Cloutier Lab

Research Proposed:

The formation of accurate connections between neurons during development of the nervous system is dependent on several biological processes that include axonal outgrowth and guidance. The somatosensory system is required for the processing of stimuli, such as heat and surface pressure, which are detected by receptors on the skin. Primary sensory neurons that detect these stimuli and convey the information to the central nervous system are clustered in the dorsal root ganglia (DRG) located adjacent to the spinal cord (SC). While neurons with different sensory modalities are intermingled within the DRG, their central afferents segregate into specific laminae of the SC according to their sensory modality. Nociceptive and thermoceptive neurons project axons to the superficial layers of the SC (laminae I and II) while mechanoreceptive neurons project their axons to deeper layers (laminae III to VI). The elaboration of precise axonal projections is therefore critical to achieve an accurate representation of environmental stimuli in the central nervous system (CNS). While we have an excellent grasp of the anatomical characteristics of DRG afferent projections in the SC, the molecular mechanisms that promote accurate segregation of these projections remain poorly understood.

The proposed research project is aimed at examining the role of the axon guidance receptor, Neogenin, and of its ligands, the RGMs, in the development of the somatosensory system. During their stay in the laboratory, the student will determine the spatio-temporal expression patterns of RGMs and Neogenin in the somatosensory system using a combination of in situ hybridization and immunohistochemical approaches. In addition, the student will perform a detailed analysis of the accuracy of targeting of different subsets of DRG neuron axons in the spinal cord of Neogenin mutant mice. These studies will shed some light on the molecular mechanisms by which RGM-Neogenin interactions regulate the formation of accurate synaptic connections in the somatosensory system. Gaining a better insight into these mechanisms will be essential in the future development of regenerative therapies to repair damage caused to the CNS by injury.

Funding for proposed experiments is available but does not include stipend.

Contact:
Jean-François Cloutier
Montreal Neurological Institute
3801 University
Montreal, QC H3A 2B4

jf.cloutier@mcgill.ca

http://cloutierlab.mcgill.ca

McGill University - Imperial College London Student Exchange
Training opportunity within the Fon Laboratory at the Montreal Neurological institute

Identifying novel substrates of parkin-mediated ubiquitination

Background: Over the past decade, the discovery of genes responsible for familial forms of Parkinson's disease (PD) has provided tremendous insight into the molecular and cell biological mechanisms involved. Mutations in the *Parkin* gene cause an autosomal recessive juvenile-onset form of PD that account for a large fraction of familial cases and probably contribute to sporadic PD as well. Importantly, several groups, including our own, have shown that the parkin protein functions as a key enzyme in the ubiquitin (Ub) system. Ubiquitin, a 76 amino acid protein, can be covalently attached to the ε-amino group of lysine (K) residues within target proteins. Ubiquitination requires the concerted activity of an E1 Ub-activating enzyme, an E2 Ub-conjugating enzyme and an E3 Ub-ligase. E3 Ub-ligases, such as parkin, form the largest family of enzymes and are involved in substrate recognition, thereby conferring specificity to ubiquitination. Despite the large amount of work carried out to understand parkin function, we still do not know the identity of most parkin substrates.

Opportunity: The aim of this proposal is to identify substrates of parkin-mediated ubiquitination in brain. This has been difficult to date because reagents (i.e anti-Ub antibodies) to purify and analyze ubiquitinated proteins from tissue have not been efficient. We plan on overcoming this limitation by crossing transgenic mice expressing HA-tagged Ub with parkin knockout mice in our lab. This will allow us to use anti-HA affinity chromatography to efficiently purify HA-Ub-protein conjugates from wild-type and parkin knockout brain samples. The samples will then be analyzed by mass spectrometry. We predict that *bone fide* endogenous parkin substrate will be recovered in samples from wild-type but not parkin KO brains. Newly identified substrates will be validated in brain and cultured neurons from parkin KO mice. As there is still much we do not know about the basic mechanisms of parkin function and its substrates, we predict this work will have major implication for our understanding of PD pathogenesis.

Role of visiting student: Under the guidance of a postdoctoral fellow, the student will be responsible for mouse brain tissue preparation and anti-HA affinity chromatography experiments. The student will optimize experimental conditions using SDS-PAGE followed by Coomassie staining prior to mass spectrometry. The student will also be intimately involved in the analysis and validation of the mass spectrometry data obtained.

