Characterizing novel neurodegenerative genes in *Drosophila*

a research proposal

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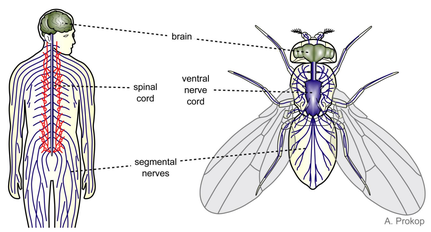
Abstract

As global populations continue to age, the prevalence of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, will continue to increase. A late onset symptom of neurodegenerative disease is locomotive defects. While many genes have been identified through whole exome sequencing as playing a role in human locomotion, little is known about what role they play in normal function. Dependable assays are needed to better understand the functions of these genes. However, modeling these diseases in vitro, to better understand, treat, and prevent them, has proven to have limitations. The isolated reality of cultures prevents an accurate simulation of the responses that would take place in the organism, such as physiological pathways and responses and non-autonomous cellular influences. Additionally, while mammalian models are important, they also can be prohibitive due to related costs and the length of time required. Fortunately, many of the issues these frameworks present have a small, but powerful answer: *Drosophila melanogaster*. Flies are easily genetically manipulated, allow for pathological observation, are inexpensive to care for, have short lifespans of about 40 days, and have an ortholog for approximately 75% of human genes known to cause disease. (Marsh, 2006)

Through the use of the GAL4-UAS system, flies can be "humanized" with a gene known to affect the brain. (Marsh, 2006). The genes will be fully characterized using behavioral and cellular assays. By observing the behavioral phenotypes of these mutants, as they age, with a negative geotaxis (climbing) assay, genetic targets of therapeutic interest can be identified. (Madabattula, 2015)



Background

Generally speaking, neurodegenerative diseases involve the degradation, and death, of neurons. Since the first description of a transgenic *Drosophila* model of a human degenerative disease in 1998, the use of fly models has grown rapidly. Diseases modeled include dominant polyglutamine-repeat diseases, tauopathies, Alzheimer's disease (AD), Parkinson's disease (PD), and expanded triplet-repeat diseases in noncoding DNA. (Marsh & Thompson, 2006) By taking advantage of flies' predisposition to climb, or **negative geotaxis**, locomotive defects can be measured and quantified over time. Using mutant *Drosophila* models allows genes of therapeutic interest to be identified as the mechanism of the climbing defect is discovered.

