Cellular plasticity in the intact and injured central nervous system

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The central nervous system was traditionally considered static, with little exchange of cells. Today we know that there are neural stem cells in both the brain and spinal cord. Most of these stem cells are quiescent and do not produce new cells under physiological conditions. Neurons are, however, continuously added throughout life in two discrete structures in the adult brain, the hippocampus and olfactory bulb, in most mammals. The generation of new neurons in the adult brain serves to maintain a pool of neurons with unique properties, present for a limited time after their birth, which enable specific types of neural processing. We have taken advantage of the massive increase in atmospheric ^{14}C by nuclear bomb testing during the cold war to birth date neurons, which has revealed a unique pattern of adult neurogenesis in humans. I will also describe how quiescent neural stem cells contribute to repair after central nervous system injuries.

Reactive oxygen species-dependent signaling for nerve 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 CX_3CR1-dependent recruitment of inflammatory cells after nerve injury induces the expression of NOX in axons via endocytosis of the cytosolic subunit Ncf1/p47phox, which is retrogradely transported in axonal endosomes via an importin ß1 and dynein depended mechanism. Endosomal NOX-p47phox is required for PTEN cysteine oxidation after injury, which leads to PTEN inactivation stimulating PI3K-pAkt signaling and DRG outgrowth. Ultimately, these early signaling vent affect transcriptional control and epigenetic reprogramming. Defying the dogma that ROS are exclusively involved in nerve degeneration, we show a specific NOX-p47phox and ROS-dependent mechanism essential for nerve regeneration.

Activating a regeneration program with hypoxia

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Permanent disabilities following CNS injuries result from the failure of injured axons to regenerate and re-build functional connections. In contrast to CNS neurons, peripheral sensory neurons successfully increase their intrinsic regenerative capacity. Activation of an axon growth program relies on the expression of regeneration-associated genes. Because individual gene based approaches have yielded limited success in axon regeneration, understanding how a large ensemble of genes can be simultaneously activated after injury could reveal strategies to stimulate robust and meaningful long-distance axon regeneration in the injured CNS.

As a model system to study the mechanisms activating a regeneration program, we use sensory neurons with cell body in dorsal root ganglia. These neurons possess two axonal branches, a peripheral axon that activates a regeneration program following injury and a centrally projecting axon that does not, thereby allowing us to study pathways that are specifically related to axon regeneration. Using this system, we previously established a link between axon injury and chromatin remodeling. In our recent studies, we identified a novel and important role for hypoxia-inducible factor 1α (HIF-1α) as a regulator of chromatin function and transcriptional responses in injured sensory neurons. We found that HIF-1α is required to promote axon regeneration via increasing histone acetylation levels and stimulating the expression of pro-regenerative genes. Consistent with a role of HIF-1α in pro-regenerative gene expression, sciatic nerve injury, but not dorsal root injury leads to the accumulation of nuclear HIF-1α in sensory neurons. HIF-1α accumulation requires the back-propagating calcium wave elicited by axon injury. Induction of HIF-1a using hypoxia enhances axon regeneration *in vitro* and *in vivo* in sensory and motor neurons. This study suggests that HIF-1α represents a critical transcriptional regulator in regenerating neurons and suggests hypoxia as a tool to stimulate axon regeneration.

Novel Therapeutic Gene Targets for Spinal Cord Injury

Murray Blackmore

Examples of successful regeneration in the peripheral and embryonic nervous systems make it clear that the regrowth of axons depends on the ability of injured neurons to initiate appropriate transcriptional programs. In contrast, many neurons in the adult central nervous system (CNS) fail to upregulate needed regeneration-associated genes and fail to downregulate growth-suppressive genes, and thus possess a limited capacity to regenerate axons after injury. We and others have shown previously that activating pro-regenerative gene expression in CNS neurons is a promising therapeutic approach, but progress is hampered by incomplete knowledge of the underlying transcription factors. To identify novel transcription factors that impact axon regeneration we employ high content assays of neurite outgrowth, followed by viral-mediated manipulation of candidate genes in a murine model of corticospinal tract injury. Because restoration of axon growth ability will almost certainly require multiple factors, we are also systematically testing combined manipulation of the most promising factors. Finally, using an optogenetic approach, we have shown that CST axons stimulated to sprout by forced overexpression of pro-regenerative factors can form functional synapses with target cells in the spinal cord. Overall these data identify novel transcription factors with the potential to regulate axon regeneration and impact functional recovery in injured CNS neurons.

