# Modeling Autism Spectrum Disorder in *Drosophila Melanogaster*

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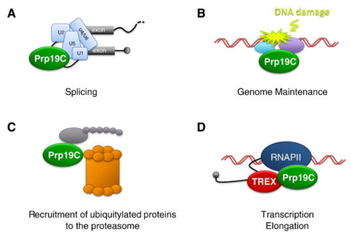
## Background

- Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental condition that impacts 1 in every 54 children eight years of age.
- Genetic components are the most frequently cited cause, but it is unclear how these genes' dysfunction lead to autism as they have a variety of other biological functions within the brain (Lord et. al 2020).




- We have collected a cohort of patients with novel neurodevelopmental syndromes that exhibit ASD symptoms and identified their genetic mutations to explore these genetic components further.
- PRPF19 is a component of the spliceosome complex in humans that is involved in pre-mRNA splicing, the process by which non-coding sequences (introns) are removed so that coding sequences (exons) can be brought together. We have identified dysfunction of this gene in our cohort (Sauerwald et. al 2017).
- The *Drosophila Melanogaster* ortholog of this gene is Prp19, allowing us to use this organism as a model for ASD.

## Hypothesis and Goal

**Hypothesis: Suppressing this gene's function in *Drosophila Melanogaster* to mimic human gene mutation will lead to the observation of altered neuromorphological and neurobehavioral phenotypes.**

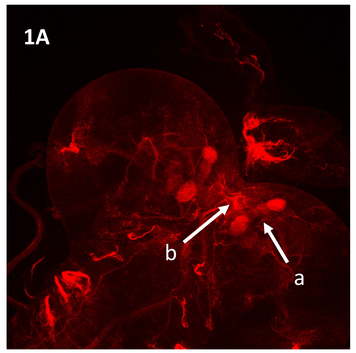
RNA interference mediated knockdown will enable us to mimic human gene mutation in order to test this hypothesis.

A variety of assays will be conducted in order to characterize the extent of these altered neuromorphological and neurobehavioral phenotypes including:

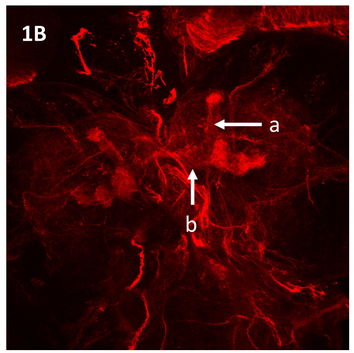
- Fas2 and pH3 staining, methods of antibody staining that allow for detection of any difference in the size and shape of the lobes of the mushroom body, as well as the number of mitotic cells in the brain respectively (Li et. al 2021, Mitiæ& McKay 2005)
- Social Space Assay that will examine the distance flies place between one another when enclosed in a specially made chamber, designed to measure potential difficulties in social situations for humans in ASD manifested in *Drosophila* (Simon et. al 2012).

**Goal: Figure out what experimental conditions will facilitate the best run-through of these assays and provide the most accurate depiction and assessment of the ASD model in Drosophila.**

## Methods and Results

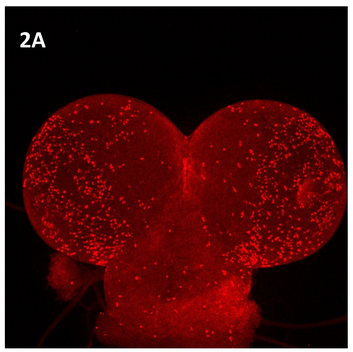



**Panel 1A:** This depicts the wildtype (WT) genotype structure of a mushroom body. Arrows a and b depict the vertical and horizontal lobes, respectively.

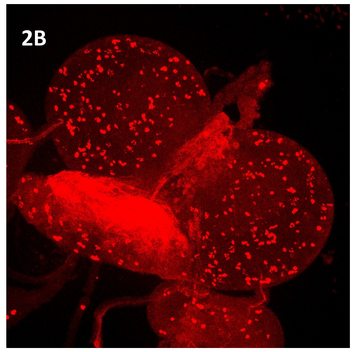



**Panel 1B:** This depicts the Prp19 RNAi genotype structure of a mushroom body. Arrows a and b depict the vertical and horizontal lobes, respectively.

**Fas2 Staining:** This method of immunostaining targets the mushroom body, the learning and memory center of the *Drosophila* brain. The horizontal lobe area and the vertical lobe area for both WT and Prp19 RNAi were quantified to see if the knockdown led to a change in brain morphology. Using a t-test, we found the difference in size of the horizontal lobe of both genotypes to not be statistically significant. However, the Prp19 RNAi vertical lobe was smaller and this finding was statistically significant with a p-value of 0.0156.



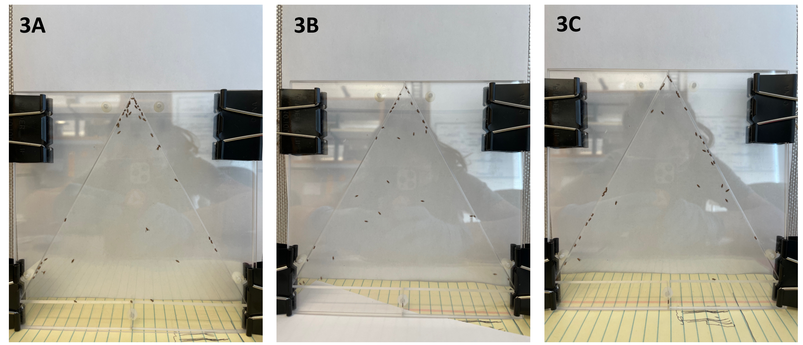

**Panel 2A:** This depicts the WT genotype structure of a *Drosophila* brain. The red dots that appear are those of mitotic (actively dividing) cells.




**Panel 2B:** This depicts the Prp19 RNAi genotype structure of a *Drosophila* brain. The red dots that appear are those of mitotic (actively dividing) cells.

## Methods and Results (Cont'd)

The average density of mitotic cells in the Prp19 RNAi was 0.002712. The average density of mitotic cells in the WT was 0.002065. The Prp19 RNAi showed a higher density. This was confirmed to be statistically significant by a t-test, yielding a p-value of 0.002862.




**Panel 3:** 3A, 3B, 3C contain female virgins, males, and females respectively in the social space assay. The assay was conducted with WT flies. For all the genders (virgins and non-virgins), a majority of the flies remained within 0-2 body lengths of one another. This was an average of 59.12% for female virgins, 49.96% for males, and 50.69% for females.

## Conclusions and Future Directions

pH3 Staining in WT vs Prp19 RNAi:

- Due to an error in mounting the brains of the WT genotype onto the microscope slides, a different magnification had to be used to capture the images of this genotype. I would conduct this experiment again and image with the same magnification for both genotypes to more accurately assess the number of cells in each lobe.
- I would also conduct this experiment again to make sure I pick larvae of the exact same size for both WT and Prp19 RNAi to ensure their brains are also of comparable sizes.

Fas2 Staining in WT vs Prp19 RNAi:

- I would conduct this experiment again to make sure I pick larvae of the same size for both WT and Prp19 RNAi to ensure their brains are of comparable sizes. There seemed to be a slight inconsistency in the overall size of the brain between the two sample sets so I would want to be even more accurate. In addition, some of the brains were torn in parts.

I want to conduct the Social Space Assay in flies that can survive to adulthood with a similar phenotype to ASD to compare to WT to see if there is a behavioral phenotype that can be accounted for.

## Contact

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