focused on understanding the central role of the spleen in regulating monocyte recruitment to the injured spinal cord.

References:

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An exercise challenge reveals severe CV dysregulation acutely after T3 or T10 contusion injuries

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Clinically, cervical and high-thoracic spinal cord injuries (SCI) result in altered autonomic control of the heart and vasculature, which negatively influences cardiovascular (CV) end-organ structure and function. Descending control of sympathetic preganglionic neurons is reduced, while parasympathetic circuitry is left largely intact. This imbalance is thought to result in severe CV dysfunction including resting and orthostatic hypotension and autonomic dysreflexia (AD). Basic research into CV function after SCI, and specifically AD, has been hampered by the lack of rodent models of incomplete injury that exhibit robust dysfunction similar to that seen in the clinic, which persist into the chronic phase. Our lab has been studying locomotor function after SCI for many years and following several failed attempts to bring about frank improvements in overground locomotion using activity-based rehabilitation, we, along with several others, hypothesized that incompletely injured rats retrain themselves by walking around in their cages (Fouad et al., 2000; Kuerzi et al., 2010; Starkey et al., 2014). We propose that this reasoning might also explain why rats show few robust indicators of CV dysfunction after incomplete SCI.

For these studies, we used female SD rats with T2, T3 or T10, moderate or severe contusions. Assessment of hemodynamic control was performed using implantable telemetry devices (Data Sciences International). In separate animals, high-resolution echocardiography (Visualsonics) was used to evaluate cardiac and vascular structure and function. Various exercise protocols, including swimming and stepping in shallow water, were implemented to assess how the CV system maintains blood pressure control during instances of increased cardiopulmonary demand (Magnuson et al., 2009; Kuerzi et al., 2010). Dobutamine (a beta-1 agonist) stress echocardiography was used to challenge cardiac function independent of exercise (Cove et al., 1995). In both T3 and T10 moderately contused animals, an exercise challenge revealed profound hemodynamic instability acutely that resolved by 5 weeks post-SCI. Cardiac output and stroke volume were also not different from pre-injury by 6 weeks post-SCI. However, in animals with severe T2 contusions, cardiac output and stroke volume were significantly decreased at 6 weeks post-injury (under isoflurane anesthesia) and they responded robustly to Dobutamine, reaching baseline levels at doses of 10 to 20µg/min.

Overall, our results suggest that rats with incomplete SCI that recover partially weight supported stepping also recover near normal CV control despite significant disruption of spinal sympathetic circuitry. In turn, this suggests that acute post-injury spontaneous in-cage activity sufficiently engages autonomic circuitry to prevent or reduce the severity of long-term dysfunction. These results lead to the hypothesis that models of SCI that incorporate reduced post-injury activity will result in more severe and persistent CV and autonomic dysfunction including spontaneous and induced AD.

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Magnuson DS, Smith RR, Brown EH, Enzmann G, Angel C, Quesada PM, Burke D (2009) Swimming as a model of task-specific locomotor retraining... Neurorehabil Neural Repair 23:535-545

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Chronic oligodendrocyte genesis and remyelination after spinal cord injury

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Oligodendrocyte loss and demyelination are common features of the acutely injured spinal cord. Several studies have shown that progenitor cells present within the adult CNS undergo robust proliferation in the first two weeks after spinal cord injury (SCI) and a portion of these cells differentiates into new myelinating oligodendrocytes. In this study, we used retroviral tracing and transgenic reporter mice to determine for how long these spontaneous endogenous reparative responses last. Surprisingly, oligodendrogenesis continued for at least 3 months post-injury in gray and white matter and newly derived myelin was formed at all times examined around axons bordering the lesions and in distal white matter closer to the pia. This long-lasting response was accompanied by chronically elevated FGF-2 and CNTF expression, which may have acted directly on NG2+ progenitor cells as they expressed pSTAT3, a downstream signaling molecule after CNTF activation, for at least 5 weeks post-injury. While new oligodendrocytes were detected at every time examined, their numbers declined with time, which corresponded to a slow rise in transcripts for inhibitors of oligodendrocyte differentiation including Id2, Id4 and BMP4. Collectively, this work reveals a surprisingly long-lasting reparative response of the oligodendrocyte lineage cells to SCI, and also suggests that the lesioned tissue remains in a highly dynamic state for several months post-injury. Ongoing studies are examining potential triggers of post-SCI progenitor proliferation and differentiation into new oligodendrocytes, such as TLR4 signaling. Overall, these results are encouraging as they indicate spinal tissue may still be responsive to therapeutic strategies for several months. Additionally, efforts should be exerted to understand the mechanisms controlling the endogenous repair such that it is not inadvertently disrupted.

References:

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Mechanisms of myelin repair and intermodal plasticity

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Spinal cord injury results in the damage of axonal and glial elements that degenerate over time. While remyelination has been a long-sought therapeutic target, limited attention has focused on improving spared or regenerated myelin. Recent work has demonstrated that chronic demyelination of spared axons is rare. Demyelinated central axons that do not become remyelinated tend to degenerate over time in a similar process observed for chronic peripheral nerve demyelination. Nevertheless, patients undergo loss of axonal conduction acutely that is only partially restored over time. Chronic injuries are characterized by slowed conduction, metabolic vulnerability and increased sensitivity to axonal fatigue. Recent anatomical work has shown that decreased conduction velocities could be a result of specific myelin parameters. For example, myelin regeneration is typically thinner and composed of shorter myelin sheaths than what is observed in myelin generated during normal development.

While there is a great deal known regarding the development of glial progenitor cells, gene regulatory networks and growth-associated pathways that control myelin differentiation are largely unknown. Recent data indicates adult myelin may exhibit plasticity and the capacity to adapt to changes in neural activity. It may be that clinical improvement in people with chronic spinal cord injury may result from augmenting the process of myelin repair by improving the number of myelin wraps or increasing myelin sheath length. Optimization of myelin characteristics in both injury and aging is a focus of our laboratory. This presentation will review the morphological and computational evidence for myelin deficits after injury that may be amenable to therapeutic approaches.

References:
Using zebrafish to study myelinated axon formation, function and repair in vivo

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The myelination of central nervous system (CNS) axons by oligodendrocytes is essential for rapid, energy efficient nerve impulse conduction, and for long-term axonal health. In recent years it has also become apparent that myelin is not a static insulator, but a dynamic structure that can be remodeled throughout life. New myelin made by newly differentiating oligodendrocytes also forms through life, and the abundance of oligodendrocyte precursor cells in the adult CNS provide the basis for the regenerative capacity of myelin (remyelination) that is seen in disease or following injuries such as SCI. Despite the importance of myelin for CNS formation, function and repair, we have a relatively limited understanding of the molecular mechanisms that underpin its construction, modulation, and regeneration in vivo. My group use zebrafish as a model organism to study mechanisms of myelinated axon biology in vivo. Zebrafish have properties that have made them a prominent vertebrate laboratory model for live cell imaging, gene discovery, and chemical biology/ drug discovery. In this presentation I will provide an overview of our work using this model, where we currently focus our studies on the biology of myelinated axons in the larval spinal cord. I will describe our ongoing work implicating neuronal activity in the regulation of myelination and neural circuit function. I will give an update on our gene and drug discovery screens that are identifying molecular mechanisms of importance in axonal growth, axonal survival and oligodendrocyte development and myelination. I also briefly introduce a transgenic demyelination model in the zebrafish spinal cord, and our plans to carry out high-throughput drug discovery screens of relevance to CNS repair in zebrafish. I hope that this work will be of interest to the SCI field.

References:
Czopka, T, ffrench-Constant C, and Lyons DA. Individual oligodendrocytes have only a few hours in which to generate new myelin sheaths in vivo. Developmental Cell (2013) 25(6):599-609

Supported by
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Neuro-monitoring for patients with acute severe spinal cord injury

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The management of acute severe traumatic spinal cord injury (TSCI) is variable [1]. The role of bony decompression and the optimal levels of mean arterial pressure (MAP) and arterial CO2 for reducing secondary cord damage are unknown. We recently developed a technique to monitor intraspinal pressure (ISP) after TSCI, by placing a pressure probe subdurally at the injury site [2], analogous to monitoring intracranial pressure (ICP) after traumatic brain injury (TBI). We compute the spinal cord perfusion pressure (SCPP = MAP – ISP). ISP waveforms are similar to ICP waveforms with three peaks and comparable Fast Fourier Transforms [3]. At the level of injury, subdural and intraparenchymal ISPs are the same [4]. Our ISP recordings have yielded several clinically important findings:

1) After TSCI, three intrathecal compartments form: above injury, injury site with spinal cord compressed against dura, below injury [4, 5]. Each compartment has a different pressure profile, with the highest pressure at the injury site when the patient lies horizontally. This suggests that draining cerebrospinal fluid using a lumbar catheter may not reduce ISP at the injury site.

2) ISP is not reduced by reducing arterial CO2, administering mannitol or changing the dose of sevoflurane [1]. Administering inotropes, such as noradrenaline or metaraminol, increases SCPP. 3) Increasing SCPP improves the limb motor score and sensory level in some TSCI patients [1]. 4) We define the spinal vascular pressure reactivity index (sPRx) as the running correlation coefficient between ISP and MAP [2, 3]. sPRx < 0 means intact reactivity and sPRx > 0 impaired reactivity. Optimum SCPP (SCPPopt) is the SCPP that minimizes sPRx. A plot of sPRx vs. SCPP is U-shaped, which suggests that spinal cord hypoperfusion and hyperperfusion are detrimental.

5) In general, SCPPopt is ~90 mmHg, but SCPPopt varies between patients [2, 6]. Hence, universal blood pressure targets are inadequate. We propose individualized treatment to achieve SCPPopt.

6) In TSCI patients who had laminectomy, compression of the wound is transmitted to the damaged spinal cord causing increased ISP, reduced SCPP and potentially further spinal cord damage [2, 6]. We thus suggest that TSCI patients are nursed on their side or supine using a ring pillow.

7) Bony decompression does not effectively reduce ISP because the swollen spinal cord remains compressed against the dura [2]. We reported that laminectomy + expansion duroplasty is required to effectively decompress the injured cord [6]. Compared with laminectomy, laminectomy + expansion duroplasty significantly reduces ISP, increases SCPP and improves sPRx. Data from 36 TSCI patients show that ISP monitoring is safe. There were no probe-related complications such as spinal cord damage, persistent cerebrospinal fluid leak, meningitis, wound infection, probe-related haematoma, deteriorating ASIA score or ascending motor/sensory level. Recently, we started microdialysis monitoring from the injury site. Our data suggest that SCPP < 70 mmHg causes spinal cord ischaemia (high lactate:glucose ratio, high glutamate) and that SCPP < 50 mmHg causes cell lysis (high glycerol). I will conclude by suggesting that ISP monitoring for TSCI should be introduced as standard of care, in the same way that ICP monitoring is routinely used to guide management of TBI.

References:


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Each year thousands of new spinal cord injuries occur that lead to long term deficits and serious quality of life issues. Although a large number of preclinical studies have been conducted to test therapeutic interventions, to date no proven therapeutic modality exist that has demonstrated a positive effect in neurological outcome. This fact emphasizes the need for continuous research in the pathophysiology and treatment of this serious clinical problem. Recent publications have provided encouraging findings for therapeutic agents that target neuroinflammation, free radical damage, excitotoxicity and ionic changes. Indeed, several treatment studies have reported improvements in sensory and motor function that indicate the translational potential for treating clinical SCI. Riluzole, a sodium-channel blocker has been tested in a phase 1/2a clinical trial with encouraging safety and neurological outcome data. Cethrin, that blocks Rho A, was tested after SCI with an application applied to the exposed dura. This safety trial showed promise and further clinical trials to test efficacy are planned. A minocycline study in acute SCI patients is also being conducted based on encouraging preclinical data. In addition to these strategies, a novel anti-inflammatory treatment that specifically targets the innate immune response and abnormal inflammasome activation is being developed to target SCI. Therapeutic hypothermia has a history of being used to treat SCI. Multiple laboratories have reported the benefits of moderate hypothermia and described temperature-dependent effects on multiple pathomechanisms. Clinical studies have now evaluated the use of systemic hypothermia in severely injured cervical SCI patients with encouraging safety and outcome results. A multicenter clinical trial for the use of acute therapeutic hypothermia in SCI has been proposed. Together with optimal medical support, early surgical interventions, and targeted temperature management strategies, the testing of novel pharmacological agents should lead to better outcomes for our patients.

References:
Translational biomarkers for acute spinal cord injury

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An inherent limitation of clinical trials is their singular reliance upon standardized functional measures of neurologic impairment for patient enrollment, stratification, and treatment assessment. The high variability in these functional outcome measures forces researchers to enroll large numbers of patients into trials and limits the ability to determine treatment efficacy in critical early-stage clinical trials. In many acute SCI patients, such measures are impossible to even perform due to concomitant injuries, thereby making such patients ineligible for clinical trials (and further slowing recruitment). Clinical trials in acute SCI are therefore extremely slow and prone to failure, as history has sadly shown.

We and others contend that biomarkers will play an important role in facilitating the translation of acute SCI therapies. Biomarkers that accurately classify injury severity and precisely predict neurologic outcome, even in patients who cannot be functionally evaluated, will not only increase the number of SCI patients that can be recruited for clinical trials, but will also reduce the number needed to complete them. Biomarkers that measurably reflect biologic responses within the injured cord would also define which patients are the best candidates for (and most likely to benefit from) specific therapies, depending upon their mechanisms of action. The translational importance of such biomarkers is also recognized in other neurologic disorders such as traumatic brain injury (TBI), Alzheimer’s, and Parkinson’s disease. For example, it has been estimated that the use of CSF biomarkers in the selection of subjects for early Alzheimer’s trials “could reduce sample size by 67% and trial costs by 60%” as compared to trials depending solely upon clinical measures. Obviously, such an effect on clinical trials of SCI would be highly desirable.