CNS axon regeneration, folate, and epigenetics

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Folic acid supplementation decreases the occurrence of congenital central nervous system (CNS) anomalies such as neural tube defects. This implies that folic acid plays an important role in CNS growth and development. Considering that cellular and molecular signals needed for growth of the embryonic CNS may be shared with the injured adult CNS that requires repair, our laboratory aims to investigate the relationship between the folate pathway and the CNS in adult rodents. Specifically, we study the anatomical, biochemical, and molecular response of the folate pathway to CNS injury, and the extent to which folate can enhance in vivo and in vitro axonal regrowth of injured CNS tissue, in particular the spinal cord. We have shown that folic acid treatment improves *in vivo* axonal regeneration into peripheral nerve grafts in the adult rat spinal cord, that the response is mediated by neuronal rather than glial mechanisms, and it follows a parabolic curve with maximal spinal regeneration occurring at 80µg/kg of folic acid, beyond which it tapers back to baseline. Furthermore, we have shown that the pro-regenerative effect of folic acid in the spinal cord is highly dependent on the folate receptor (folbp1) and specific enzymatic steps within the folate pathway leading to DNA methylation. Lastly, ongoing work is demonstrating that folate-induced axonal growth is epigenetic with heritable consequences. We conclude that the folate pathway plays a crucial role in the regeneration and repair of the adult CNS after injury, and is not restricted to the embryonic period. Such a response is mediated by specific modifications in the folate and methionine-methylation cycles leading to epigenetic adaptations.

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What do reactive astrocytes do?

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Astrocytes undergo profound changes in morphology and gene expression in response to brain injury and disease. But whether reactive astrocytes are harmful or helpful has been unclear. We recently found that the genes induced in reactive astrocytes depends on the nature of the inducing injury. After ischemia, reactive astrocytes upregulate neurotrophic factors suggesting they may be beneficial, whereas after systemic injection of lipopolysaccharide (LPS) they strongly upregulate multiple complement cascade components needed to drive synapse destruction suggesting they may be detrimental. These findings suggest that, like macrophages which exist on a spectrum from bad (M1) to good (M2) states, reactive astrocytes also exist in bad (A1) and good (A2) states. Here we show that LPS-induced M1 microglia are sufficient to induce A1 reactive astrocytes. M1 microglia do this by releasing IL1α, TNFα and C1q, which together are sufficient to induce A1 (bad) reactivity in purified astrocytes within 24h and are all required for M1 microglia to induce the A1 state. Using IL1α, TNFα and C1q together, allowed us to create the first defined serum-free cultures of pure A1 reactive astrocytes enabling us to investigate their function. By directly comparing the function of normal astrocytes with A1 astrocytes in vitro, we found that A1 astrocytes are unable to promote neuronal survival, axon outgrowth, synapse formation or synapse function, and have lost the ability to phagocytose synaptosomes and myelin debris. In addition to loss of their normal functions, A1 reactive astrocytes gained a powerfully neurotoxic function, releasing a toxic protein that specifically induces apoptosis of neurons and oligodendrocytes. Drugs that prevent the formation of A1 reactive astrocytes or inhibit this toxic protein may have great potential to treat neurodegenerative diseases and promote regeneration after spinal cord injury.

Tools for anatomical and functional analysis of widely distributed biological networks

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Our research interests focus on developing tools and methods for neuroscience (optogenetic actuators and sensors; tissue clearing and imaging) as well as on investigating the mechanisms underlying deep brain stimulation (DBS) and on the long-term effects of DBS on neuronal health, function, and ultimately behavior. Methods such as Optogenetics (for precise function control) and CLARITY (for intact circuit mapping) enable scientists to understand nervous system circuit anatomy, function, and dysfunction at a depth previously impossible, vastly expanding knowledge of the nervous system and associated psychiatric, neurological, and peripheral organs disorders. CLARITY renders tissue transparent for visualization and identification of cellular components and their molecular identity without slicing therefore improving the likelihood to detect and accurately map sparse populations or projections. This method complements Optogenetics in that it can reveal circuit-wide effects of optogenetic manipulations and also aid in mapping novel circuits that need tuning in disease. The mastery, improvement, and implementation of both these methods require a large-scale cross-disciplinary effort. To this end, our projects range from optimizing CLARITY for non-brain tissue, to developing actuators and sensors of electrical activity via protein engineering and mining of natural sources, to studying the impact of neuromodulation of selected pathways in the central and peripheral nervous system on behavior.

