NS Independent Study Proposal

Name of Lab: X Lab at NYU School of Medicine

Number of Credits Desired: 2

Number of Hours Estimated to Work in the Lab per week: 30

Brief Description of the Project:

The lab investigates the complex, reciprocal interactions between axons and myelinating glia, i.e., Schwann cells (SCs) in the PNS and oligodendrocytes in the CNS. In the lab, I am working under the guidance of a 6th year MD/PhD student studying the signaling pathways that link SCs to both axons and other cell types in the PNS, such as fibroblasts, endothelial cells, and perineurial glia. In particular, we have generated conditional knockout mice for the key myelination transcription factor *Egr2* (aka *Krox20*) in which SCs are arrested at a pro-myelinating stage. Using this model, we are studying the downstream consequences of blocking myelination on neuronal gene expression. In complementary studies, we address the role of the Sonic hedgehog pathway, which is thought to be regulated downstream of *Krox20* in Schwann cells, in non-autonomous signaling between SCs and other PNS cells.

My role in the project focuses on a number of elements that will help form a better understanding of the role *Krox*-20 plays in normal nerve development, demyelinating neuropathies, nerve injury, and in non-autonomous signaling among adjacent axons/SCs. To this end, we have generated a *Krox-20* chimeric knockout mouse by aggregating a wild type embryo with a *Krox-20* conditional knockout embryo. This novel approach allows for the creation of a PNS mosaic in which mutant SCs are placed adjacent to wild type SCs in the same nerve bundle. This experiment will provide exciting and novel information on whether, and perhaps how, adjacent Schwann cells interact and signal to one another, as well as some possible outcomes of such interactions. Moreover, this experiment will shed light over the mechanisms by which the

node of Ranvier operates in the localization and clearage of necessary proteins through the asymmetric formation of paranodes (i.e., nodes at the boundary between mutant and WT SCs will be normal; or perhaps a lack of paranode on one side will lead to nodal defects). Finally, as the *Krox-20* knock out mice do not exhibit proper saltatory conduction (as the PNS nerves fail to myelinate and form proper nodes/paranodes), the very nature of electrical and saltatory conduction in chimeric nerves will be investigated. To complement this, we will utilize lentiviral vectors to attempt to restore normal *Krox-20* expression in mutant nerves and assay for morphological or functional rescue of the mutant phenotype.

Over all, the results of this study will increase our understanding of PNS glial biology and will provide the knowledge basis for future clinical therapies for inherited neuropathies such as Charcot-Marie-Tooth Disease, in which segments of the peripheral nerve de-myelinate and re-myelinate. Understanding non-autonomous signaling between neighboring SCs will be critical for such developments.

<u>My Specific Role in the Project and General Responsibilities in Lab:</u>

Regarding the project described above, my work will follow the day-to-day operations of hypothesis-driven basic science—that is, the formation of a hypothesis, the appropriate design of experiments to test it, inclusion of relevant controls, analysis of the resulting data, modification of the current hypothesis in light of new findings, and design of future experiments. Specifically, my responsibilities will include:

(1) Maintaining a large mice colony (approximately 500 mice) – this work entails managing the appropriate genotypic lines of mice that we use for an array of experiments (such as the *P0*-Cre, *Krox-20* line); genotyping new born litters (using PCR); weaning mice and maintaining the survival of mice who present debilitating phenotypes (such as the *Krox-20* KO).

(2) Investigating scientific questions relating to both the project described above and other projects in the lab alongside my supervisor, Brendan. This entails surgical and biological methods such as survival surgeries, maintaining and preparing lab equipment, injections, perfusions, tissue collection, teased nerves, embedding tissue, sectioning, staining, and quantitative and qualitative analysis on imaged tissue samples. My work also entails basic cell culture, cloning, and virus preparation.

(3) Quantification work that entails working with EM images and demands understanding the biological characteristics and structure of nerves and glia to be able to pinpoint cells of interest and any abnormalities; as well as building and maintaining MATLAB scripts to allow for analysis of the raw data retrieved, proper statistical analysis, and creating graphical displays.