We have been interested in utilizing cerebrospinal fluid from acute SCI patients to evaluate the measurable secondary injury responses after human SCI. Our initial studies identified a series of inflammatory cytokines and structural proteins within the CSF that accurately classified injury severity. We have continued to collect CSF in a multicenter initiative and have conducted further analyses to demonstrate the ability to classify injury severity and predict neurologic recovery with CSF biomarkers. More recent work using ‘omics’ platforms will shed broader insights into the pathophysiologic responses to acute human SCI.

References:

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Clinical neuroprotection and spinal cord physiology during acute spinal cord injury care

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Spinal cord injury (SCI) is a major unsolved challenge in medicine. Impact trauma to the spinal cord shears blood vessels, causing an immediate ‘primary hemorrhage’. During the hours following trauma, the region of hemorrhage enlarges progressively, with delayed or ‘secondary hemorrhage’ adding to the primary hemorrhage, and effectively doubling its volume. The process responsible for the secondary hemorrhage that results in early expansion of the hemorrhagic lesion is termed ‘progressive hemorrhagic necrosis’ (PHN). PHN is a dynamic process of auto destruction whose molecular underpinnings are only now beginning to be elucidated. PHN results from the delayed, progressive, catastrophic failure of the structural integrity of capillaries. The resulting ‘capillary fragmentation’ is a unique, pathognomonic feature of PHN. Recent work has implicated the Sur1-Trpm4 channel that is newly upregulated in penumbral microvessels as being required for the development of PHN. Targeting the Sur1-Trpm4 channel by gene deletion, gene suppression, or pharmacological inhibition of either of the two channel subunits, Sur1 or Trpm4, yields exactly the same effects histologically and functionally, and exactly the same unique, pathognomonic phenotype – the prevention of capillary fragmentation.

Three treatments that show promise for reducing PHN following traumatic SCI are: riluzole, systemic hypothermia, and glibenclamide, the last selectively targeting Sur1-Trpm4. Each has demonstrated efficacy in multiple studies with independent replication, but there is no way to compare them in terms of efficacy or safety, since different models were used, different laboratories were involved, and different outcomes were evaluated. Using a model of lower cervical hemicord contusion, we compared safety and efficacy for the three treatments, administered beginning 4 hours after trauma. Treatment-associated mortality was 30% (3/10), 30% (3/10), 12.5% (1/8) and 0% (0/7), in control, riluzole, hypothermia and glibenclamide groups, respectively. For survivors, all three treatments showed overall favorable efficacy compared to controls. On open-field locomotor scores (modified Basso, Beattie and Bresnahan scores), hypothermia- and glibenclamide-treated animals were largely indistinguishable throughout the study, whereas riluzole-treated rats underperformed for the first 2 weeks; during the last 4 weeks, scores for the three treatments were similar, and significantly different from controls. On beam balance, hypothermia and glibenclamide treatments showed significant advantages over riluzole. After trauma, rats in the glibenclamide group rapidly regained a normal pattern of weight gain that differed markedly and significantly from that in all other groups. Lesion volumes at 6 weeks were: 4.8 0.7, 3.5 0.4, 3.1 0.3 and 2.5 0.3 mm$^3$ in the control, riluzole, hypothermia and glibenclamide groups, respectively; measurements of spared spinal cord tissue confirmed these results. Overall, in terms of safety and efficacy (lesion volume), systemic hypothermia and glibenclamide are superior to riluzole. With regard to safety, efficacy, general wellbeing, and ease of administration, glibenclamide is superior to riluzole and systemic hypothermia.

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Maximising recovery of function after spinal cord injury by combining electrical stimulation, locomotor training and LV-Chondroitinase-ABC


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Electrical epidural stimulation (ES) of the lumbar spinal cord (L2 to S1) has previously been shown to improve locomotor function in complete transection models of rat spinal cord injury (SCI) in conjunction with monoaminergic and serotonergic agonists and bipedal locomotor training (Ichiyama et al., 2008; van den Brand et al., 2012). However, this functional improvement does not translate into recovery of overground locomotion. We have also recently demonstrated that exercise up-regulates inhibitory chondroitin sulphate proteoglycans (CSPGs) in the lumbar spinal cord (Smith et al., 2015) potentially restricting synaptic plasticity and previous evidence has demonstrated functional improvements in reaching and grasping tasks following application of ChABC following SCI (Garcia-Alias et al., 2009). It was therefore our premise for this study that addition of lentiviral chondroitinase (LV-ChABC) locally after injury would enhance synaptic plasticity thus allowing for improved functional recovery. Adult Sprague-Dawley rats received a severe spinal contusion injury (T9/10), epidural implantation at segmental levels L2 and S1 and intra-spinal injections of LV-ChABC or saline (control) ~1mm above and below the level of the lesion. Rats were then randomly assigned to one of four groups: cage control, training only, ES only (40 Hz; L2) or ES+training. Rats in trained groups stepped bipedal-to-quadrupedally on a body weight supported treadmill (5-16 cm/s) (5 days/week, 20 mins/day) for 8 weeks. By the end of the 8-week period rats in the Saline+ES+training group showed improvements not only in supported treadmill stepping ability but also in open field locomotion (BBB), with combination saline/LV-ChABC treated animals achieving the highest overall increase in mean BBB score compared to Saline/LV-ChABC controls. Furthermore kinematics analysis also revealed subtle differences in stepping characteristics and pattern following 8 weeks of training. We did not observe any electromyography responses of hindlimb muscles following cortical stimulation in any animal from any group, and no increased sensitivity to mechanical pain stimulation. Therefore these results suggest that a combination of step training and epidural stimulation in an incomplete model of SCI successfully improved locomotor function further than either therapy administered alone. Combination treatment animals not only improved in treadmill stepping performance but were also able to transfer this skill to an open field task without the presence of stimulation. Interestingly, addition of LV ChABC produced differences in both kinematic profiles and withdrawal responses to mechanical paw pressure. These promising results indicate that the combination of ES, locomotor training and LV-ChABC is a viable treatment for functional recovery after severe SCI.

References:

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Novel cell autonomous modulators of CNS axon growth

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Axons in the adult central nervous system (CNS) fail to regenerate after injury, therefore the prognosis for patients with severe spinal cord injury (SCI) is unfavorable. While full recovery is rare, a modest amount of spontaneous recovery is observed acutely after trauma that is titrated by the extent and location of the injury, in both patients (1) and models of SCI (2). The mechanisms that mediate spontaneous functional recovery are unknown and represent a significant barrier to therapeutic design. To this end we used an unbiased in vivo functional screening approach and identified 5-poly-phosphatases (5PPs) as significantly enriched in a subset of corticospinal motor neurons (CSMNs) that had initiated a spontaneous functional growth response after CNS injury.

Previously we have shown that intact corticospinal (3) and intact rubro-fugal (4) neurons spontaneously sprout de novo arbors and form new functional circuits after partial SCI. These data highlight the pivotal role of uninjured motor circuit plasticity in supporting functional recovery after trauma and strongly support a focus of experimental strategies on enhancing intact circuit rearrangement to promote functional recovery after SCI. To gain insight into the molecular mechanisms driving spontaneous functional sprouting in adult CNS neurons we completed differential expression profiling of intact CSMNs in an active and quiescent growth mode. We found 5-poly-phosphatases (5PPs) was significantly enriched in intact CSMNs undergoing functional sprouting. To confirm a role for 5PPs in promoting axon growth we transfected acutely dissociated embryonic day 17.5 mouse cortical neurons in vitro with 5PPs. 5PPs significantly enhanced average and total neurite outgrowth after 3 days in vitro (DIV) in comparison to controls. Additionally over expression of 5PPs in mature cortical neuron cultures enhanced growth of neurites that had been lesioned in vitro, and furthermore siRNA mediated knockdown of 5PPs attenuated growth of lesioned neurites below basal levels. These results compliment our screening data and confirm the capacity of 5PPs to support CSMN axon growth. Understanding the molecular mechanisms by which 5PPs enhances axon growth and whether specific subsets of CNS neurons are sensitive to its effects are crucial to the design of otherapeutics that aim to exploit these new targets. We are currently assessing whether AAV-mediated over expression of 5PPs in vivo is capable of stimulating functional plasticity from intact CSMNs after unilateral corticospinal tract lesion and from lesioned CSMNs after contusion injury.

References:

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High-throughput proteomics reveal alarmins as amplifiers of tissue pathology and inflammation after spinal cord injury

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Spinal cord injury is characterized by acute cellular and axonal damage followed by aggressive inflammation and pathological tissue remodelling. The mediators underlying these processes are still largely unknown. Here we used an innovative proteomics approach to identify novel bioactive mediators of pathological tissue remodelling after spinal cord injury by studying the enriched extracellular proteome. Proteomics revealed multiple matrix proteins not previously associated with injured spinal tissue, including small proteoglycans involved in cell-matrix adhesion and collagen fibrillogenesis. Network analysis of transcriptomics and proteomics datasets uncovered persistent overexpression of danger-associated molecular patterns (DAMPs or alarmins) that can trigger inflammation via pattern recognition receptors. In mechanistic experiments, inhibition of toll-like receptor-4 (TLR4) and the receptor for advanced glycation end-products (RAGE) revealed the involvement of alarmins in inflammatory gene expression, which was shown to be dominated by IL1 and NFκB signalling. Extracellular high-mobility group box-1 (HMGB1) was identified as the likely endogenous regulator of IL1 expression after injury. These data reveal a novel tissue remodelling signature and identify endogenous alarmins as amplifiers of the inflammatory response that promotes tissue pathology and impedes neuronal repair after spinal cord injury.

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Neural circuitry that produces the basic rhythm and pattern of walking is situated in the spinal cord. Following spinal cord injury, locomotor training can lead to significant improvement in gait in animals and humans, demonstrating that spinal networks undergo rearrangement in order to restore fundamental locomotor function. Understanding mechanisms of this spinal plasticity is important for the further development of strategies aimed at improving motor function following spinal cord injury.

Genetic techniques have provided a window through which to study spinal motor circuits and their plasticity. dI3 interneurons (INs) are a population of glutamatergic spinal INs characterized by the expression of the homeodomain transcription factor IsI1. We have previously shown that dI3 INs are involved in spinal processing of sensory afferents and are essential for grasping function. In studying the role of dI3 INs in locomotion, we have found that in addition to receiving direct sensory input from the limbs, they receive input from spinal locomotor circuits. That is, they are situated as spinal “comparators,” comparing the predicted and actual movements. In mice in which neurotransmission by dI3 INs has been genetically removed (dI3 \textit{OFF} mice), mild locomotor deficits are seen. However, after complete spinal transections, treadmill locomotor training led to limited functional recovery in dI3 \textit{OFF} mice compared to controls. These results demonstrate that dI3 INs are required for locomotor recovery, and provide insight into the mechanisms underlying motor functional recovery after spinal cord injury.

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Recent developmental studies have begun to provide an outline of the key pathways that control the specification and functional properties of the neurons that contribute to the spinal sensorimotor circuitry. Using a comprehensive approach that combines genetics, neuroanatomical, electrophysiological and behavioural analyses, we have begun to assess the contribution that molecularly identified neuronal cell types make to the control of movement in mice with the goal of identifying potential targets for therapeutic intervention for spinal cord injury. Recent efforts in the lab have focused on two classes of inhibitory neurons that are essential for flexor-extensor driven motor behaviors and locomotion. By mapping the presynaptic inputs to these cells from cutaneous and proprioceptive sensory pathways, we have begun to identify interneuron cell types in the dorsal horn that play key roles in modulating locomotion. Our analyses indicate that excitatory interneurons in the dorsal horn that transmit cutaneous information play key roles in controlling locomotion, and as such are potential targets for therapeutic intervention.
Stop and go: a matter of excitation

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For locomotor behaviours, like walking, motor circuits in the spinal cord itself generate the actual timing and coordination of the rhythmic muscle activity. These circuits are at the core of generating locomotion and understanding the operational organization of these circuits is key to understand how the behaviour is generated. In this talk, I will discuss findings from our lab that have revealed the role of designated populations of neurons that serve key functions in the spinal locomotor network in generating the rhythm of locomotor movements. I will also address how locomotor networks are selected to secure appropriate coordination of movements at different speeds of locomotion and discuss how command signals from the brain may initiate and stop the behavior.

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Clinical trial in SCI: are we ready?

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Three clinical trials of neural stem cells for spinal cord injury are in progress. But are we ready for these trials? The field of modern neural stem cell biology is young, and ongoing experiments have a great deal to teach us about the basic molecular biology, physiology, development and function of these cells. In addition, progress is evolving nearly daily in the tools for generating and driving the fate of neural stem cells to specific lineages tailored to repair of the injured spinal cord. These are important considerations to take into account with regard to the translation of neural stem cells for SCI. Premature clinical trials may lead to early failures and disillusionment among researchers, patients and funding agencies. Prolonged delays in exploiting this new biological tool for nervous system repair could deprive patients of therapies that may be effective only in limited time windows after SCI. Striking the proper balance remains a challenge.