Molecular and Cellular Mechanisms of Spinal cord Regeneration in Amphibians

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Xenopus laevis at tadpole stages (stage 50-54, R-stages) regenerate in response to spinal cord injury (SCI) a capability that is lost at the metamorphic climax (stage 56-66, NR-stages), providing a unique model system to study spinal cord regeneration. Here we will present results from two approaches aiming to understand the genetic and cellular mechanisms of spinal cord regeneration in Xenopus laevis. First we studied the role of Sox2+ ependymal cells. We have found that in R-stages Sox2+ cells have a rapid and transient activation in response to injury followed by migration of Sox2+ cells into the ablation gap and restoration of the ependymal canal. Importantly, no activation of Sox2+ cells and no migration to the ablation gap occur in NR-stages. Reduction of Sox2 levels by morpholino electroporation diminishes regeneration suggesting that Sox2+ cells are necessary for spinal cord regeneration. Furthermore, cells isolated from spinal cord at stage 50 and transplanted into non-regenerative animals restore axon growth. In addition, we have performed a transcriptomic profile using RNA-SEQ of the response to SCI in R- and NR-stages. We have found extensive changes in the transcriptome of regenerative tadpoles already at 1 day after injury, which was only observed in non-regenerative froglets at 6 days after damage. In addition, we found differential regulation of the following when comparing R- and NR-stages: 1) genes related to neurogenesis and the axonal growth cone; 2) gene ontology enrichment analysis revealed differences in genes from biological processes including cell cycle, response to stress, metabolism, development and immune response and inflammation and 3) we have also identified previously uncharacterized transcripts regulated differentially after SCI. We have validated differential expression of several genes involved in these processes using low-scale validation (RT-qPCR). Currently we are testing by gain and loss-of-function studies the role in spinal cord regeneration of a subset of genes.

Successful regeneration of the zebrafish spinal cord

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In contrast to mammals, adult zebrafish can regenerate their spinal cord. During regeneration supraspinal neurons extend an axon over the lesion site and progenitor cells around the lesion generate new spinal inter- and motor neurons. Regeneration in adult fish takes 6 weeks until full functional recovery. For observation purposes, a larval regeneration paradigm would be advantageous. We have now established a mechanical lesion paradigm in larval zebrafish. Complete mechanical transection of the spinal cord at 3 days post-fertilisation leads to paralysis of the fish. Within 48 hours after injury, the lesion site closes, axons regrow and larvae show recovery of swimming activity. This is accompanied by a reaction of the immune system, involving influx of neutrophils, macrophages and microglia from 6 hours after lesion. In two different mutant lines of the innate immune system or under immune-suppression with dexamethasone, locomotor recovery is significantly impaired, indicating an essential role of the immune response for functional recovery. We are currently analysing, which of the crucial events in spinal cord repair, such as wound closure, fusion of the spinal cord, axon regrowth and neurogenesis, are affected in the mutants to determine the role of the innate immune system in successful repair.

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Regeneration of the adult zebrafish brain: the role of lineage conversion

