Lewy body dementia is characterized by motor impairment, visual hallucinations and memory loss caused by protein accumulation in neurons throughout the cerebral cortex (1). These aggregates consist of misfolded alpha-synuclein proteins, encoded by the SNCA gene, ultimately leading to neuron cell death (2). SNCA has also been linked to proper dopamine release and regulation, which is essential for normal memory and movement (2). SNCA overexpression leads to a disruption in dopamine signaling (5), *yet it is unclear how this affects brain function.* Understanding how SNCA affects dopamine regulation is critical for understanding neurodegenerative disease pathogenesis and treatment development.

My **primary goal** is to determine how SNCA mediates dopamine production and brain function. I **hypothesize** that alpha-synuclein is used to regulate machinery used in dopamine release. When apha-synuclein is overexpressed, these proteins may aggregate in synaptic terminals, impairing proper transporter protein activity, interaction and movement. I will use the zebrafish (Danio rerio) as model organisms because they share similar brain structure, neuron function, and have behavioral phenotypes that are easy to observe.

**Aim 1: Identify conserved amino acids in SNCA important in dopamine release.**

**Approach:** I will use Clustal Omega to identify amino acids necessary for dopamine release. I will then use zebrafish and CRISPR/Cas9 to edit targeted, conserved zebrafish amino acid to determine which amino acids are important for behavior and dopamine levels. Behavior will then be assayed by observing fish swimming patterns, and dopaminergic neurons will be assayed through immunofluorescence imaging of neurons. **Rationale:** Conserved amino acids are likely to play a key role in dopamine regulation. **Hypothesis:** Editing specific conserved amino acids that regulate dopamine expression will cause an increase in dopamine expression and any behavioral defects in zebrafish.

**Aim 2: Identify small molecules that rescue SNCA mutant behavioral phenotypes**

**Approach:** I will use the amino acid edited zebrafish generated in aim 1 that cause behavioral defects, and screen mutant zebrafish against a focused chemical library of small molecules in 96 well plates. Behavior will then be assayed by observing fish swimming patterns, and dopaminergic neurons will be assayed through immunofluorescence imaging of neurons. **Rationale:** Molecules that rescue the behavioral defects will likely function in the release of dopamine. These molecules could then be used in treatments for Lewy body dementia. **Hypothesis:** Molecules that rescue behavioral defects are likely to function in dopamine release.

**Aim3: Identify proteins that interact with SNCA that are important for mediating behavior and dopamine.**

**Approach:** I will collect brain cells from WT zebrafish and mutant zebrafish generated in aim 1. These cells will be analyzed via co-immunoprecipitation and mass spectrometry. **Rationale:** the comparison of WT protein interactions to mutant protein interactions allows for the analysis of interacting proteins that could play a vital role in dopamine release and regulation. By determining interacting proteins, we can better explain the mechanisms and influences of dopamine release **Hypothesis:** Protein interactions present in wild type tissue will differ from protein interactions in mutant tissue.

**References:**

1. Science-The Lewy Body Society. (2016). Retrieved from https://www.lewybody.org/about-dlb/science/

2. SNCA Gene-Genetics Home Reference. (February, 2018). Retrieved from https://ghr.nlm.nih.gov/gene/SNCA

3. Desplats, P., Spencer, B., Coffee, E., Patel, P., Michael, S., Patrick, C., Adame, A., Rockenstein, E., Masliah, E. (February, 2011). α-Synuclein Sequesters Dnmt1 from the Nucleus- A NOVEL MECHANISM FOR EPIGENETIC ALTERATIONS IN LEWY BODY DISEASES. Journal of Biological Chemistry, 286(11): 9031–9037. doi:10.1074/jbc.C110.212589

4. Funahashi, Y., Yoshino, Y., Yamazaki, K., Mori, Y., Mori, T., Ozaki, Y., Sao, T., Ochi, S., Iga, J., Ueno, S. (2017). DNA methylation changes at SNCA intron 1 in patients with dementia with Lewy bodies. Psychiatry and Clinical Neuroscience, 71: 28-35. doi:10.1111/pcn.12462

5. Butler, B., Sambo, D., Khoshbouei. (June, 2016). Alpha-synuclein modulates dopamine neurotransmission. Journal of Chemical Neuroanatomy, 83-84: 41-49. https://doi.org/10.1016/j.jchemneu.2016.06.001