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Preclinical studies of AST-OPC1 (formerly GRNOPC1) in animal models of traumatic SCI have shown that these cells survive, migrate throughout the injury site, and improve locomotor function. Therefore, a phase 1 clinical trial was initiated to assess the safety of AST-OPC1 in patients with neurologically-complete T3-T11 thoracic SCI. Five subjects received a low dose of $2 \times 10^6$ AST-OPC1 cells between 7 and 14 days following their injury. The cells were administered via a single injection into the spinal cord lesion using a dedicated syringe positioning device. Subjects received a low dose of tacrolimus immunosuppression for 46 days, which was then tapered and discontinued at day 60. Subjects were followed for 1 year under the main study protocol and are being followed for an additional 14 years under a long-term follow up protocol. To date, all five patients have been followed for over 3 years. There have been no serious adverse events related to AST-OPC1, tacrolimus, or the injection procedure. Serial MRI scans indicate that lesion cavity formation at the AST-OPC1 injection sites was substantially reduced through 3 years of follow up in 4 of 5 subjects. There were no unexpected changes in neurological function. The data to date suggest that AST-OPC1 can be safely administered to patients in the subacute period after thoracic SCI. The next phase of development will be a Phase 1/2a trial to evaluate the safety and activity of escalating doses of AST-OPC1 in patients with subacute sensorimotor complete cervical SCI. The first participant in this trial received AST-OPC1 in June, 2015.
What's in a name? Progress and pitfalls in stem cell clinical trials for spinal cord injury

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The potential of cell-based therapeutic strategies to treat neurological disease and traumatic injury to the central nervous system (CNS) is an area of intense investigation. The pathophysiological evolution of spinal cord injury (SCI) is complex, including both primary damage at the epicenter of the trauma, and secondary damage spreading rostral and caudal to the injury site. The potential efficacy of stem cell strategies aimed at either cell replacement or tropic effects at the lesion site is likely to be dependent on injury level (cervical, thoracic or lumbar) and the associated specific motor neuron pools affected, degree of axonal preservation, and efficiency of endogenous remyelination and circuitry reconstruction. Key issues include how transplanted cell fate and integration are modulated, transplantation timing, transplantation location, the immunological niche of the injured SCI microenvironment, the use of pharmacological immunosuppressant regimens as a part of translational strategies, and validation and characterization of clinical cell lots. We have tested the ability of research grade human Central Nervous System Stem Cells propagated as neurospheres (hCNS-SCns) to engraft, migrate, differentiate, and promote locomotor recovery after thoracic contusion SCI in multiple animal models, including genetically immunodeficient mouse and rat models in which there is no xenograft rejection, as well as pharmacologically immunosuppressed mouse and rat models. hCNS-SCns transplantation immediately, 9 days after SCI, or 30 days after SCI yielded unique characteristics of migration and differentiation by engrafted cells. Delayed hCNS-SCns transplantation resulted in functional locomotor improvements in the absence of tumor formation or allodynia, in association with terminal differentiation into myelinating oligodendrocytes and synapse-forming neurons in the SCI environment. Selective ablation of human cells using Diphtheria toxin (DT) abolished locomotor recovery, demonstrating that continued survival of engrafted cells is necessary for sustained functional improvement in this paradigm. No evidence of host-mediated mechanisms of recovery, e.g., reduction in lesion volume or glial scar, or increase in tissue sparing or host regeneration, was observed. In this presentation, we report the results of transplantation of research (RCL) and clinical (CCL) lots of these cells into a unilateral cervical model of SCI at 9 and 60 days post-SCI. Although recovery of function was detected with the RCL at both transplantation timepoints, CCL cells failed to demonstrate improvements in motor function at either transplantation timepoint. These data raise questions as to whether in vitro/in vivo demonstration of potency and comparability of cell lines, in the absence of analysis of intended functional endpoints, is adequate to support translation to the clinic.

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Why is regeneration of CNS axons so feeble? What can we do to energise them

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CNS axons fail to regenerate because of the inhibitory environment and because of an intrinsic lack of regenerative ability. Why does this happen, and how can regeneration be stimulated? We believe that there are two problems; 1) as CNS axons become specialized they set up a selective transport filter which excludes many molecules needed for axon regeneration, including integrins and trks; the end result is that the axon shaft becomes a signalling-deficient zone, 2) even in axons such as sensory axons that do not have a selective transport block there is a mismatch between the neuronal integrin repertoire and the integrin ligands in the environment and in addition CSPGs and NogoA inactivate integrins.

Much of what we now understand about CNS axons came through studying integrin biology in regeneration. We chose the focus of integrin-mediated axon as our focus for two reasons; 1) Integrins can be a therapeutic and can enhance regeneration, 2) Changes in integrin biology between growing and non-regenerating axons can indicate what has gone wrong with growth mechanisms in mature CNS axons. Neurons in the CNS lose regenerative ability with maturity. As axons grow during development they transport integrins to the growth cone, but as they mature integrins are excluded from the axons and only go to the somatodendritic domain. We find the same for trks, and other receptors including IGF receptors are also reported to be excluded from axons. The result of this is that antibodies that recognize PI3 signalling show that mature axons are signalling-deficient; it is not surprising that there is little regenerative response when they are cut. How does this transport block occur? Integrin transport and trafficking in axons relies on the GTPases Rab11 and GTPase Arf6 whose activation state regulates transport and trafficking. Two Arf6 GEFs are upregulated during maturation to reverse integrin transport to retrograde, so preventing integrin entry into axons. There is also a role for the axon initial segment.

Sensory axons do not have a selective transport block, yet they also regenerate poorly in the CNS. The main matrix glycoprotein in the damaged CNS is tenascin-C, but the tenascin-C binding integrin alpha9beta1 is absent in DRG and CNS neurons. Alpha9 integrin transfection allows axons to grow prolifically on tenascin, but in vivo the effect is modest. The problem is that the CNS inhibitory molecules CSPG and NogoA inactivate integrins, and for integrin manipulation to be effective it has to be combined with an integrin activator, kindlin1. Coexpression of alpha9 integrin and kindlin1 in sensory neurons enables profuse long distance axon growth in the spinal cord with sensory and behavioural recovery. We conclude that the main reason for failure of axon regeneration in the CNS is because mature axons become increasingly specialized, with exclusion of many molecules needed for axon growth. They therefore lack the ability to interact with their environment, and to generate signals to drive regeneration.

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Gene therapy approaches to promote axonal regeneration

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Gene therapy can be defined as the introduction of a gene encoding a therapeutic protein to treat a disease. Neurosurgical repair of avulsed spinal ventral root or a peripheral nerve lesion rarely results in complete return of function. A key question addressed here is: can axon regeneration in an injured spinal nerve root and in the peripheral nerve be stimulated by combining neurosurgical repair with gene therapy for neurotrophic factors. The effects of constitutive lentiviral vector-mediated expression of a number of neurotrophic factors expressed in either reimplanted spinal ventral roots or in sciatic nerves repaired with nerve bridges was tested. Lentiviral vector-mediated overexpression of BDNF, GDNF and NGF in nerve autografts exhibited a spectrum of effects, including early stimulatory effects on axons entering the graft and excessive axon growth and Schwann cell proliferation in the graft at 20 weeks post-surgery. Persistent expression of these factors in autografts interfered with target cell reinnervation and functional recovery in a modality specific way. Therefore, we created a novel, immune-inert, regulatable lentiviral vector and present preliminary data with this regulable vector in the spinal root avulsion model. As a first step to overcome nerve fiber trapping at sites of high level neurotrophic factor expression, a regulatable lentiviral vector for GDNF was applied in a spinal root avulsion model. Initial results suggest that timed expression of GDNF promotes regeneration of motor axons and diminishes axon trapping. So far, PNS-gene therapy in rodent models largely relied on lentiviral vector-mediated gene delivery. These vectors integrate their genetic information into the host cell genome and therefore carry the risk of insertional mutagenesis. Adeno-associated viral (AAV) vectors are gaining increasing acceptance as a clinical gene delivery platform. The translatability of gene therapy as an approach to promote repair in human patients has been explored by investigating the transduction properties of 9 common AAV serotypes in cultured rat and human nerve segments. AAV vectors based on serotypes 1 to 9 were compared for their capability to transduce cultured primary rat and human Schwann cells and nerve segments. AAV1 is the best serotype to transduce rat Schwann cells, whereas AAV2 and 6 performed equally well in human Schwann cells. Transduction of monolayers of cultured rat and human Schwann cells did not accurately predict the transduction efficiency in nerve segments. Rat nerve segments could be genetically modified equally well by a set of four AAV vectors (AAV1, 5, 7, 9), whereas AAV2 was superior in human nerve segments. The transduction of rat and human Schwann cells and nerve segments by entirely different AAV serotypes, as documented here, highlights one of the challenges of translating gene therapy from experimental animals to human patients.

References:
Development of integration-deficient lentiviral vectors for spinal injury

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Safe methods are required to deliver gene therapies to localised areas of the spinal cord for therapeutic purposes in spinal cord injuries (SCI). Integration-deficient lentiviral vectors (IDLVs) are obtained through the use of mutations affecting residues in the catalytic active site of integrase. In the absence of integration the vectors become episomal circles but still deliver transgenes efficiently, leading to stable transduction of quiescent tissues and transient expression in dividing cells. IDLVs have been used in a variety of CNS disease models, including rescue of retinal dystrophies and a Parkinson disease model. They have already been used in the intact spinal cord, demonstrating effective expression in localised areas and strong transgene expression in motor neurons.

Our CHASE-IT consortium (http://www.spinal-research.org/chondroitinase/) pursues the delivery of Chondroitinase ABC (ChABC) through non-integrating vectors, including adeno-associated viral (AAV) vectors and IDLVs. In our prior work ChABC has already shown powerful activity in contusion SCI at cervical and thoracic levels, including partial rescue of function when delivered through a standard integrating lentiviral vector. Regarding IDLVs, we are exploring the use of a variety of promoters, including constitutive, tissue-specific and tetracycline-regulated, after initial development of a deimmunised version of the latter.

IDLVs encoding ChABC driven by constitutive and tissue-specific promoters have been initially tested in cortical neurons in vitro. Using the Morgan-Elson assay we detected strong ChABC activity in the culture supernatants, similar to that observed in parallel experiments with integrating lentiviral vectors. In vivo testing of these IDLVs encoding ChABC in the spinal cord is in progress.

Regarding the use of a tetracycline-regulated promoter, no prior demonstration exists of delivery of an inducible system through IDLVs. In this case we have used a luciferase-based system to test whether IDLVs would support delivery of either standard or de-immunised tetracycline reverse transactivators in growth-arrested Chinese hamster ovary (CHO) cells and cortical neurons in vitro. Our results indicate that IDLVs are suitable delivery vectors, but that higher induction levels can be achieved with the standard reverse transactivator in some cases. In vivo testing will follow soon.

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Barriers and solutions to combination therapeutics strategies in spinal cord injury

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Combinations of therapeutics are critical to the successful treatment of diseases such as cancer, infectious diseases such as tuberculosis, and in psychiatry. The rationale for combinations is molecular redundancy and evolution of resistance. The combination of implanted medical devices with drugs and biologics has recently established clear value in vascular disease. It is evident that a single therapeutic agent is unlikely to lead to substantial neurological recovery after SCI. Given the numerous mechanisms and consequences of injury, it is important to design treatment combinations targeting key processes additively or synergistically. One combination with accumulating evidence is that targeted rehabilitative activity can increase the effect of some biological therapeutics. However, the majority of SCI therapeutics identified and tested experimentally have been done so individually. Another desirable combination is to target therapeutics to successive stages of SCI with early neuroprotection, followed by augmented tissue repair and neuroplasticity, in concert with optimized rehabilitative activity. Clinical trials tend to exclude subjects that are concurrently receiving another experimental treatment. The lack of SCI therapeutics whose efficacy has been established as single agents is an obvious impediment to designing rational combinations. However, efficacy may remain elusive until the potency of combined activity can be determined.

There are several practical impediments to combined approaches. They include the added complexity in preclinical testing, the design and execution of clinical trials, and business and regulatory challenges. A central problem is the challenge to isolate the effects of individual treatment components, both for their contribution to efficacy and to side effects, and to understand dose optimization. Computational models and in vitro screens may be helpful to both select combinations and assess molecular interactions. Technological innovations in biological sensors that accurately convey temporal changes in multiple molecules and processes may enrich datasets to reveal critical interactions. This data will require compression, analysis, and interpretive methods. Both systemic and CNS specific information may aid understanding of the impact of combinations. Enrichment of assessment tools including biomarkers, imaging, functional assessments, and statistical methods that can test linkage in multiple domains is needed. Recognizing the necessity for this progress it is important to encourage the most feasible and interpretable combinations. Thus, agents with well-defined and specific mechanisms of activity that can be measured would be rational to combine. The less clear our understanding of the effects of a therapeutic, the more difficult it will be to interpret how it is working in a combination.
Use of individualized medicine techniques to focus the translational value of the canine clinical SCI ‘model’

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Pet dogs that incur spinal cord injury in the course of their everyday lives have potential as models in which to test the ability of putative therapies to translate from laboratory to clinic. The most important role that this population of spinal cord-injured subjects can play is in modeling the heterogeneity of clinical patients. To some extent each spinal cord injured patient – whether human or dog - has a unique combination of factors that contribute to the observed functional loss.

The question then arises as to how to take account of this individuality when analyzing the effects of a novel intervention. A similar question arises frequently in other fields of medicine, particularly clinical chemistry, and has contributed to the development of personalized medicine.

Methods to measure any biological analyte have inherent sources of variation, associated with variability in measurement, variability within an individual (from one recording time to another) and variability between individuals. Together this variation will create an inevitable uncertainty about reported (numerical) results, which can make it difficult to be sure whether a change in value from one timepoint to another represents a ‘real’ difference. We have been investigating the application of personalized medicine techniques to overcome this problem when analyzing function after spinal cord injury in dogs.