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Severe traumatic brain injury (TBI) of the adult mammalian central nervous system (CNS) leads to life-long loss-of-function, and neuronal regeneration does not occur. In contrast, adult zebrafish have a remarkable ability to regenerate adult brain, retina and spinal cord. Neurogenesis in adult rodents is limited to only two subregions of the telencephalon, but in adult zebrafish occurs along the entire length of the neuraxis, suggesting a mechanistic link to its regeneration ability. The cellular and molecular mechanisms that enable or prevent adult CNS regeneration are little known. To study these mechanisms in adult zebrafish, we developed TBI lesion assays, and analyzed cellular reactions to TBI. We find that adult zebrafish can efficiently regenerate brain lesions and lack permanent glial scarring. Several cell types proliferate as a consequence of the TBI. Using conditional Cre-loxP-based genetic lineage tracing, we asked which stem/progenitor cell types react to injury, proliferate and which contribute to neuronal replacement. We previously described that a subtype of ventricular radial glial stem cells proliferates and generates neuroblasts that migrate to the lesion site. The newly generated neurons survive for at least 3 months, are decorated with synaptic contacts and express mature neuronal markers. Here, we used hematopoetic stem cell transplantation and Cre/loxP lineage tracing to determine if proliferating non-neuronal stem cell lineages convert to generating neuronal-marker expressing cells after TBI, as was reported after HSC transplantation in mammals. So far, we find no new neurons of donor descent. Leukocytes, oligodendrocyte progenitors, mature myelin, endothelial cells and pericytes appear not to convert to a neuronal lineage following TBI. In vivo lineage conversion is currently discussed as a possible therapeutical strategy for neurodegenerative conditions in mammals, using misexpression of lineage converting transcription factors. Our results indicate that lineage conversion is rare in regeneration-capable adult zebrafish brain, in spite of the challenges of a traumatic brain lesion.

Convergence of Nutrient and Injury Response Pathways in Xenopus CNS Repair

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We have developed an assay for brain injury and recovery with a behavioral readout using the visual system of *Xenopus laevis*. Focal ablation of part of the optic tectum in Xenopus tadpoles prevents a visual avoidance response, yet animals recover the visual avoidance behavior gradually over a week following injury (McKeown, et al., 2013). We have demonstrated that focal injury to the optic tectum results in a burst of cell proliferation proximal to the injury site, and that these injury-responsive progenitor cells generate mature neurons. We also showed that recovery of the visual avoidance behavior is inhibited by drugs that block cell proliferation, and that visual deprivation, which expands the neural progenitor pool in the optic tectum, facilitates behavioral recovery from injury. These data indicate that the generation of new neurons is critical for recovery from injury in the developing tadpole brain. Here we report that nutritional status plays a key role in neurogenesis and the integration of new neurons into the damaged circuit. Xenopus tadpoles initially live off their yolk, but at later developmental stages limiting external food sources decreases neural progenitor cell (NPC) proliferation in the CNS and Sox2-expressing NPCs appear to enter a quiescent state. Nutrient restriction- (NR) induced quiescent progenitor cells can be triggered to re-enter the cell cycle upon the re-exposure to food via an mTOR-dependent mechanism. Moreover, the increased proliferative responses to food and injury are additive, indicating that the developing CNS possesses greater proliferative capacity than triggered by either single neurogenic stimulus. Furthermore, increased access to food after injury accelerated behavioral recovery from injury. Electrophysiological recordings indicate that NR decreased the intrinsic excitability of newly generated neurons and impaired their integration into the tectal circuit. These data indicate that nutrition plays a key role in neurogenic proliferation, differentiation, and circuit assembly, and that nutritional status is critical in recovery from CNS injury.

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Cell-type specific rules for neural plasticity underlying a stereotyped behavior.

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Skilled behaviors may persist for decades, but little is known about the stability of the underlying neural control patterns. We demonstrate that stable song behavior in zebra finches is associated with stable ensemble activity in pre-motor cortex. These ensemble patterns persist after peripheral nerve damage, revealing that normal sensory-motor correspondence is not required to maintain the cortical pattern. Using new optical and electrical methods capable of tracking cells for many days, we are examining the rules underlying this ensemble stability. Excitatory projection neurons, but not inhibitory interneurons reveal day-to day drift in firing patterns. These observations suggest a hypothesis for maintenance of motor skills in cortex: inhibitory interneurons can provide a stable mesoscopic dynamical pattern while individual excitatory neurons explore fine-grained variations in motor commands.

Rewiring the damaged pathways via a neural interface

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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.

Plasticity in the Corticospinal System after Spinal Cord Injury

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The corticospinal tract is an important target for motor recovery after spinal cord injury (SCI) in humans. Using noninvasive electrophysiological techniques we have demonstrated the presence of plasticity in corticospinal projections targeting spinal motoneurons of muscles located close and at a distance from the injury site in individuals with chronic anatomically incomplete cervical SCI. We developed tailored protocols for precisely timing the arrival of descending and peripheral volleys at corticospinal-motoneuronal synapses of hand muscles. We found that the arrival of presynaptic volleys prior to motoneuron discharge enhanced corticospinal transmission and hand voluntary motor output. These findings are the first demonstration that spike timing-dependent plasticity of residual corticospinal-motoneuronal synapses provides a mechanism to improve motor function after SCI. Modulation of residual corticospinal connections may present therapeutic target for enhancing voluntary motor output in motor disorders affecting the corticospinal tract.