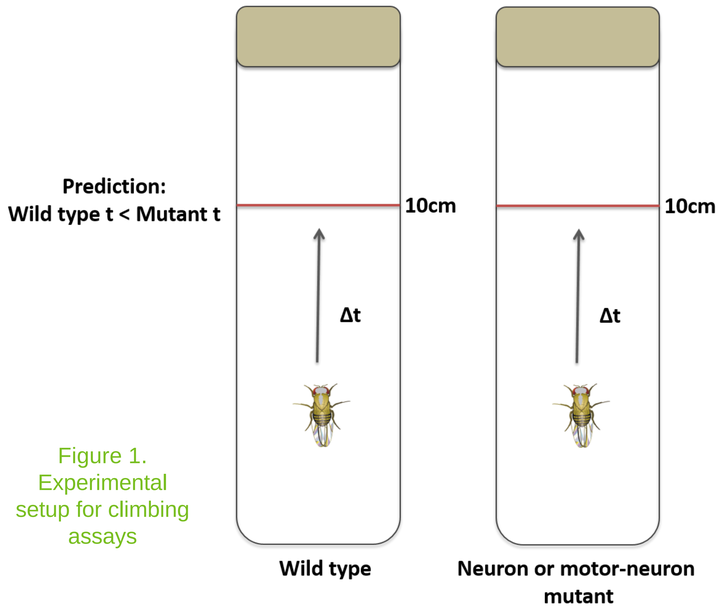


Figure 1. Experimental setup for climbing assays

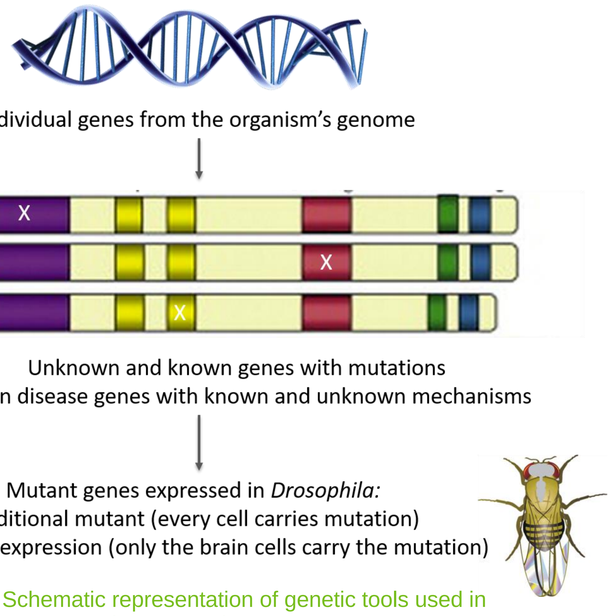


Figure 2. Schematic representation of genetic tools used in these experiments

Experiments Proposed

The experiments proposed include both a forward and reverse genetic screen, using both unknown mutants and known neurodegenerative mutants, respectively, to search for locomotory defects. The trial results will be compared to wild type and known locomotory-defective mutants. We will perform statistical analysis, using students t-test and ANOVA methods. We will also look for reduced lifespan in forward screened mutants, and this will be assessed separately. Once data is collected, we will further characterize these genes in our *Drosophila* model of neurodegeneration. We hope to uncover a novel gene involved in neurodegeneration with our project.

Methods

- Obtain flies for blind mutant screen
- Obtain flies with known genetic mutations
- Rear flies to obtain correct genotype for assay
- Collect flies of correct genotype, sex, and age
- Setup climbing assay
  - Transfer flies into climbing tube and seal with barrier
  - Setup camera focused 10 cm from the bottom
  - Begin recording
  - Gently tap tube on foam pad to displace flies to bottom
  - Conduct each trial for 2 minutes from last tap
  - With fresh tube, repeat 10 times for each genotype
- Average numbers of flies per trial to pass 10 cm
- Analyze the videos by recording the total number of flies that pass the target line
- Analyze performance using statistical analysis, such as student t-test and ANOVA
- Add candidate genes to list for further characterization

Predicted Results

We anticipate the forward genetic screen will result in a collection of neurodegenerative mutants. Our reverse genetic screen will confirm previous hypotheses as to the nature of the mutation and its effects on behavior. We will further analyze general behavior, including mating, socializing, feeding, and lifespan, of all of these mutants. Our long-term goal is to characterize these genes in working models of physiology and morphology associated with neurodegeneration, and ultimately to provide therapeutic insights for mammalian models of brain diseases.

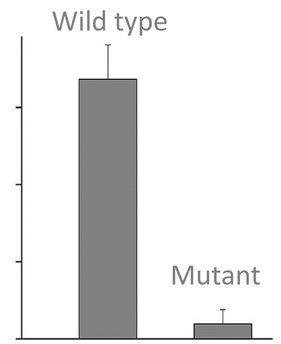


Figure 3. Predicted results: a decrease in climbing ability due to the mutation of a gene putatively involved in neurodegeneration

References

Madabattula, S.T., Strautman, J.C., Bysice, A.M., O'Sullivan, J.A., Androschuk, A., Rosenfelt, C., Doucet, K., Rouleau, G., Bolduc, F. (2015). Quantitative Analysis of Climbing Defects in a Drosophila Model of Neurodegenerative Disorders. *Journal of Visualized Experiments, 100*. doi:10.3791/52741

Marsh, J.L. & Thompson, L.M. (2006). *Drosophila* in the Study of Neurodegenerative Disease. *Neuron, 52,* 169-178. Neuron 52, 169–178, October 5, 2006 ª2006 Elsevier Inc. doi: 10.1016/j.neuron.2006.09.025