We have recorded repeated data measurements on a series of dogs in order to quantify the coefficients of variation associated: i) with the analytical technique itself; ii) within an individual animal; and, iii) within the group. This permits calculation of the ‘reference change value’, which defines the threshold of difference from baseline that must be crossed to define a ‘real effect’ of an intervention.

This preliminary investigation has suggested that changes of ~70% from baseline value must be achieved to imply a real effect of an intervention on measured bladder function or gait coordination. Furthermore, reanalysis of a previously acquired dataset would suggest that this more stringent definition may reduce the observed rate of ‘successful’ intervention, whether applied to clinical or laboratory subjects.

References:

Supported by the International Spinal Research Trust
Making the most of the best: adjuvant rehabilitation interventions to maximize function-related neuroplasticity

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Our recent growth in understanding of neuroplasticity has had tremendous implications for people with spinal cord injury and other central nervous system disorders. At this time, exercise, practice, and training are the best approaches available to reduce motor impairment and physical limitations that result from spinal cord injury. The interventions promote adaptive plasticity that supports restoration of movement function. There are a number of non-invasive forms of stimulus energy, such as electrical, magnetic, and vibration stimuli that are clinically available and seem to augment the effects of training. Evidence indicates that stimulation activates the same neural circuits that are activated by voluntary effort and training. Therefore, when used in combination with training, stimulation has the potential to promote neuroplasticity beyond that achieved by practice or training alone. In neurologically healthy individuals studies have shown these approaches to enhance neural excitability and improve motor performance. In persons with neurological disorders, clinically available forms of non-invasive stimulation seem to be of value for modulating neural excitability as adjuncts to programs designed to improve hand/arm function or to improve walking function.

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Case report: functional recovery in a patient with transplantation of autologous olfactory bulbar olfactory ensheathing cells combined with peripheral nerve bridging

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We recently reported on a patient where autologous bulbar olfactory ensheathing cells were transplanted into the stumps on either side of a mid thoracic spinal cord knife injury and the 8 mm separation gap was bridged by autologous peripheral nerve strips. The pattern of recovery suggests that severed nerve fibres had regenerated across the bridge provided by the transplant and had restored connections that allowed motor and sensory function.

(1) This is parallel with our rat work over many years in which we compared mucosal and bulbar OECs, and where behavioural recovery was correlated with histology showing that severed fibres had crossed the lesion sites.

(2) The transplantation was carried out after 21 months of general rehabilitation, including 8 months of intense rehabilitation (5 hours a day, 5 days a week). MEP and EMG studies performed at the beginning and end of the pre-operative period, together with regular neurological assessments did not show any signs of neurological recovery. Prognostic signs of recovery, such as sacral pin-prick sensation or even a flicker of movement in the leg muscles were totally absent at the time of operation.

(3) The highly specific spatial and temporal recovery of crossed motor and sensory function observed in the patient fits accurately with the known motor and sensory spinal pathways.

The nerve strips were implanted on the left and slightly across the midline. The radiological evidence indicates that they had survived and were well vascularised. The major motor recovery was on the left, descending from T8/9 to L3 progressively over 2.5 years after operation.

Superficial sensation started to return within five months on the right. This is consistent with the view that the regenerating fibres had followed the known normal crossed pathway of the primary dorsal root axons carrying superficial sensation fibres across the midline below the lesion, and had regenerated across the transplanted cell/nerve tissue bridge that was created at operation on the left. By making contact with ascending spinothalamic neurons in the cord immediately above the injury the regenerating fibres would have had only a short distance to travel before an ascending sensory pathway for perception was restored.

Deep sensation returned late and bilaterally, starting at 6 months after surgery and becoming more evident in the period after 10 months. The nerve grafts spanned the midline region of the dorsal columns on both sides. The late return of deep sensation is consonant with dorsal column fibres regenerating at 1mm/day reaching the dorsal column nuclei (an ascent shown in our rat studies).

It is difficult to ascribe this spatial and temporal pattern to mere chance. However, it fits well with the normal pattern of spinal pathways.

It is possible that, over the 2.5 year period that was needed to obtain T11 to L3 return of function, fibres regenerating at 1mm/day could have crossed the distance of 6 cm from the T8 to the L3 segments. But this is not necessary. Control of L2-3 motoneurons does not have to be monosynaptic. If corticospinal or even propriospinal fibres simply crossed the lesion and made contact with neuropil below the lesion, the descending corticall motor message could have been carried via a multisynaptic pathway to the L3 motoneurons. We do not believe that the pattern of re-connection was normal, but that the new connections were sufficient to carry motor and sensory messages across the injury and that function returned as a result of plasticity during the learning process occurring during rehabilitation.

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Benefits of surgical implantation of an investigational biodegradable neuro-spinal scaffold in various animal models of acute spinal cord contusion injury: clinical translation

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The pathophysiological sequelae of the primary spinal cord injury (SCI) include edema, spinal cord swelling, reduced blood flow, and local tissue ischemia resulting in further cellular necrosis culminating in the appearance of a tissue void (cavity). Biodegradable scaffolds can be implanted within the necrotic lesion to fill this void and provide structural support to the surrounding viable tissue while serving as a locus for appositional healing.1,2 This work focuses on translational animal studies using clinically-relevant contusion injuries in both rats and pigs to support current clinical investigation.

A spinal T10 contusion injury was created in Sprague-Dawley rats and cylindrical scaffolds (1.0 mm diameter, 2.0 mm length) were surgically implanted at the lesion site between 24 and 72 hours later. Histological analysis at 12 weeks revealed that rats in the non-treated control group developed large cavities surrounded by a thin rim of spared tissue. In contrast, in rats implanted with scaffolds, cavity volume decreased by 86% and spared tissue width increased by 44%. Although scaffolds were fully resorbed by 12 weeks after implantation, the amount of remodeled tissue at the site of implantation in the lesion epicenter increased by 111%. These results demonstrate that biodegradable scaffold implantation in the acutely injured spinal cord can reduce cavitation and promote tissue sparing and remodeling.

To translate the use of biomaterial scaffolds for acute SCI treatment, evaluating the neurosurgical techniques used for implanting a device into the recently injured spinal cord in a clinically relevant, large animal model is needed. Four Gottigen pigs were contused at T10 with a 50g weight dropped from 40cm, followed by a 100g compression for 5 minutes as described previously3. At 4, 6 and 24 hours following injury, the necrotic injury center was gently debrided following internal decompression via myelotomy and a scaffold was implanted. Further, at 24 hrs post-injury (n=2) intraparenchymal pressures were measured using a 1F pressure catheter. In each animal, the intraparenchymal cavity accommodated a cylindrical scaffold of at least 2mm diameter and 7mm length. In addition, the surgical technique provided the added benefit of reducing intraparenchymal pressures below pre-surgery levels. Our findings demonstrate the feasibility of biodegradable scaffold implantation within 4 to 24 hours in a clinically relevant large animal model of acute SCI.

Multimodal therapeutic interventions are needed to provide next generation spinal cord injury treatments. Acute parenchymal surgical decompression and scaffold implantation to provide structural support to residual tissue and serve as a permissive environment for appositional healing is currently under clinical investigation. In an ongoing pilot clinical trial, 3 subjects have been enrolled to date. Two of the three subjects converted from complete (AIS A) to incomplete injuries (1 subject to AIS B and 1 subject to AIS C) at 1 month post-implant.

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Suppression of AMIGO3 promotes dorsal root ganglia neuron axon regeneration after spinal cord injury

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Unlike the peripheral nervous system, the spinal cord which forms part of the central nervous system (CNS) is unable to regenerate. Nogo-A, myelin associated glycoprotein (MAG), and oligodendrocyte-derived myelin glycoprotein (OMgp) are some of the major inhibitory proteins in the CNS which contribute to the non-regenerating nature of CNS axons. They do this by binding with a common Nogo-66 receptor and activate axon growth cone collapse through the RhoGTPase pathway. However, manipulating the extracellular environment in the CNS after injury can induce limited axon regeneration. In this study we demonstrate by manipulating expression of extracellular protein molecules -AMIGO3 (an amphoterin-induced gene open reading frame), that axon regeneration in the CNS is possible. Data from immunohistochemistry in regenerating and non-regenerating spinal cord injury models showed that low levels of AMIGO3 expression correlated with regenerating sciatic nerve (SN) and preconditioning SN+DC lesion models. Knockdown of AMIGO3 in dorsal root ganglion neuron (DRGN) cultures promotes DRGN neurite outgrowth. Non-viral delivery of an AMIGO3 shRNA plasmid to knock down AMIGO3 expression in injured dorsal columns (DC), demonstrated significant DC axon regeneration. Moreover, AMIGO3 when tagged with GFP, targeted large diameter DRGN after injection and was anti-inflammatory revealing a novel function of AMIGO3 in regulating inflammation in the CNS after injury. The mechanisms by which AMIGO3 supresses or promotes axonal regeneration is not yet known but we conclude that AMIGO3 plays a major role in DRGN axon regeneration and could be harnessed to promote regeneration of injured neurons after spinal cord injury.
Human olfactory derived mesenchymal stem cells as a candidate for spinal cord repair

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Previously we have identified and characterised a source of adult MSCs from the uniquely regenerative human olfactory mucosa (OM-MSCs) and shown that they have enhanced clonogenicity, proliferation rate and in vitro CNS myelination compared to bone marrow derived MSCs (BM-MSCs, Lindsay et al., 2013). For comparison of the two MSC types, microRNA-based (miRNA) fingerprinting was carried out, which demonstrated 64% homology between the two. Interestingly, 26 miRNAs were differentially expressed, and of these, we focussed on miR140a-5p due to its reported role in the regulation of chemokine production. We found that CXCL12, a chemokine regulated by this microRNA was differentially secreted by OM-MSCs. Addition of CXCL12 to myelinating cultures promoted myelination and a selective CXCL12 receptor blocker and anti-CXCL12 prevented the promyelinating effect. Transduction with the miR-140-5p antagonist and mimic produced inverse changes in CXCL12 RNA, confirming the regulatory role of miR-140-5p for CXCL12. To assess the repair potential of human OM-MSCs GFP tagged-cells were transplanted 3 weeks post-injury into the spinal cord of rats with a thoracic level 9 injury. Cells filled the lesion surviving until at least 4 weeks post-transplant and resulted in reduced levels of cavitation, and a greater amount of neurofilament positive fibres within the lesion site. Data obtained using a treadmill based gait analysis system suggested that transplanted animals recovered co-ordinated stepping earlier than control animals with immunohistochemical assessment revealing enhanced peripheral myelination within the cavity and within ventral and lateral areas of the cord by invading Schwann cells. These data suggest that OM-MSCs promote both de novo myelination and re-myelination of injured/spared axons which could account for the quicker restoration of co-ordinated stepping that was found within transplanted animals. Thus tissue niche may play an important role in determining how beneficial a particular MSC type might be in terms of SCI repair and that OM-MSCs which promote myelination could be a better MSC choice for use in the clinic.

References:

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Chondroitinase gene therapy for repair after spinal cord injury

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Spinal cord extracellular matrix is densely packed with growth inhibitory chondroitin sulphate proteoglycans (CSPGs), which become more abundant after injury. Thus, matrix modification has become a leading experimental strategy for promoting repair following spinal cord injury (SCI). Despite the beneficial effects that have been achieved by digesting CSPGs with the bacterial enzyme chondroitinase ABC (ChABC), the potential for achieving long term efficacy in traumatic injuries that mimic a human SCI has been limited, due to suboptimal delivery methods and issues of enzyme instability. However, we have recently demonstrated promising efficacy of a ChABC gene therapy approach in a clinically relevant contusive SCI model using viral vector delivery of a mammalian compatible ChABC gene. Here we demonstrate the efficacy of ChABC gene therapy in contusion injury models at differing spinal levels. When used to treat a contusion injury at either C5 or T10 spinal level, ChABC gene therapy resulted in increased spinal conduction through the injury epicenter, improved functional performance in skilled locomotion, significant neuroprotection and enhanced plasticity of intact spinal circuitry. Moreover, we compared the efficacy of ChABC gene therapy using different viral vectors (both adeno-associated viral vectors and lentiviral vectors) and different promoters to drive gene expression (CMV and PGK), in order to determine the optimal vector properties necessary for significant repair. We find that the use of different promoters results in differing patterns of ChABC expression. The use of a PGK promoter primarily leads to transduction of neuronal cells, resulting in widespread CSPG degradation throughout the spinal cord and the most dramatic improvements in functional and anatomical outcome measures. Although the most potent effects were observed with a PGK-lentiviral vector, we also observed some efficacy with a PGK adeno-associated viral vector, which is promising for future clinical development. Thus, we demonstrate the therapeutic potential of ChABC gene therapy to treat clinically relevant injuries at different spinal levels and present findings on optimizing the delivery paradigm for chondroitinase gene therapy.

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Among the range of motor and sensory deficits that occur following cervical spinal cord injury (SCI), respiratory dysfunction is one of the most devastating and life-threatening. With an increase in the incidence of high- to mid-cervical SCI, there is a need for treatments capable of restoring voluntary control of breathing in injured individuals. Functional electrical stimulation (FES) has been widely employed to treat non respiratory (e.g. locomotor, lower urinary tract dysfunction) and respiratory (diaphragm pacing) deficits in people with SCI. Diaphragm pacing (DP) not only reduces or eliminates the need for mechanical ventilation, but also promotes plasticity and recovery of independent respiration. Such benefits may not be limited to stimulation of respiratory muscles, however, as non-respiratory afferents are also known to modulate respiratory function. The goal of the present study work was to test whether a close-loop stimulation of either respiratory or non-respiratory muscles can enhance phrenic motor output following high cervical SCI in the adult rat.