Human cortex re-organization when interacting with a BCI

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Brain-computer interface is typically a completely novel task. When driven off of a motor-associated control signal, the BCI makes use of native motor systems. As the user becomes proficient with the BCI, changes are seen in performance and in brain signals, both at the control electrode and in remote cortical regions.

Reviewing our experience with invasive BCI in human subjects with subdural electrodes implanted for diagnostic clinical purposes, we will first look at the behavioral and performance changes that occur with learning. The activity at the control electrode shows an initial, strong, increase in local activity to achieve control, followed by a tapering of power but with a marked decrease in variability, reflecting a more learned state. Multiple brain regions are active at the start of learning, but decrease involvement in the learned state, paralleling observations from learning of native motor tasks. Interactions between these remote areas and the control electrode also show changes over time.

Typically, we are using motor cortex regions to drive the BCI. When we look at the motor tasks originally used to identify these areas, a fascinating pattern emerges. Rather than a fixed mapping to a motor task (e.g., hand movement), cortical areas can repurpose to the BCI and appear to drop involvement in the original motor task. Changes in the homunculus happen rapidly. Resting networks similarly show changes within minutes of BCI performance.

Taken together, these results show a very fluid map of brain function when users interact with this novel task. The cognitive neuroscience of BCI use will be of strong interest to both basic research and ultimate implementation of clinical BCIs.

Endogenous bioelectrical networks in non-excitable tissue control regeneration of the CNS and complex organ patterning

Michael Levin

The ability to program growth and form at the organ level offers an escape from the complexity ceiling that hinders current efforts in regenerative medicine. How can regeneration of whole organs such as appendages or brains be induced without having to micromanage the construction at the level of cell specification or individual gene expression? In this talk, I will discuss my lab's efforts to understand and tame developmental bioelectricity. All cells, not just excitable nerve and muscle, communicate with each other via slow bioelectric gradients and neurotransmitters. We developed methods to observe and specifically manipulate the bioelectric conversations among cells that are coordinated towards the creation and repair of large-scale body structures. Using optogenetics, pharmacology, computational modeling, and molecular perturbation of ion channel genes, we can rationally control the behavior of endogenous bioelectric circuits that set up voltage distributions that are instructive for morphogenesis. After introducing the toolkit available for manipulating bioelectric signaling and functionally linking it with gene expression and epigenetic pathways, I will review some exciting recent data (focused on amphibian and planarian models) that suggest a new roadmap for regeneration of neural and other structures. For example, appropriate modulation of voltage gradients can reprogram tissues into complete eyes, induce repair of spinal cord and amputated limb, regulate innervation of implanted tissues, and repair genetic birth defects of brain formation. I will give a perspective on the biomedical applications of these findings, and discuss the future of electroceuticals - a plethora of human-approved ion channel drugs that can be used in regenerative medicine, birth defects, and bioengineering. Remarkably, bioelectric signals are often non-cell-autonomous (implementing distributed representations of anatomical shape). Thus, I will conclude with a controversial hypothesis for how to best crack the bioelectric code: exploring the parallels between the ways that information and memories are represented in the brain and the encoding of target morphologies in regenerating somatic tissues.

Manipulating Intra-Axonal Ca2+ Stores to Limit Secondary Axonal Degeneration Following SCI

