**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 neurofibromas on or under the skin [1]. Children with NF1 also have learning disabilities [2,3]. NF1 is caused by an autosomal dominant mutation in 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 an excellent model organism to study neuron function and learning since zebrafish are transparent in their embryonic stage along with having their neural circuits developed enough for learning assays by 5 days post fertilization [4]. My **long-term goal** is to understand how NF1 functions in a pathway that leads to learning.

**Aim 1: Identify which amino acids in NF1 are important for learning.**

**Rationale**: Once the necessary amino acids of NF1 for learning are identified we will have a better understanding of how NF1 learning disabilities occur.

**Approach**: The NF1 protein is highly conserved across species with advanced learning (i.e. humans, chimps, gorillas, etc.) and simple learning (i.e. mice, rats, zebrafish, frog, and fruit fly). To determine which amino acids in this protein 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, knockout zebrafish for these amino acids will be created using CRISPER to test with learning assays [4] to identify learning mutants. The mutant learners will be the ones associated with amino acids necessary for learning in NF1.

**Hypothesis**: There will be certain amino acids that are similar in simple learning organisms that differ from advanced learning organisms, these amino acids will be important for learning.

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

**Rationale**: Knowing small molecules that effect learning in *Nf1* mutants will lead to a better understanding of pathways that NF1 acts in during learning.

**Approach**: With the above learning mutant zebrafish a phenotype-based forward chemical genetic screen will be completed. 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.

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

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