Adult Sprague-Dawley rats received a complete lateral hemisection at the C2 (C2Hx) level. This proof-of-principle injury model removes all descending input to the ipsilateral phrenic motoneuron pool, resulting in hemi-diaphragm paralysis. Closed-loop electrical stimulation (50Hz bursts) of diaphragm or forelimb (biceps brachii) ipsilateral to the injury was delivered 4-6 hours post-C2Hx. Stimulus was triggered by the contralateral phrenic/hypoglossal nerve activity and delivered at the onset of inspiratory phase. Different animal preparations were used to optimize the stimulation parameters for each treatment strategy. Animals receiving DP were anesthetized and spontaneously breathing. In contrast, decerebrate, unanesthetized animals were artificially ventilated for stimulation of biceps. Biceps stimulation was selected based on the overlap between motoneuron pools innervating the biceps (C3-5) and diaphragm (C3-5/6).

These experiments have revealed that stimulation of the either respiratory or non-respiratory muscles can improve breathing function following C2Hx. Stimulation of biceps brachii acutely following a C2Hx resulted in increased phrenic motor output ipsilateral to injury. As would be predicted from ‘open-loop’ clinical use of DP, stimulation of the paralyzed hemi-diaphragm restored contractile activity and increased tidal volume acutely following C2Hx animals. Recording of intact ipsilateral phrenic nerve activity during diaphragm stimulation confirmed activation of afferent feedback from diaphragm muscle for each stimulus. Ongoing studies are now investigating long-term functional benefits achieved with these strategies and associated alterations in the underlying circuitry.
Developing a method to study functional plasticity and synaptogenesis following experimental spinal cord injury

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The CNS has a poor intrinsic capacity for regeneration, although some functional recovery does occur. This is mainly in the form of sprouting, dendritic remodeling and changes in neuronal coding, firing and synaptic properties; elements collectively known as plasticity. Following spinal cord injury (SCI), a fundamental approach to repair the injured CNS is therefore to harness, promote and refine plasticity. This is partly limited by some components of the extracellular matrix such as chondroitin sulphate proteoglycans, which are important inhibitory molecules that can be manipulated by therapeutics such as chondroitinase ABC. The corticospinal tract is an important descending motor pathway involved in locomotion, posture and voluntary skilled movements. Therefore regeneration and anatomical reorganisation of this projection is often examined in studies of experimental SCI. The use of genetically encoded fluorescent reporters of presynaptic release or neuronal activity represents a novel approach to gain insight into the anatomical and functional status of new connections arising from this pathway. Here we used viral vectors to express such functional probes in neuronal populations of interest and an acute ex-vivo cervical spinal slice preparation from adult rats was developed with the aim of imaging changes in fluorescence following electrophysiological stimulation in real-time using two photon microscopy. We are currently optimizing this preparation in naïve rats to study spinal connectivity in the uninjured situation and will then apply this technique to rats which have undergone a unilateral pyramidotomy lesion. This will allow us to investigate functional synaptogenesis and plasticity of a known, spared population of fibres following injury and furthermore how this may change following delivery of therapeutics which promote plasticity such as chondroitinase gene therapy.

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Human iPSC-derived corticospinal neuron grafts following cervical contusion injury

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Traumatic spinal cord injury (SCI) can result in a multifaceted array of behavioral and biochemical disturbances that pose both an immediate and long-term burden on the patient and health care system. Studying cervical injury is of particular clinical significance as it accounts for over half of SCI and represents a growing proportion in presented injuries.1,2 Whereas the predominant amount of grafts in preclinical trials to date use undifferentiated neural stem cells and uncharacterized progenitor cells, we have chosen to use human induced pluripotent stem cells (iPSCs) driven towards a corticospinal motor neuron (CSMN) fate as they have the capacity for long distance axonal growth and for functional synaptic connectivity. Herein, human iPSCs were cultured and differentiated to a CSMN fate and implanted in immunodeficient rats following a C5 contusion injury. Cells were phenotypically characterized and monitored within the host corticospinal tract following implantation for growth, migration, and synapse formation. Using a combinatorial treatment (iPSC graft with a forelimb-specific rehabilitation regimen), we hope to eventually facilitate functional recovery of electrophysiologically mapped corticospinal neurons after sub-acute and chronic spinal cord injury.

References:

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Inhibiting cortical PKA activity in spinal cord injured rats enhances corticospinal tract plasticity and rehabilitative training efficacy via EPAC


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Elevated levels of the cellular messenger molecule cAMP have frequently been associated with the ability of neurons to sprout and extend neurites, even in the growth inhibitory environment of the central nervous system. A prominent downstream target of cAMP that has been associated with neurite outgrowth is Phosphokinase A (PKA). Here we attempted to demonstrate that also the neuroplasticity promoting effect of rehabilitative motor training following spinal cord injuries is mediated via PKA activation. However, when we inhibited cortical PKA using its inhibitor Rp-cAMPS during the phase of rehabilitation in spinal cord injured rats to prove this principal, we found the opposite effect. In two independent experiments we discovered that blocking cortical PKA in parallel to rehabilitative training, increased functional recovery and collaterals sprouting of injured corticospinal tract fibers, an indicator of neuroplasticity. When we evaluated the effects of the PKA inhibitor in vitro, we found that increasing cAMP levels still resulted in increased phosphorylation of a prominent downstream target (i.e., CREB). This however was not found when instead of increasing cAMP a specific PKA agonist was utilized, suggesting that an alternate cAMP dependent pathway was involved. This was proven in an in vitro neurite outgrowth essay, where blocking PKA increased neurite outgrowth, however when this was combined with an inhibitor for another downstream target of cAMP (EPAC), outgrowth was significantly reduced. It appears that by blocking PKA activity, higher cAMP levels are available for EPAC to increase neurite outgrowth. Accordingly, when we applied an EPAC agonist we found significantly increased neurite outgrowth.

In conclusion this study shows that although PKA and EPAC act synergistically to translate rehabilitative training induced neuronal plasticity, the increase in EPAC activation is key to enhanced plasticity. This finding offers new specific pharmacological gateways to boost rehabilitative training.
Omega-3-polyunsaturated fatty acids as novel therapeutics for chronic central neuropathic pain following spinal cord injury

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Introduction: Central neuropathic pain (CNP) presents in about 41% of spinal cord injury (SCI) patients, and has a major impact on daily functioning. It affects rehabilitation, and often leads to anxiety, depression and even suicide. Currently there is no effective treatment for this condition. Spinal cord microglia are key players in the development and maintenance of SCI-CNP by releasing proinflammatory mediators such as TNF-α and IL-1β. Activated microglia express the nuclear peroxisome proliferatoractivated receptors (PPARs) and the retinoid X receptors (RXRs). Evidence shows that the omega-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) attenuate microglial activation in a rat compression model of SCI. EPA has been shown to attenuate TNF-α expression in activated macrophages in vitro. DHA and EPA are natural ligands to the PPARs and RXRs. Here, we hypothesized that in vitro treatment with DHA and EPA would a) prevent microglial activation when treatment is given simultaneously with the inflammatory agent lipopolysaccharide (LPS); b) attenuate microglial activation when treatment is given with a 4h delay following LPS exposure.

Methods: Primary rat microglial cell cultures were prepared using Wistar neonates (P3-5). After mechanical and chemical dissociation of the spinal cord and cortex tissue, cells were seeded in poly-Dlysine coated dishes and kept at 37°C. At DIV4 the dishes were manually shaken and the floating microglia collected, pelleted, re-suspended and plated onto uncoated coverslips. We examined the effects of DHA and EPA on microglial activation using different concentrations, i.e. 0.8, 4, 8, and 32 µM. After 4h treatment with DHA or EPA, cells were fixed with 4% PFA and processed for immunocytochemistry. Microglial proinflammatory phenotype specificity and levels of iNOS expression were confirmed using double immuno-labelling with Iba-1 (a pan microglial marker) and iNOS (a proinflammatory marker). The dynamic morphological changes of microglia in response to each treatment were photographed prior to fixation. Finally, mRNA levels of TNF-α and IL-1β in microglia were examined using quantitative RT-PCR.

Results: Over 95% of cultured cells were microglia, as assessed by Iba-1 and Hoechst (a nuclear dye). The data from the simultaneous treatment experiments showed that DHA at 0.8 µM and EPA at 32 µM significantly reduced iNOS expression in microglia when compared to the controls (LPS only). In the delayed treatment experiments, we only observed a significant reduction of iNOS by DHA at 8 µM when compared to the controls, whilst EPA was not effective at all concentrations. Microglia at the above effective concentrations of DHA and EPA showed typical quiescent bipolar shape in comparison to enlarged cell body with cytoplasmic granular vacuoles and a loss of bipolar shape in the controls. The quantitative RT-PCR data further showed significantly reduced expressions of TNF-α and IL-1β mRNA in microglia at the above effective concentrations when compared to the controls.

Conclusion: Our data have revealed potent effects of DHA at low concentrations in modulating microglial activation when given simultaneously at LPS exposure or at 4h delay following LPS exposure. In contrast, EPA at high concentration showed potent effects in modulating microglia when given simultaneously at LPS exposure. Future studies will investigate the mechanisms underlying DHA and EPA effects and their potential to prevent or attenuate SCI-CNP using clinically relevant in vivo models.

References:

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An outbreak of antimicrobial resistance: challenges faced on a spinal injuries rehabilitation unit

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Spinal Injury Rehabilitation is sometimes faced with unusual challenges. We describe a unique situation in the Welsh Spinal Injury Rehabilitation Centre in 2014 during which there was an outbreak of OXA 48 CPE. We present our experience in controlling this outbreak and the difficulties we faced. These difficulties include problems in patient isolation, contact with therapy services, incontinence related hygiene, cleaning of equipment and transfers to and from acute care.

Antimicrobial resistance is a worldwide issue due to increasing mortality attributed to it, increasing healthcare costs, difficulty treating infectious diseases and damage to trade and economies. Carbapenemase-producing Enterobacteriaceae (CPE) are a group of bacteria that have formed resistance to some of our most powerful antibiotics Carbapenems. These are usually last resort antibiotics for serious infections caused by drug resistant Gram-negative bacteria (including Enterobacteriaceae). Carbapenemase have become endemic in the United States of America and Greece, but are being increasingly reported worldwide over the past 14 years. OXA-48, a subtype of Carbapenemases was originally found in Turkey in 2003 from a Klebsiella pneumonia. This is now found throughout Europe, southern and eastern parts of the Mediterranean and Africa. It is usually produced by K. pneumoniae and E. coli.

In 2013 Public Health England produced a toolkit on how to detect, manage and control CPE. This advice applicable for acute healthcare settings contained no specific guidance for long stay rehabilitation units such as ours. Using recommendations from the guidance we were able to contain the outbreak without compromising on the ongoing successful rehabilitation program of our patients. This was achieved using universally increased infection prevention and control measures (without patient segregation), antibiotic stewardship, robust communication during transfers to acute care and patient education.

From February 2014 for a duration of 12 months 62 patients were screened. 7 tested positive for OXA-48 containing organisms. On subsequent rescreening only 2 patients remained positive. Importantly there were no incidences of active infection from these organisms during this period.

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Public Health England “Acute Trust toolkit for early detection, management and control of carbapenemase-producing enterobacteriaceae” PHE publications gateway number 2013314, Published December 2013.


NOX2 and reactive oxygen species are essential regulators of axonal regeneration

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Understanding the fundamental biological mechanisms responsible for nerve injury-dependent signals controlling the regenerative programme is key to our ability to design strategies for the enhancement of regeneration and recovery after nerve damage. Here, we show that NOX2-dependent production of reactive oxygen species (ROS) that have been classically shown to promote axonal degeneration, are required for axonal regeneration after nerve injury. Specifically, we found that CX3CR1-dependent recruitment of inflammatory cells after nerve injury induces axonal NOX2, which is retrogradely transported in axonal endosomes via importin β1 and dynein. Endosomal NOX2 inhibits PP1 via oxidation in the catalytic cysteines, which decreases the dephosphorylation of Rb, resulting in E2F1-dependent regenerative gene expression. Defying the dogma that ROS are exclusively involved in nerve degeneration, we show a specific NOX2 and ROS-dependent mechanism essential for nerve regeneration.

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Understanding translational failure: a systematic review and meta-analysis of animal studies of central neuropathic pain following contusion spinal cord injury

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Spinal Cord Injury inflicts profound and chronic impairment of sensation, including central neuropathic pain in 41% of patients – a debilitating and largely treatment resistant condition. Studying pain poses a number of practical and ethical difficulties. As such, pain research has mainly been performed using animal models. Despite advancements in the methodology of these studies, the failure of translation of research to the clinic remains a prominent issue, in particular in the development of effective analgesics for central neuropathic pain following spinal cord injury. In other neurological diseases, the failure to translate pre-clinical findings to effective clinical treatments has been partially attributed to bias introduced by shortcomings in the design of animal experiments. The aim of this study was to perform a systematic review and meta-analysis of published studies using an animal model of contusive spinal cord injury induced central neuropathic pain. Methodological design and quality and the impact of both on the reported effect sizes of behavioural outcomes in model characterizing and drug treatment studies were assessed. Additionally evidence of publication bias was sought. Data were extracted for reported study quality, design and for neurobehavioral outcomes and weighted mean difference meta-analysis was used. Egger regression and trim and fill analysis were used to identify publication bias. 64 publications met the inclusion criteria and were included in the meta-analysis. Reported methodological quality was low; less than half of publications reported blinded assessment of outcome or random allocation to group. No publication reported a sample size calculation. Studies with a higher quality score reported smaller differences in behavioural outcomes between intervention and control animals. Factors of external validity such as age, sex, time of assessment, severity of injury and the use of post-operative analgesia had an impact on reported effect sizes. Publication bias was found in studies assessing drug efficacy. In summary, our data suggest that the translational impact of animal models of spinal cord injury induced central neuropathic pain research may be enhanced by improving methodological quality. Factors of external validity should be considered to provide the most clinically relevant model of spinal cord injury.