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Protecting white matter is a major goal to improve neurological recovery following SCI. Whether the axoplasmic reticulum (AR), an endomembrane system within axons, contributes to secondary axonal degeneration following SCI remains unclear. Furthermore, the individual role of the AR's major Ca2+ release channels, ryanodine receptors (RyR) and inositol 1,4,5-trisphophate receptors (IP3R) in axonal injury remains poorly understood. Here we utilize two-photon excitation microscopy to image axonal swelling, axonal retraction, axonal spheroid formation in *Thy1*YFP transgenic mice, axonal Ca2+ wave generation and Ca2+ accumulation in axons (fluorescent Ca2+ indicators), and changes in AR structure as these dynamic events are unfolding in real-time following a laser-induced SCI (LiSCI) *ex vivo*. We found that transected axons formed endbulbs and underwent slow sigmoidal retraction away from the lesion site or underwent pan fragmentation over 6 hours of direct observation following LiSCI. Axons undergoing slow retraction had peak Ca2+ increases within the endbulbs whereas axons undergoing pan fragmentation were associated with robust Ca2+ increases along the length of the axon. Reducing intra-axonal Ca2+ release mediated by RyR significantly reduced axonal retraction in both zero Ca2+ and normal Ca2+ aCSF perfusate. In contrast, targeting IP3R was only effective in zero Ca2+ aCSF. Overall, our data indicate that targeting RyR may reduce secondary axonal injury following SCI.

Cervical Spinal Stimulation and Respiratory Recovery after Upper Cervical Spinal Cord Injury

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Over half of all spinal cord injuries (SCI) occur at the cervical level leading to disruption of bulbospinal pathways to respiratory motor neurons, respiratory muscle paralysis, and diminished breathing capacity. Of the 6,000 new cervical spinal injuries per year, ~20% will require mechanical ventilatory support. Even when mechanical ventilation is not necessary, breathing impairment greatly increases susceptibility to life-threatening lung infections. In fact, the leading cause of morbidity and mortality in cervical SCI is respiratory impairment. Our goal is to restore respiratory function in a rat model of cervical spinal injury by utilizing cervical spinal epidural electrical stimulation in combination with serotonergic agonists (e.g. quipazine). By inducing respiratory plasticity after synergistic modulation of the phrenic motor network (e.g. cervical epidural stimulation plus quipazine), we will enhance the contributions from spared motor neurons, thereby preserving ventilatory capacity. This project may lead to novel therapies to extend and improve life for patients with cervical spinal cord injury by preserving breathing."

Resolving Intracellular Mechanisms of Neurotrophin-Mediated Signal Transduction Via Optogenetics

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Neurotrophin-mediated signaling pathways regulate a diverse spectrum of neuronal functions including cell survival, differentiation, neurite outgrowth, apoptosis, and synaptic formation and plasticity. How exactly one set of signaling modality is responsible for multiple cell responses remains elusive. Evidence suggests that distinct cell fate determination can be achieved via modulation of neurotrophin signal transduction in both space and time. A quantitative delineation of intracellular mechanisms, however, is limited primarily due to lack of tools that allows precise spatiotemporal controlling of the signaling processes. Optogenetics, an emerging technique that combines the power of light and molecular cell biology, is a remarkable tool to achieve this goal. Here, we construct an optogenetic system that uses light to regulate neurotrophin-mediated signaling pathways. We show that distinct downstream pathways differentially control cell fate determination in neuronal cell lines. We find that light-activated Raf/MEK/ERK pathway alone is sufficient to stimulate significant neurite outgrowth in PC12 cells in the absence of nerve growth factors. Intermittent on/off light control reveals a memory effect in light-induced neurite outgrowth. Light-controlled neurotrophic signaling enables precise dissection of individual subsets of signaling pathways with superior resolution in space and time. Results from our research will help resolve intracellular mechanisms of neurotrophin-mediated signaling in developmental and axonal regeneration processes.

