**Specific Aims**

Neurofibromatosis (NF1) is a genetic disorder resulting in tumor growth in the brain, spinal cord, nerves and skin [1]. Typically NF1 manifests itself with café au lait spots, freckling in armpits or groin area, and fibromas on or under the skin [1]. Children with NF1 also have learning disabilities [2,3]. NF1 is caused by an autosomal dominant mutation anywhere along the *Nf1* gene coding for neurofibromin (NF1) that plays a role in the cAMP pathway, necessary for cell-cell communication. Loss of NF1 leads to disregulation of the cAMP pathway leading to learning disabilities [3]. The role for NF1 in the cAMP pathway as it relates to learning is unclear.

I **hypothesize** that mutations in *Nf1* dis-regulate the cAMP pathway, which is necessary to maintain proper neuronal connections. Zebrafish are excellent model organism to study neuron function and learning since zebrafish are transparent and easy to preform learning assays by 5 days post fertilization [4]. My **long-term goal** is to understand how NF1 functions in learning.

**Aim 1: Identify conserved amino acids in NF1 protein necessary for learning.**

**Hypothesis**: Amino acids that are conserved in both simple learning and advanced learning organisms, and those that are specific to advanced learning organisms, will be important to understand how NF1 functions in the learning process.

**Approach**: The NF1 protein is highly conserved across species with advanced learning (humans, chimps, gorillas, etc.) and simple learning (i.e. mice, rats, zebrafish, frog, and fruit fly). To determine which amino acids are important for learning, Clustal Omega will be used to align amino acid sequences and analyze the areas that are differing in polarity or subgroup of amino acid. Once candidate amino acids have been identified, transgenic knockout zebrafish will be created using CRISPER and then the fish will be assayed for learning ability to [4] to identify the conserved amino acids important for learning.

**Rationale**: Identifying key amino acids important for learning will lead to a better understanding of how mutations in NF1 lead to learning disabilities.

**Aim 2: Identify small molecules that rescue learning deficits in NF1 zebrafish.**

**Hypothesis**: Learning function will be rescued in *Nf1* mutants by certain small molecule(s) associated with the cAMP pathway.

**Approach**: Transgenic zebrafish NF1 learning mutants from Aim1 will be used in a phenotype-based forward chemical genetic screen. A focused chemical library will be developed to screen the small molecules associated with the cAMP pathway. Our phenotype of interest would be rescued function of learning. Controls will be the percentage of wild type fish that preform the learning assays.

**Rationale**: Small molecules that rescue *Nf1* mutants with learning disabilities will lead to a better understanding of signaling pathways that NF1 acts through in the brain.

**Aim 3: Determine protein phosphorylation levels in NF1 mutants in different tissues.**

**Hypothesis**: In *Nf1* learning mutants there will be lower phosphorylation levels in the cAMP pathway’s proteins due to inactivation by the mutant NF1 protein.

**Approach**: Quantitative phosphorylation with mass spectrometry will be completed on the learning mutant *Nf1* zebrafish in neuronal tissue vs. other tissue types. Proteins with differing phosphorylation levels in the neuronal tissue vs. other tissues will be analyzed for what pathway they participate in through STRING interaction networks to determine if proteins associated with part of the cAMP pathway are affected in neuronal tissue.

**Rationale**: Phosphorylation levels during disease state can illuminate which pathways are not functioning.

Regarding *expected outcomes*, finding where in the cAMP pathway that the NF1 protein is disregulating the cAMP signaling will help us understand NF1 learning disabilities. Understanding how NF1 affects learning disabilities will contribute to knowledge of how learning works through the brain/neuronal connections, along will possible drug targets to NF1 patients living with learning disabilities.

**References**

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[3] Wolman MA, de Groh ED, McBride SM, Jongens TA, Granato M., Epstein JA. 2014. Modulation of cAMP and Ras Signaling Pathways Improves Distinct Behavioral Deficits in a Zebrafish Model of Neurofibromatosis Type 1. Cell Reports.  8(5):1265-70.

[4] Wolman, M. and Granato, M. (2012), Behavioral genetics in larval zebrafish: Learning from the young. Devel Neurobio, 72: 366–372. doi:10.1002/dneu.20872