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

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It is well established that exposing rodents to environmental enrichment (EE) increases neural activity, leading to structural and synaptic plasticity in the brain.\(^1,2\) Given the similarities in transcriptional signaling between neuronal plasticity as well as neurogenesis and axonal outgrowth, we hypothesised that EE could promote axonal outgrowth and regeneration by shifting the transcriptional program towards a regenerative profile. We demonstrate that 10 days exposure to EE alone or in combination with a conditioning injury dramatically increases DRG outgrowth on both permissive and inhibitory substrates in vitro. Strikingly, exposure to EE for just 10 days primed DRG neurons to provide a lasting increase in their outgrowth potential in vitro, which was retained for at least 4 weeks after the mice were placed back into standard housing. Following nerve injury in vivo, 10 days pre-exposure to EE leads to enhanced axon regeneration and significant electrophysiological and behavioral improvements, which were again further enhanced when combined with a conditioning injury. After observing no changes in the levels of neurotrophic factors or cytokines in the extrinsic DRG environment following EE we hypothesised that EE causes a lasting increase in the intrinsic growth potential of DRG neurons via epigenetic modulation and/or changes to gene expression. Combinational analysis of RNAseq from laser captured large-diameter DRGs and proteomic analyses from axoplasm demonstrate that EE increases neuronal activity and axonal transport. Furthermore, we show that EE modulates the epigenome of DRG neurons via activation of CBP and increased levels of H4K8ac and H3K27ac, both markers of transcriptional activation. Ongoing experiments aim to elucidate the link between enhanced neural activity and the observed changes to epigenetic modifications and gene expression following EE induced outgrowth and regeneration. Ultimately, while EE has been shown to be a promising post-injury rehabilitative therapy, here we demonstrate that pre-exposure to EE enhances axon regeneration by increasing the intrinsic regenerative potential of DRG neurons. This could be used to identify novel regenerative molecular targets for future gene therapies or drug-based approaches to treat SCI.

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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. 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. 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 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.

References:

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Astrocyte remodeling in response to GSK3β inhibitors and chondroitinase ABC ex vivo in a spinal cord slice culture model

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Astrocytes constitute a major element of the mature glial scar that forms as a result 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 combinatorial treatment with GSK3β inhibitors and chondroitinase ABC (ChABC) has a synergistic effect on regulating the astroglial scar to one that is more permissive for axon growth. To test multiple combinatorial treatments with numerous GSK3β inhibitors that have different modes of action is not feasible in vivo, whilst in vitro cultures of astrocytes do not reflect the complex three-dimensional multicellular environment of the spinal cord. To help overcome these issues, we have developed ‘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 or optic nerves from mice aged 3-weeks were maintained in culture for 3 to 7 days in vitro (DIV) and treated with a range of GSK3β inhibitors (lithium chloride, ARA014418, tideglusib or TWS119) or ChABC. Inhibition of GSK3β and treatment with ChABC induced morphological changes in astrocytes in the spinal cord, 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. 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. To examine this, we plan to seed neuronal cultures onto the spinal cord slice culture and measure neurite outgrowth. These experiments will allow us to determine the optimal combinations of GSK3β inhibition and ChABC for regulating the astroglial scar to one that is more permissive for axon growth, prior to determining whether they promote reformation of connections and recovery of function in vivo in SCI.

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Neurotrophin-3 normalises spinal reflexes after central nervous system injury

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Neurotrophin-3 (NT3) promotes the survival and neurite extension of specific neuronal populations including Ia proprioceptive fibers. We are investigating the mechanism by which NT3 modulates spinal reflexes mediated via this neuronal population and motor neurons in vivo.

Firstly, we evaluated retrograde trafficking of NT-3 towards the spinal cord following AAV-NT3 overexpression in forelimb muscles. We have detected increased levels of NT3 protein in dorsal root ganglia connecting to the treated muscle groups. We are currently investigating transcriptional and post-translational changes of downstream molecules of its receptor trkC in dorsal root ganglia.

Secondly, we use electrophysiology to functionally test the effects of NT3 overexpression in the muscle on the modulation of spinal reflexes involving proprioceptors and motor neurons. Rodents, like humans, develop spasticity after spinal cord injury or stroke, caused by the hyper-excitability of the spinal reflex pathway. Thus, we developed an animal model, which allows for repeated electrophysiological assessment of this monosynaptic reflex. More specifically, we recorded the H-reflex from the abductor digiti quinti, a forepaw muscle, in rats every two weeks up to 10 weeks after a bilateral pyramidotomy. Rats were treated 24 hours post-injury by injection of an AAV-NT3 (or AAV-EGFP as a control treatment) into the forelimb flexor muscles on one side. Naive animals show a frequency-dependent depression of the H-wave at higher stimulation frequencies. After injury, this effect is reduced. However, neurotrophin-3 treated animals recover to baseline levels 6 weeks post-injury. Furthermore, rats treated with neurotrophin-3 have normalized polysynaptic reflexes and recover some motor function in their forelimbs whereas the control treated animals do not.

Due to the profound effects observed after spinal cord injury following NT3 treatment, our data shows that spinal reflexes can be positively modulated after CNS injury with intramuscular neurotrophin-3 treatment.

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Using in vivo conditional silencing to dissect the central pattern generator: emerging hierarchies

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Models of the intrinsic spinal cord circuitry responsible for stereotypic stepping, the central pattern generator for locomotion or CPG, have evolved to include rhythm generation and pattern formation components, with the former being responsible for right-left alternation. The powerful new tool of conditional silencing developed by Tadashi Isa and colleagues has allowed exploration of the roles played by anatomically-defined propriospinal neurons (PN) in the central generation of locomotion in the intact, alert and behaving adult rat. We have targeted two populations of PNs with cell bodies at L2 that project to either the L5 or C6 spinal segments. Conditional silencing of L2-L5 PNs disrupted hindlimb right/left alternation during stepping resulting in a hindlimb hopping gait with no disruption of forelimb alternation or flexor/extensor (intralimb) coordination. Conditional silencing the L2-C6 long ascending PNs disrupted right/left alternation in both forelimbs and hindlimbs without significantly altering stepping frequency or intralimb coordination. The phenotypes were robust, reversible and repeatable and occurred in all animals silenced. Approximately 20-30% of steps showed a disrupted phase relationship during silencing, and the entire range of possible phases, from 0.5 to 1 (or 0) were represented in the data set. These results suggest that silencing L2-L5 and L2-C6 propriospinal neurons functionally de-couples the right and left sides of the pattern generating circuitry of the hindlimbs or of all four limbs, respectively, without disrupting either the CPG “clock” or the flexor/extensor half-centers. Thus, these data argue that CPG circuitry involves a “half center” responsible for R/L alternation that is distinct from the rhythm generation and pattern formation components.

Interestingly, swimming, as a bipedal mode of locomotion, was not disrupted by silencing either class of interneuron, supporting the concept that swimming is a default, hard-wired pattern that is relatively refractory to perturbation by sensory input, in stark contrast to stepping that is powerfully modulated by afferent input. Taken together, our results lead us to speculate that these two classes of L2 propriospinal interneuron integrate CPG activity and sensory input to ensure R/L alternation during normal overground stepping.

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Systematic investigation of the epigenetic regulation of axonal regeneration

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Insight into the molecular mechanisms that control axonal regeneration after spinal cord injury has revealed a number of signaling cascades including IGF1, PI3K and PKA-dependent pathways. These signaling events ultimately culminate in the recruitment and activation of specific transcriptional machinery, leading to the downstream activation of regenerative associated genes, needed for long-term regenerative reprogramming. This implicates that successful regeneration is likely to depend on the interplay and cross-talk of regenerative signaling networks. Given that transcriptional control is regulated by specific epigenetic signatures including after nerve injury, we hypothesize that defined epigenetic marks provide a specific transcriptional environment that determines whether regeneration occurs or fails.

Here we provide insight into a systematic investigation of the genes, epigenetic modifications and upstream signaling pathways that are associated with regeneration or regenerative failure after sciatic peripheral or central spinal cord injury, respectively in dorsal root ganglia (DRG). To this end, we employ a combinatorial large scale H3K27ac and H3K9ac ChIP-seq, RNA-seq and proteomic analysis of the DRG cell body-axonal unit after central or peripheral nerve injury. We identify injury responsive genes that are targets of the histone acetyltransferases CBP/p300 and PCAF or conversely, that are negatively regulated by histone deacetylases such as HDAC3 and are enriched in pro-active histone marks, including H3K9ac and H3K27ac. We also provide a bioinformatic analysis correlating H3K27ac and H3K9ac enrichment with injury responsive gene and protein regulation, describing how different quantitative/qualitative responses are induced when comparing peripheral versus central axotomies.

In order to clarify the functional implications of this differential molecular response, we are currently working towards identifying upstream signaling cascades that may affect CBP/p300 and HDAC3 through post-translational modifications, altering their enzymatic activity, upon peripheral and central injuries. Ultimately, this work aims to identify the specific events that converge on the epigenome to promote or inhibit regeneration, optimising the identification of new drug targets for treatment following spinal cord injury.

References:

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Plasticity of spared thoracic interneurones rostral to a lateral spinal cord section

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The regenerative capacity of the neurones of descending tracts, axotomised in the spinal cord, is generally quite limited and is usually restricted to short range sprouting above the injury. There has, therefore, been a recent focus on the role of local interneuronal systems in receiving this short range sprouting from descending tracts and creating detours around the injury (Stelzner and Cullen 1991, Arvanian et al. 2006; Bareyre et al 2004, Courtine et al 2008). Relevant to this, we investigated plasticity of ventral horn interneurones (INs) in the ipsilateral segment immediately rostral to a spinal lateral hemisection, but with dorsal columns spared (segments ranged from T5-T12, median T8).

The data come from 14 adult cats of either sex, initial weights 1.9 – 3.6 kg. Control data came from acute experiments on uninjured animals previously described (Saywell et al. 2011). At 6-8 or 16–18 weeks post-injury, non-axotomised thoracic INs (mostly contralaterally projecting) were intracellularly labelled with Neurobiotin. Fourteen INs were labelled in the lesioned cords and successfully reconstructed, either for their somadendritic, or for their axonal morphologies or both. A number of abnormal features were observed in the dendritic tress of the INs including thickened proximal dendrites, tortuous structures, increased right-angled branching, asymmetry of the dendritic tree, increased numbers of varicosities along dendritic processes and dendritic processes crossing the midline.

Axons were recovered for 14 interneurones from 12 animals, 6-8 weeks post-lesion (n= 4) and 16-18 weeks post lesion (n= 10). Axons from INs of the lesioned cords had significantly more collateral branches in the first 4mm of the main axon than controls (2.31 vs 1.07, P =0.0379 Mann-Whitney, n = 13 lesion, 14 control). Individual collateral branches from the same axons were more likely to overlap in the rostral-caudal plane than controls (P=0.0457, Fishers exact test n = 14 lesion, 16 control). Areas containing boutons were measured and expressed a percentage of the total ventral horn area. From this is could be seen that axon collaterals from INs in the lesioned cords terminated in a significantly larger percentage of the ventral horn than controls (13.7% vs 4.9% P= 0.0006, Mann Whitney, n=12 lesion, 12 control). The ventral horn (including the intermediate areas) was divided into 12 specific zones. Consistent with the previous result, it was found that collateral branches from INs in the lesion group terminated in significantly more zones than controls (5 vs 7.4, P= 0.0064, T-test, n=12 lesion, 12 control). The zones with the largest increase in projections were the intermediate areas including Clarke’s Column, the intermediolateral columns and the intercalated nucleus, suggesting implications for autonomic function.

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An integrin strategy for corticospinal tract regeneration

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Integrin receptors form a trans-membrane link between the intracellular and extracellular compartments of cells. These cell-surface receptors mediate cell adhesion to the extracellular surface and signalling across the cell’s plasma membrane. To date, 24 types of integrins have been identified and each receptor bears its own binding affinity for extracellular matrix (ECM) substrates. Importantly, integrins can enhance the intrinsic regeneration potential of axotomized neurons.

My PhD training aims to explore the therapeutic potential of integrins to promote axon repair of the corticospinal tract after spinal cord injury (SCI). The poster will outline the ‘integrin strategy’ and highlights three issues that limit axonal regeneration in the injured central nervous system (CNS). Firstly, the adult CNS fails to up-regulate the correct integrins that interact with the ECM at the lesion site. Secondly, integrins become inactivated at the lesion site. Thirdly, integrins are excluded from mature CNS axons.

Based on recent data in our laboratory, we have selected three molecules to overcome these problems:
- Alpha9 beta1 integrin
- Kindlin1
- shRNA Ankryn G

Hopefully, this integrin approach will lead to successful regeneration of the corticospinal tract and promote motor recovery after SCI.