Neuregulin-1 Controls an Endogenous Repair Mechanism After Spinal Cord Injury

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Following traumatic spinal cord injury, acute demyelination of spinal axons is followed by a period of spontaneous remyelination. However, this endogenous repair response is suboptimal and may account for the persistently compromised function of surviving axons. Spontaneous remyelination is largely mediated by Schwann cells, where demyelinated central axons become associated with peripheral myelin. This phenomenon is particularly prominent in the dorsal columns which contain long tracts of ascending myelinated axons. However, the molecular control, functional role and the origin of these central remyelinating Schwann cells is currently unknown. The growth factor neuregulin-1 (Nrg1) is a key signalling factor controlling myelination and remyelination of axons in the PNS, via signalling through ErbB tyrosine kinase receptors. Here we examined whether Nrg1 is required for Schwann cell-mediated remyelination of dorsal column axons and whether Nrg1 ablation influences the degree of spontaneous remyelination and functional recovery following spinal cord contusion injury. We used a tamoxifen inducible Nrg1 mutant mouse to achieve conditional ablation of Nrg1. Following spinal contusion injury in adult mice, Nrg1 ablation was associated with a complete absence of Schwann cells within the spinal cord and profound demyelination of spinal axons. Nrg1 null mice also exhibited poorer levels of spontaneous locomotor recovery than injured control littermates. There was no compensatory oligodendrocyte remyelination in Nrg1 null mice, and removal of peripheral input to the spinal cord also revealed that the majority of remyelinating Schwann cells originated within the injured spinal cord. We also examined the role of specific Nrg1 isoforms, using mutant mice in which only the Ig-containing isoforms of Nrg1 (types I and II) were conditionally ablated, leaving the type III Nrg1 intact. We found that the IgNrg1 isoforms were dispensable for Schwann cell mediated remyelination of central axons after spinal cord injury, indicating that the type III isoform (cysteine-rich domain containing) has a critical role in this repair process. However, IgNrg1 null mice also demonstrated impaired recovery compared to control mice, which likely reflects the role of IgNrg1 in muscle spindle maintenance. Our data provide novel mechanistic insight into endogenous regenerative processes after spinal cord injury, demonstrating that Nrg1 signalling regulates central axon remyelination and functional repair and drives the trans-differentiation of central precursor cells into

PNS-like Schwann cells that remyelinate spinal axons after injury. Manipulation of the Nrg1 system could therefore be exploited to enhance spontaneous repair after spinal cord injury and other CNS disorders with a demyelinating pathology.

Molecular Determinants of Axonal Regeneration

Bhagat Singh

Deficits in axonal regeneration impose a significant burden on patients and typically result in life-long disabilities. Axonal regeneration essentially fails to occur in the CNS, primarily due to a lack of intrinsic neuronal growth competence and extrinsic inhibition. Earlier work conducted in the Woolf lab on 9 mouse strains ***has identified a remarkable and specific CNS regenerative capacity of the inbred CAST mice strain***, strongly suggesting that strain-specific genetic factors determine this growth potential (Takao et al., *Neuron* 2015). However, the precise molecular and genetic determinants of this regenerative capacity now need to be determined. Dorsal root ganglion (DRG) neurons have two branches: peripheral axons, which regenerate after injury, and central that projects to the spinal cord, does not regenerate. Preconditioned injury – an injury preceding the spinal cord injury (SCI) – to peripheral nerves, enhances the intrinsic growth capacity of central axons to regenerate in the dorsal columns beyond the lesion site in the spinal cord (Neuman and Woolf, *Neuron* 1999). Besides, after preconditioned injury, under similar *in vitro* culture conditions, only a selective population of injured sensory neurons grows on myelin compared to other neighbouring non-growing neurons, strongly suggesting that injury and neuron specific genetic factors determine growth potential in the SCI. ***We believe that expression and activation of a defined core set of transcription factors (TF) or genes in the CAST strain controls the overall regeneration network***. Once I identify these gene/TFs, I will determine if activation of these gene/TFs can change a neuron's status into an actively growing one and whether forced expression of the identified candidates could enable us to convert C57/B6 neurons (poor CNS regenerators) into CAST-like neurons (strong CNS regenerators). My work includes isolation of DRG sensory neurons from CAST and C57/B6 mice before and after preconditioned injury by FACS followed by NextGen sequencing to identify differential expression of genes and TFs in the two strains and of their networks of regulated genes. I am also analyzing C57 and CAST specific miRNAs, which may create the inter-strain specific transcriptional profiles. Using transcription factor binding site analysis in promoter regions of genes in different regeneration network modules has identified ASCL1 (aka Mash1), a basic helix loop-helix transcription factor. Ascl1 was selectively upregulated in CAST injured DRG neurons and its overexpression increases growth substantially in c57 uninjured neurons. Knock down of Ascl1 in CAST neurons significantly decreased neurite outgrowth on an inhibitory myelin substrate. Through RNASeq data I have also identified Type 1 Interferon (IFN) pathway selectively upregulated after injury in CAST sensory neurons. Now, I am manipulating this pathway to assess its impact on the preconditioning response and neurite outgrowth. My next step would be to test Ascl1 and IFN pathway *in vivo* in an optic nerve injury and SCI animals models for axonal outgrowth and behavioral outcomes. This work will define and validate the role of critical pathways driving enhanced regenerative capacity of CAST-like neurons, which has translational repercussions in developing new therapeutic opportunities for CNS injuries and repair.