Please send inquiries to:
Dr Ted Fon
Montreal Neurological Institute
McGill University
ted.fon@mcgill.ca

McGill University - Imperial College London Student Exchange

Training opportunity within the Kania Laboratory at the Institut de recherches cliniques de Montreal

Background

During embryogenesis, spinal motor neurons are born in excess and approximately half of them die an apoptotic death. In mice lacking the LMX1b gene, in which the limb extensor muscles are absent and flexors are duplicated, there is a more severe loss of motoneurons that innervate extensors following apoptosis. In contrast, the number of flexor-innervating neurons remains the same as in controls. This leads to the question: what is the pattern of flexor muscle innervation in the LMX1b mutant limb?

Opportunity

By staining limbs from WT and LMX1b null mice for aBTX, a marker of the neuromuscular junction, and myosin, a marker of muscle fibers, the student will be able to assess differences in density of innervation of muscle fibers, gaining insights into the targeting and branching of motoneurons into the ectopic flexor tissue.

Contact:
Dr. Artur Kania
Institut de recherches cliniques de Montreal
110, avenue des Pins Ouest
Montréal (Québec) H2W 1R7 Canada
Email: artur.kania@iecm.qc.ca

Regulation of the netrin-1 receptor DCC by Ser/Thr phosphorylation

Nathalie Lamarche-Vane, Dept of Anatomy and Cell Biology, McGill University

Netrins are a small family of secreted proteins that guide growing axons during neural development. They are found in multiple vertebrate and invertebrate species and show highly conserved functions as axon guidance cues. In vertebrates, netrin-1 was identified on the basis of its ability to promote commissural axon outgrowth from explants of embryonic spinal cord. It is a bifunctional molecule attracting and repelling different classes of axons. The Down's syndrome Cell Adhesion Molecule (DsCAM) and the Deleted in Colorectal Cancer (DCC) have been identified as netrin-1 receptors mediating attraction of axons, however DCC can also participate in repulsion. The UNC5 family of proteins plays a role in the repulsive effects mediated by netrin-1, either alone or in combination with DCC. The signaling pathways that mediate the response of axons to netrin-1 are still incompletely understood. The importance of these guidance molecules in the development of neurodegenerative diseases, such as Parkinson and Alzheimer's diseases, mental retardation, and spinal cord injuries prompted us to investigate the intracellular machinery regulated by netrin-1. We are interested to investigate the molecular mechanisms underlying the effects of netrin-1 leading to a coordinated and directed response of growth cone navigation.

The receptor DCC is a tyrosine kinase-associated receptor, but it is also phosphorylated on Serine and Threonine residues in response to netrin-1. By mass spectrometry analysis, we mapped four phosphorylated Ser/Thr residues in the cytoplasmic tail of DCC. This project will be to determine the role of these phosphorylated residues in the regulation of DCC function using site-directed protein mutants and cell biology approaches.

Contact:
Dr. Nathalie Lamarche-Vane
Dept. of Anatomy and Cell Biology
McGill University
Email: nathalie.lamarche@mcgill.ca

McGill University - Imperial College London Student Exchange

Training opportunity within the McKinney Laboratory at the Bellini Life Scince Complex, McGill University

Dr Anne McKinney's laboratory has focused on studying excitatory synapse formation and maintenance under physiological and pathological conditions such as epilepsy and Alzheimer's Disease. She has made seminal contributions to the field including that AMPA receptor activation is necessary for dendritic spine maintenance. The McKinney lab uses a multidisciplined approach transgenic animals, electrophysiology, EM, immunohistochemistry and 4D imaging to address their questions of interest. It is also one of the few laboratories which combine high resolution 4D imaging with electrophysiology. One area of particular relevance for the McGill-Imperial student exchange program is related to the involvement of lipid metabolism in synaptic dysregulation in Alzheimer's Disease.

Background: Alzheimer's disease (AD) is the most prevalent form of dementia leading to a progressive cognitive decline that currently cannot be cured. The typical signs of AD include upregulation of inflammatory markers, early synapse loss followed by neuronal death, intracellular neurofibrillary tangles and accumulation of extracellular senile plaque that is made up mainly of amyloid-β peptide (Aβ). Based on this evidence, researchers have shown the abnormal accumulation of oligomeric Aβ as an important process in AD progression. However, another hallmark of AD, namely an increase of "adipose inclusions", suggests aberrant lipid metabolism in the brains of AD patients has largely been overlooked. This disruption in lipid homeostasis may aggravate the interference of synaptic connections induced by aberrant oligomeric Aβ accumulation. Large-scale epidemiological studies have also revealed several AD risk factors related to lipid homeostasis, such as the ε4 allele of the apolipoprotein E (APOE) gene. There is also a prevalence of AD in the obese population and a significant reduction of AD in long-time statins users to lower blood cholesterol. All indicate the importance of lipid dysregulation in AD progression.