References:

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Restoring voluntary walking in individuals in severe spinal cord injury via a closed-loop spinal stimulation

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Gait disturbance in individuals with spinal cord injury (SCI) is attributed to the interruption of descending pathways to the spinal locomotor center, whereas neural circuits below and above the lesion maintain their functional capability. An artificial neural connection (ANC), which bridges supraspinal centers and locomotor networks in the lumbar spinal cord beyond the lesion site, may restore the functional impairment (Sasada et al., 2014). To achieve an ANC that sends descending voluntary commands to the lumbar locomotor center and bypasses the thoracic spinal cord, upper limb muscle activity was converted to magnetic stimuli delivered noninvasively over the lumbar vertebra. Five individuals with severe SCI at thoracic level participated in the experiment. All participants were able to initiate and terminate walking-like behavior and to control the step cycle through an ANC controlled by volitional upper limb muscle activity. The walking-like behavior stopped just after the ANC was disconnected from the participants even when the participant continued to swing arms. The induced walking-like behavior became significantly larger by additional voluntary effort of walking. These results demonstrate that the ANC induces volitionally controlled, walking-like behavior of the legs in severe SCI individuals. This paradigm was able to compensate for the dysfunction of descending pathways by sending commands to the preserved locomotor center at the lumbar spinal cord and enable individuals with paraplegia to regain volitionally controlled walking.


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Modeling cervical ventral root injury in vitro

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During high impact traumas such as motorcycle accidents, spinal nerve roots of the brachial plexus can be torn away or ‘avulsed’ from the spinal cord surface resulting in a longitudinal spinal cord injury with debilitating and permanent paralysis of the shoulder, arm and hand. Despite recent advances in the surgical reconstruction of injured nerve roots (i.e. nerve root re-implantation), adjunctive therapies for ventral root avulsions are required if functional recovery to the arm and hand is ever to be reliably achieved. Incomplete repair after ventral root avulsion and re-implantation may be due to several factors. Despite trophic support provided by the re-implanted nerve root, almost 50% of motoneurons in the ventral horn of the spinal cord undergo chromatolysis and apoptosis (Eggers et al. 2010). Motoneurons that do survive must send axons through disorganized and gliotic white matter to reach the re-implanted nerve root. Therapeutic interventions that promote ventral horn motoneuron survival and enhance axon regeneration and targeting into the re-implanted ventral root are required to improve functional recovery.

Olfactory ensheathing cells have shown significant potential for the treatment of spinal cord injuries (Raisman, Barnett, and Ramón-Cueto 2012). Our laboratory is currently developing autologous human olfactory ensheathing cell (hOEC) transplants from patient biopsies of olfactory mucosa and plan to trial these therapeutic cultures in patients with brachial plexus ventral root avulsion injuries. Prior to clinical application, characterization of the biological activity of our hOEC therapy product must be completed for product specification and regulatory compliance using a range of complimentary in vivo and in vitro bioassays. We have developed an in vitro organotypic rat spinal cord slice culture assay to characterize the neuroprotective and neurite outgrowth effects of hOEC cultures on postnatal cervical ventral horn motoneurons. This culture system models proximal axotomy of relatively mature ventral horn motoneurons and maintains many aspects of the in vivo spinal cord architecture, local neuronal connections and astrocytes.

Our initial experiments indicated that rat olfactory bulb OECs enhanced neurite outgrowth over collagen control substrates in serum containing medium. We have now conducted further validations to characterise the response of the model to control substrates and soluble factors in serum free medium. Our results show the model is capable of producing a dose response to soluble growth factors (GDNF) quantified as the number of choline acetyltransferase positive ventral horn motoneurons per slice, and distinguishes between mildly (collagen) and highly (matrigel) permissive growth substrates quantified by Scholl analysis of neurofilament positive neurite outgrowth. Thus, the model will provide an excellent system for assaying the neuroprotective and neurite outgrowth effect of our hOECs. We plan to use this model to assess the impact of variations in the proportions of OECs to fibroblasts in our hOEC cultures.

References:


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A multinutrient preparation designed to enhance synapse formation and function improves outcome in experimental spinal cord injury

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Spinal cord injury (SCI) leads to major neurological impairment, associated with significant tissue loss. Endogenous repair processes occur following SCI, but they are limited. Recent clinical trials in Alzheimer’s disease have demonstrated the efficacy of Fortasyn Connect (FC), a specific multinutrient combination that was designed to compensate for the loss of neuronal membranes and synapses in dementia patients (Wurtman et al., 2006; Wurtman et al., 2009a, b), and that contains docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), choline, uridine monophosphate, phospholipids, folate, vitamins B6, B12, C, E and selenium. We tested if this multinutrient combination countered the tissue destruction occurring after SCI and supported regenerative processes, improving the neurological outcome. Adult rats received an injury induced by cord compression at thoracic level (Huang et al., 2007), and immediately after SCI they were fed daily with a control diet or a diet supplemented with different doses of the specific FC multinutrient combination (low dose FC, medium dose FC, or high dose FC) for 4 or 9 weeks. At 4 weeks, only 50% of rats that were fed the control diet were able to plantar place their paws, and only 2 rats had recovered gait coordination. In contrast, 6 out of 7 rats fed the diet with the high dose of FC had recovered a coordinated gait. Five of them showed a normal position of the paws and full recovery of toe clearance, and 2 of them showed a gait that was undistinguishable from that of uninjured rats. The BBB score was 17.1 ± 1.6 in this group, in comparison with the BBB score of 8.8 ± 1.3 in rats fed the control diet. This was accompanied by significant protection of oligodendrocytes and myelin in the injured tissue, a decreased microglial neuroinflammatory response, and an increase in pre- and postsynaptic markers. The medium dose of FC that did not show efficacy after 4 weeks of treatment led to improved motor score, increased neuronal and oligodendrocyte survival, decreased microglial activation, and better axonal preservation after 9 weeks of supplementation. These results suggest that a diet supplemented with this specific multinutrient combination has marked therapeutic potential in SCI.

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Supported by Nutricia Research
Combining electrical stimulation with ibuprofen overcomes MAG-induced inhibition of nerve growth

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A phase I human clinical trial proved the safety of oscillating electric field stimulation (OFS) for SCI and suggested improved sensory function (Shapiro et al., 2005). Despite this promising result the conditions were probably not ideal since no systematic study optimized the electric field strength and the electrical wave form (e.g., oscillation frequency). Furthermore, it is probable that combining growth promoting drugs with OFS would improve outcome, as shown in a SCI model using OFS combined with the natural nucleoside inosine (Bohnert et al., 2007). Here, we used time lapse microscopy to compare growth responses of amphibian spinal neurons in a steady 150 mV/mm DC electric field (DCEF) and oscillating electric fields (OFS) of various frequencies with and without the non-steroidal anti-inflammatory drug ibuprofen. We chose ibuprofen because it improved axon regeneration in rodent SCI by preventing elevation of rho A (Fu et al., 2007). We tested the ability of these treatments to overcome the action of the natural inhibitory molecule myelin associated glycoprotein (MAG) because it contributes to the failure of spontaneous recovery following spinal cord injury (SCI) by causing growth cone collapse.

Our results confirmed previous observations that a steady DCEF of 150 mV/mm increased neurite growth rates and caused growth cones to deflect toward the cathode (negative pole) of the DCEF (Rajnicek et al., 2006). When the electric field polarity was reversed every 30 minutes growth cones deflected toward the new cathode during each interval, leaving a ‘wavy’ path as they extended, but electrical stimulation did not affect the overall growth rate. However, when the OFS frequency was 50 Hz neurites extended more rapidly than controls. Therefore, steady DCEF stimulation and 50 Hz OFS stimulation each improved neurite growth. Adding 100 µM ibuprofen to the culture medium accelerated growth rates to 276% of controls (no drug). When a steady DC EF of 50 mV/mm or 150 mV/mm was applied in addition to 100 µM ibuprofen neuron growth rates were significantly higher than for DCEF treated cells without the drug. Importantly, ibuprofen enhanced the sensitivity (extent of cathodal reorientation) of growth cones to the DCEF. This implies that ibuprofen could permit use of smaller stimulation intensities during SCI electrotherapy, thus reducing unwanted side effects near the electrodes.

We confirmed that MAG (0.1 µg/ml) slowed neuron growth and that a 150 mV/mm DCEF applied during MAG exposure restored growth rates to the same level as no drug, no EF controls. When ibuprofen was added to MAG-treated cells it improved growth rates to control (no drug, no EF) levels. Most interestingly, when an EF was applied to MAG-exposed cells in combination with ibuprofen the growth rate was faster than MAG with a DCEF and also faster than MAG with ibuprofen but no EF. Therefore, the combination of ibuprofen and DCEF stimulation overcame the growth inhibitory influence of MAG more than either alone. These data will have impact upon new combined electrotherapies for central nervous system repair.

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Intravenous immunoglobulin therapy attenuates complement activation and improves recovery from spinal cord injury

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Introduction: Spinal cord injury (SCI) leads to entry of blood proteins and circulating immune cells into the neural parenchyma. The resulting inflammatory response, which involves robust activation of the complement system [1], contributes to secondary injury and impairs recovery. This study aimed to determine whether intravenous immunoglobulin (IVIg) therapy, which is already used clinically for treating a variety of inflammatory conditions, is also effective in contusive SCI.

Methods: C57BL6/J or Emx1-creERT2:Rosa26-tdTomato mice were subjected to SCI as detailed previously [2]. One hour later, IVIg (0.05-2 g/kg), vehicle or albumin (protein loading control) was administered via the femoral vein. Recovery was monitored via the Basso Mouse Scale (BMS) for locomotion [3]. MRI/DTI was used to assess lesion development [4,5]. Routine biochemical and histological techniques were used to assess complement activation and tissue pathology.

Results and Conclusions: IVIg administration attenuated complement system activation based on acute reductions in tissue C3/C3b, C3a and C5a levels (1 day post-injury). Functional and histopathological outcomes were also improved for IVIg doses of 0.5 g/kg and above (35 days post-SCI). The therapeutic effect of IVIg was detectable via non-invasive diffusion tensor imaging (DTI), in particular its amelioration of SCI-induced increases in radial diffusivity (RD) in the ventrolateral white matter. In summary, this study highlights the prospect of using IVIg as an immune-modulatory treatment in SCI, and also the potential of DTI to assess tissue damage and screen for the efficacy of promising candidate interventions in preclinical studies.

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Characterization of a novel axon growth repellent and its role in spinal cord injury

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In vertebrate embryogenesis, the developing nervous system undergoes a segmentation process through the formation of the mesodermal somites. In particular, motor axons outgrowing from the neural tube follow a segmented pattern controlled by several mechanisms, including growth cone repulsion, that ensure that the peripheral nervous system develops without obstruction within the future cartilage and bones of the vertebral column. In this context, repellent molecules guide navigating axons by excluding them from “no-go” areas in the embryo. Following injury to the adult brain and spinal cord, growth cone repulsion may also block regeneration, with serious clinical consequences. Among the candidate molecules, PNA-binding glycoproteins, chondroitin sulphate proteoglycans (CSPGs), Eph/Ephrins and semaphorin 3A have been proposed as repellents acting on different receptor systems expressed by primary sensory axon growth cones.

Recent work in the lab has identified a protein in embryonic somites that generates spinal nerve segmentation by contact-repulsive axon guidance. The protein is expressed selectively on the surface of somite cells, and cause collapse of axon growth cones when applied to neuronal cultures. In addition, detergent extracts of mammalian (rat) grey matter and of a cultured line of human astrocytes have been shown to possess growth cone collapse-inducing activity. My experiments have indicated that this central nervous system (CNS)-derived activity has molecular properties closely 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. The overall aim of the project is to examine this novel CNS-derived system in more detail to confirm its molecular identity, elucidate how it is related to the somite-derived axon repellent, and assess its role in spinal cord injury.

The experimental objectives are: 1) to further identify the somite protein responsible for the segmented pattern of the developing spinal cord, using the chicken somite system (in ovo experiments, molecular cloning and RNA-sequencing); 2) to identify the protein responsible for this activity in human astrocyte extracts; in addition to live immunostaining, this includes purification of the protein using biochemical techniques and functional testing using collapse assays; 3) to test the most effective in vitro inhibitor of protein for its ability to promote axon regeneration and functional recovery (in collaboration with Pr James Fawcett).

References:

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Short- and long-term effects of neural precursor transplantation after spinal cord injury

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Mid-cervical contusion injuries result in devastating functional consequences, including impaired respiration. Disruption to the phrenic circuit can cause permanent paresis or paralysis of the diaphragm - the major muscle of inspiration. For spinal cord injury patients, this can mean ventilator dependency, which incurs significant financial cost and diminishes quality of life. Additionally, respiratory failure and related complications are two of the highest contributing factors to morbidity and mortality in the clinical population. Thus, there is an urgent need for improved therapeutic approaches to improve respiratory function following spinal cord injury. Recent work in our lab has demonstrated that spinal interneurons may contribute to ongoing plasticity in the injured cord that results in modest functional improvement. We hypothesize that this population of cells could serve as an ideal therapeutic target for enhancing this endogenous plasticity and optimizing recovery. The present studies examine the therapeutic potential for the transplantation of precursors cells derived from developing spinal cord tissue. The primary goal in using this tissue - inherently rich in interneuronal progenitors - is to reconnect the phrenic circuit and improve respiratory function following mid-cervical spinal cord injury.