microRNA-155 deletion improves spinal cord repair

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Spinal cord injury (SCI) disconnects central axons, which fail to regenerate. Axon regeneration is limited by insufficient neuronal growth responses, and by damaging SCI-induced inflammation. Despite our mechanistic understanding of SCI pathology, successful repair therapies remain elusive. microRNA (miR)-155 is a key inflammatory mediator in macrophages that could also hinder axon growth. Here, we reveal a novel dual role for miR-155 in restricting spinal cord repair. Isolated miR-155 knockout (KO) macrophages had reduced inflammatory capacity. When cultured with dorsal root ganglion (DRG) neurons, miR-155 KO macrophages supported improved neurite outgrowth. miR-155 also directly restricts axon growth potential: compared with cultured WT neurons, miR-155 KO neurons exhibited enhanced outgrowth capacity. After peripheral conditioning lesion, miR-155 KO neurons had increased regeneration-associated gene expression. In the SCI epicenter, miR-155 KO mice had reduced inflammation and enhanced axon regrowth. Therefore, miR-155 has detrimental roles in both macrophages and neurons that limit CNS repair.

Brian Kaspar – N/A

Impairment of glymphatic pathway function in the aging brain: sleep, waste and neurodegeneration

Jeffrey Iliff, PhD

Aging is the strongest risk factor for virtually every neurodegenerative disease, including Alzheimer's disease (AD), yet the features of the aging brain that render it vulnerable to protein mis-aggregation and neurodegeneration are not know. The glymphatic pathway is a brain-wide perivascular network that supports the recirculation of cerebrospinal back through the brain, supporting the clearance of interstitial solutes including amyloid β during sleep. Perivascular CSF recirculation and amyloid β clearance is impaired in the aging mouse brain, and this impairment is associated with changes in localization of the perivascular astroglial water channel aquaporin-4 (AQP4), which is localized primarily to perivascular astrocytic endfeet. In a study carried out in human frontal cortical tissue, we report that loss of perivascular AQP4 localization is a feature of the aging human brain as well, and that these changes in AQP4 localization are associated with worsening AD pathology and cognitive decline. These findings suggest that changes in perivascular AQP4 localization with age may underlie regional and age-related vulnerability to amyloid β deposition in the AD brain.

My travels with Schwann cells, from bench to bedside

Mary Bartlett Bunge

The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Miami FL

When cells (including Schwann cells, SCs) of the rat PNS could be purified and expanded in number in tissue culture, Richard Bunge in 1975 envisioned that the SCs could be introduced to repair the CNS as SCs enable axons to regenerate after PNS injury. The SCs could be implanted autologously into human CNS lesions after acquiring them from a PN biopsy. Development of the new culture systems by Richard and Dr. Patrick Wood to study interactions between sensory neurons, SCs and fibroblasts led to increased knowledge of SC biology in the 70s and 80s. Joining the Miami Project to Cure Paralysis in 1989 brought the opportunity to use this knowledge to initiate spinal cord repair studies. Development of a rat complete cord transection/SC bridge model allowed the demonstration that CNS axons regenerate into a SC bridge. Together with study of contused rat cord, it was concluded that implanted SCs reduce cavitation, protect the tissue around the lesion from secondary injury, support regeneration of axons, and form myelin around these axons. Human SCs support axon regeneration as do rat SCs. The outcome of SC transplantation was improved when combined with the addition of neurotrophins, elevation of cyclic AMP levels, transplantation of olfactory ensheathing cells, administration of a steroid or introduction of chondroitinase into the SC implant. Increased efficacy meant higher numbers of axons, particularly from the brainstem, and more SC-myelinated axons in the implants and improvement in hindlimb movements. Astrocytes at the SC bridge/host spinal cord interfaces play a key role in determining whether axons cross these interfaces to enter SC territory. The SC work described here contributed to gaining approval from the FDA for an autologous human SC, subacute clinical trial in spinal cord injured persons now being completed at the Miami Project.