Opportunity: This project aims to (i) to investigate how an imbalance in lipid homeostasis and the formation of lipid deposits in response to oligomer Aβ induce adverse morphological and functional changes in hippocampal dendritic spines in Aβ-model of AD and (ii) to test nanodelivery systems with drug designed to correct and induce circuitry repair. We have already preliminary data showing that there is change in lipid composition in AD hippocampus and modifying lipid composition can prevent structural and functional changes induced in AD. We will now investigate the mechanisms involved.

Contact: Anne McKinney PhD
Department of Pharmacology and Therapeutics
Tel: 1-514-398-5685
e-mail: anne.mckinney@mcgill.ca

McGill University - Imperial College London Student Exchange

Training opportunity within the Rao Laboratory at the Centre for Research In Neuroscience at McGill University

Project Title: Elucidation of the neuronal circuit controlling movement decision

Project description: Normal brain function relies on the establishment of neuronal circuits and the proper communication between neurons within each circuit. These neuronal circuits translate sensory signals such as olfactory, mechanical, sense of oxygen or gravity into changes in behavior. The exact mechanism by which animal behaviors are controlled by specific neuronal circuits at cellular and molecular levels, however, remains largely undefined. The Drosophila Turtle (Tutl) gene encodes for a novel IgSF-superfamily cell surface receptor, which is the fly homolog of KIAA1355 in humans and Dasm1 in mice. Tutl is expressed in a subset of neurons in the central nervous system (CNS). Mutations in the tutl gene affect larval behaviors such as tactile escape response, decision making and coordinated righting behaviours. The exact function of Tutl, however, remains unknown. The goal of this project is to investigate the molecular and cellular mechanism by which Tutl regulates complex behaviours in Drosophila, which can serve as an excellent model to understand neural basis of animal behaviours. The objectives include behavioural analysis of the mutants and identify important neurons in the circuit required for controlling the behavior.

Contact:
Dr. Yong Rao
Centre for Research in Neuroscience
McGill University Health Centre
Email: yong.rao@mcgill.ca

McGill University - Imperial College London Student Exchange

Training opportunity within the Stifani Laboratory at the Montreal Neurological Institute at McGill University

Background

Motor neuron (MN) diseases are pathological conditions of severe morbidity and mortality. For instance, amyotrophic lateral sclerosis (ALS) results in severe paralysis and death, with no successful pharmacological treatment currently available. Recent progress in stem cell biology and regenerative medicine is highlighting the therapeutic potential of undifferentiated neural stem or progenitor cells to replace MN cells lost during disease. However, the successful application of stem cell-based cell replacement therapies requires a detailed characterization of the molecular mechanisms controlling the normal genesis, correct localization and target connectivity of the MNs that are lost in MN diseases.

Opportunity

Cell intrinsic mechanisms of transcriptional regulation play fundamental roles in neural cell development. In that regard, recent work has shown that transcriptional corepressors of the Groucho (Gro)/TLE family are expressed in MN progenitor cells in the developing spinal cord where they participate in the generation of the correct number and types of spinal MNs. Gro/TLE proteins are also expressed in post-mitotic MNs in the spinal cord, where they are hypothesized to form transcription complexes with one of their DNA-binding partners, the transcription factor Runx1. The Stifani lab has shown that Runx1 is important for the development of subtypes of postmitotic MNs that innervate specific forelimb muscles [lateral motor column (LMC) MNs] or that are critical for mastication and swallowing (hypoglossal MNs). Notably, those are among the MNs most susceptible to degeneration in ALS, which led us to hypothesize that Gro/TLE and Runx1 act as regulators of the development and/or target muscle connectivity of MNs affected in ALS. The visiting student will perform studies aimed at testing hypothesis. More specifically, s/he will conduct experiments that will determine precisely the pattern of axonal innervation of the MNs expressing Gro/TLE and Runx1.

These studies will provide new information on the mechanisms controlling the generation and target connectivity of different MN subtypes in the hindbrain and spinal cord and will help advance our understanding of cell replacement therapy for MN disease.

Contact:
Dr. Stefano Stifani
Montreal Neurological Institute
Email: stefano.stifani@mcgill.ca