Adult, female Sprague Dawley rats received lateralized, contusion injuries (200 kilodynes; Infinite Horizon Impactor). One week post-injury, animals received an injection of dissociated developing spinal cord tissue (derived from E13.5 Sprague-Dawley or F344-EGFP Fisher rats) directly into the lesion epicenter. Animals receiving transgenic GFP allografts were immunosuppressed with CSA beginning 5 days after injury and continuing throughout the duration of the experiment. At either 1 or 12 months following transplantation, pseudorabies virus (a retrograde transynaptic tracer) was applied either to the ipsilateral hemi-diaphragm or injected into the transplant. Animals recovered for 72 hours, at which time terminal electrophysiological recordings of bilateral diaphragm activity were made under baseline (normoxic) or challenged (hypoxic, 10% oxygen) conditions.

Anatomical studies reveal long-term survival of FSC tissue one year following transplant, comparable to that seen at one month post-transplant. However, there is a reduction in the number of transplanted cells that synaptically integrate with the host phrenic circuit over this time point. Despite this apparent reduction in graft-host connectivity electrophysiological results consistently show greater phrenic activity in the ipsilateral hemidiaphragm compared to the contralateral side. Furthermore, the response to respiratory challenge was improved in transplant treated animals compared with untreated or vehicle treated controls. Ongoing studies are exploring the short- and long-term fates of transplanted cells, and how these phenotypes might change over time.

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Investigating the therapeutic and biochemical effects of local hypothermia for spinal cord injury

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Therapeutic hypothermia for spinal cord injury (SCI) has been intermittently studied since the 1960s when it was first shown to greatly improve the level of recovery in experimental models of SCI. Despite promising replication studies, post 1980s further investigation was sidelined in favour of systemic hypothermia and pharmacology. As a protective agent, hypothermic benefits are likely derived from downstream effects of reduced metabolism and blood flow alterations that attenuate some aspect of secondary injury. In theory, these benefits could be magnified at lower temperatures, but complications associated with systemic cooling including coagulopathies and bradycardia are exacerbated sub-33°C. Localised hypothermia in which only the injury site is cooled can circumvent these issues while achieving greater cooling depths.

We have developed a novel methodology to cool the adult rat spinal cord to as low as 5°C after contusion injury in a steady, controllable and safe manner. This technique allowed us to perform the first direct comparison of either no cooling or 2 hours of profound (10°C), moderate (20°C) or mild (33°C) hypothermia administered to the exposed injury site, 15 minutes after a cervical level contusion. Motor and sensory tasks revealed that mild hypothermia significantly improved behavioural recovery after injury with a modest improvement in tissue sparing, but no benefit was evident with moderate or profound hypothermia. Hypothermia was noted to effect infiltration and inflammation in the acute stages after injury in a temperature dependent manner. While mild hypothermia is beneficial, any benefit to moderate and profound hypothermia may be undermined by some reperfusion injury associated with the rapid rate of spinal cord rewarming on cessation of cooling. Controlling the rate of rewarming along with investigating whether hypothermic suppression of pro-inflammatory mediators underlies the benefits of mild local hypothermia is the subject of ongoing work.

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Re-establishment of cortical motor output maps and spontaneous functional recovery via spared dorsolaterally projecting corticospinal neurons following spinal cord injury

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Regeneration of lesioned fibers is limited in the adult mammalian CNS, but many individuals that sustain incomplete spinal cord injuries undergo spontaneous functional recovery. Cortical plasticity contributes to spontaneous recovery following spinal cord injury (SCI) but the mechanisms underlying re-formation of cortical output maps or functional recovery are incompletely understood. We performed optogenetic mapping of motor cortex in ChR2 expressing mice to assess the capacity of the cortex to re-establish motor output in the weeks following a C3/C4 dorsal column SCI, which bilaterally ablates the dorsal corticospinal tract (CST) containing >95% of corticospinal fibers. The remaining <5% corticospinal axons run in the dorsolateral column and there are no ventral corticospinal axons in the mouse. Optogenetic mapping revealed extensive early deficits followed by re-establishment of motor cortical output maps to the limbs at the same latency as pre-operatively by four weeks post-injury. Behavioural analysis of skilled locomotion on the horizontal ladder revealed initial deficits followed by partial spontaneous recovery at 42 and 56 days post-injury. Transient silencing of spared corticospinal neurons projecting in the dorsolateral spinal cord (site of AAV-cre injection at C7) via activation of the inhibitory DREADD receptor hM4Di injected into the cortex abrogated spontaneous recovery and resulted in a greater change in skilled locomotion than in control uninjured mice using the same silencing approach. Thus, relatively few dorsolaterally projecting corticospinal neurons are able to substantiate remarkable motor cortical plasticity and partial spontaneous recovery.

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

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Introduction
There is no cure currently available for spinal cord injury. A silk-based biomaterial (Spidrex®) has been shown to support neurite outgrowth of adult rat dorsal root ganglion (DRG) neurons in vitro and to promote excellent axonal regeneration in a rat model of sciatic nerve injury (1). One of the key features of Spidrex® is the presence of numerous repeated sequences of arginine-glycine-aspartic acid facilitating cell attachment to the material by integrins. 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 CNS axonal regeneration has not yet been investigated.

Hypothesis
In this study we hypothesised that 1) Spidrex® silk fibres would support neurite growth of embryonic Xenopus spinal cord neurons and postnatal rat cortical neurons in vitro; 2) a combination of Spidrex® and DHA would further enhance neurite outgrowth of postnatal rat cortical neurons when compared to either Spidrex® or DHA alone; 3) Spidrex® silk fibres would induce minimal immune response from microglia.

Methodology
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 on PDL coated glass coverslips or uncoated tissue culture dish. 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. HCA-vision and Image J were used to quantify total neurite length, longest neurite per neuron, direction and speed of neurite outgrowth. 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. Expression of iNOS inflammatory marker and levels of nitrite release were determined using immunocytochemistry and Griess assays respectively.

Results
We showed that Spidrex® silk fibres supported excellent outgrowth of CNS neurons. Particularly, there was a significant proportion of Xenopus and rat cortical neurons engaging with the silk within 100 and 200 μm from the edge of the fibre by growing along, towards or across the biomaterial. We found that DHA promoted neurite outgrowth of rat cortical neurons in a concentration-dependent manner. Furthermore, incubation of 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 with levels of iNOS and nitrite release similar to controls and significantly lower when compared to LPS treated cells. Conclusion 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.

References:

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Utilizing residual voluntary pelvic floor muscle control to improve continence in incomplete spinal cord injury: a pilot study

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Objectives: to determine whether 16 weeks of regular pelvic floor muscle training (PFMT) using a biofeedback device could improve the strength and endurance of voluntary PFM contractions in incomplete spinal cord injury (iSCI) so as to suppress neurogenic detrusor over-activity (NDO) and reduce incontinence.

Setting: The London Spinal Cord Injury Centre, Stanmore, UK.

Methods: An 8-week programme of pelvic floor muscle training was conducted in 5 males and 1 female subject with stable supra-sacral motor iSCI (AIS C&D). Clinical evaluations before and after training comprised assessments of voluntary control of the sphincters and PFM; urological function and quality of life impact questionnaires related to continence. Neurogenic detrusor over-activity (NDO) was determined by standard cystometrogram (CMG) tests of bladder function. At the assessment points repeated CMGs were performed. In a second set of CMGs participants were instructed to perform sustained PFM contractions when NDO presents. Participants stopped their bladder medication five days before each CMG tests and resumed their medication immediately afterwards. The primary outcome measure was determined from the ability to suppress NDO using 10 seconds PFM contractions calculated from the peak detrusor pressures before and after the PFM contractions. Secondary outcome measures included pelvic floor muscle strength and endurance and QoL. Wilcoxon sign rank test was used to analyse the suppression of NDO.

Preliminary results: All 6 subjects presented with residual bladder sensations and motor function of the PFM but with NDO, DSD and incontinence. After 8 weeks of PFMT with biofeedback, all six subjects improved their ability to suppress NDO from 10% to 126%, the suppression of NDO was statistically significant. PFM strength using the modified Oxford score showed an improvement of up to 34% in three subjects and no changes in the other three. The ICIQ-UI score was applicable in 5 subjects of which 2 showed improved continence (10% and 30%).

Conclusion: The interim data analysis from six subjects provide evidence that an 8 week programme of pelvic floor muscle training with biofeedback can have a significant effect on promoting voluntary control over neurogenic detrusor over-activity related to an increased control over the PFM and can reduce incontinence in selected individuals with an iSCI. Further analysis will be performed when all subjects have completed the 16 weeks programme.

References:

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Appraisal and restoration of respiratory patterns following cervical contusion

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Injury and disease can cause profound and robust alterations to inherent respiratory function and capacity. Indeed, cervical spinal cord contusions cause significant anatomical damage. However, compensatory mechanisms act to conceal the extent to which the respiratory motor system is adversely affected. In this study, we examined how respiratory function was altered in acute and chronic injuries through assessment of respiratory physiologic endpoints, ventilatory pattern variability, and the electromography of multiple inspiratory muscle groups. Cervical spinal cord injury in rats substantially altered tidal and minute volume, sample entropy, mutual information, and the magnitude of muscle output. Typical respiratory activity was not regained in animals at both acute and chronic stages of injury, possibly due to a dearth of serotonin. Further, we demonstrate that there was minimal endogenous recovery of respiratory function. When isolated, these contused pathways were insufficient to maintain respiratory activity. These data reveal the remarkable effect cervical contusion has upon respiratory motor activity and the need to enhance endogenous plasticity following injury to promote functional recovery. To this end, application of chondroitinase ABC (ABC) facilitated the rescue of respiratory motor function through contused pathways at both acute and sub-chronic time points post injury. Enzyme activity was necessary and sufficient to robustly evoke recovery of respiratory function regardless of time post-injury. Collectively, these exciting results further our comprehension of cervical contusion injuries and the effects upon respiratory motor function. They hold the potential to develop a methodology whereby clinical respiratory outcomes may be predicted immediately following a cervical injury and thus a physiologically appropriate treatment strategy applied. To this end, these data have the potential to aid development of a specific and personalized clinical treatment strategy for respiratory dysfunction following both acute and chronic cervical spinal cord contusion.

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Human iPSC-derived corticospinal neuron grafts following cervical hemisection injury

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Cervical SCI accounts for over half the current injuries and contributes significantly to the overall life-long morbidity and mortality. The transplantation of neural stem cells and neuronal progenitors has the potential to rebuild circuitries and repair functional deficits after SCI. Implantation of embryonic neural tissue has been well established over decades of research but it is a mixture of cells that require further elucidation in their survival, integration and promoting axonal elongation. In addition, NPCs derived from embryonic stem cell and induced pluripotent stem cells (iPSC) derived cortical pyramidal neurons and implanted these cells which are enriched for corticofugal neurons into a cervical hemi-lesion in immunodeficient rats. Cells were characterized prior to transplantation and following 6-12 weeks. Using a combinatorial treatment (graft with forelimb-specific rehabilitation regimen), we hope to facilitate functional recovery. We find that the vast majority of transplanted cells survive and express markers of cortical neurons. In addition, extensive axon outgrowth with the cord can be observed.

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Development of interneuronal progenitors transplanted into the injured spinal cord

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Spinal cord injury (SCI) results in a vast range of motor and sensory deficits that reduce quality of life and increase the risk of mortality. More than half of these injuries occur at the cervical level which results in some degree of respiratory deficiency. Although there has been increasing evidence for spontaneous neuroplasticity and improvements in respiration, functional recovery remains limited, yielding an urgent need for the development of therapeutics targeted at restoration of respiratory function following SCI. One therapeutic strategy gaining recognition for repair of the injured spinal cord is the transplantation of neural precursor cells (NPCs) as a source of new neurons and glia. NPC transplants have been shown to survive, differentiate, form functional synaptic connections between donor and host cells, and enhance functional recovery. However, little is known about the development of donor neurons transplanted into the injured spinal cord, and whether the injured adult spinal cord selects for specific neuronal phenotypes that could limit therapeutic potential. The present work begins to address this gap in knowledge by tracking the development of interneuronal phenotypes from donor NPCs transplanted into the injured cervical spinal cord of adult rodents.

Adult female Sprague Dawley rats received C3/4 lateralized contusions (200 kilodynes, Infinite Horizon Impactor) and were allowed to recover for one week. At that time, the injury site was re-exposed and donor NPCs were transplanted directly into the lesion cavity. NPCs were obtained from E13.5 rat spinal cord, cultured for 3 days to reach confluency (resulting in ~40% and 60% neuronal and glial precursors), and stereotaxically injected into the lesion epicenter one week following a lateral contusion at C3/C4 level. Tissue was collected at 3 days, 1 week and 2 weeks post-transplantation. NPC development over this period is compared with normal rat spinal cord development. Terminal bilateral diaphragm electromyograms (EMGs) were used to assess phrenic motor function 1 and 2 weeks post-transplantation, and compared with injured, untreated control EMGs.

Results from these ongoing studies reveal that grafted cells survive and differentiate yielding mature neurons and glia within the injured cervical spinal cord two weeks post-transplantation. Grafted donor neuronal precursors differentiated into excitatory (vGLUT expressing) neurons, but no inhibitory (GAD67 expressing) donor neurons were identified. Ongoing analysis is further defining the phenotypes of these donor neurons. Terminal EMG recordings revealed that transplantation of NPCs alters the pattern of diaphragm activity ipsilateral to injury seen 2 weeks post-injury. A substantial increase in muscle activity ipsilateral to injury and transplant during the early phase of inspiration was observed two weeks post-transplant. Consistent with previous studies using neuronal and glial restricted progenitors, these experiments confirm that transplantation of embryonically derived NPCs survive, proliferate, differentiate and extend processes into the host spinal cord. Furthermore, these ongoing experiments are beginning to identify the phenotypes of donor cells and offer insight into how the internal milieu of the injured spinal cord might select for specific neurons.

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