

# Female *Drosophila melanogaster* do not exhibit differences in *Dop2R* gene activity in the brain after exposure to GenX

Rodriguez D.<sup>1</sup>, Phelps S.<sup>2</sup>, Tyagi S.<sup>3</sup>, Maharem O.<sup>4</sup>, Stewart C.<sup>5,6</sup>

<sup>1</sup>Palo Alto High School, <sup>2</sup>Portola High School, <sup>3</sup>Woodbridge Academy, <sup>4</sup>Alexandria STEM High School, <sup>5</sup>Boz Life Science Research and Teaching Institute, <sup>6</sup>UC San Diego Extension.

## Summary

- Exposed both female and male *Drosophila melanogaster* to chemical GenX.
- Quantified expression changes of *Dop2R* in the brain using qRT-PCR and assessed fold changes between sexes.
- *Dop2R* expression showed no significant change across any of the conditions.

## Abstract

GenX is a chemical replacement for PFOA, a possible human carcinogen. The adverse health effects of GenX are unknown. Here, we explored the impact of GenX exposure on *D. melanogaster* by measuring changes in expression of *Dop2R*, a gene involved in stress regulation and circadian rhythms, using qRT-PCR. We observed a 61.6-fold increase in female ( $p = 0.301$ ) and 27.4-fold increase in male *D. melanogaster* of expression of *Dop2R* after exposure to GenX. At control conditions, males express *Dop2R* 209.3-fold more than females. We did not find any significant changes in expression of *Dop2R* between any of the conditions.

## Introduction

### Hypothesis

Exposure of *D. melanogaster* to GenX chemicals will increase the gene expression of the *Dop2R* gene in the brain. *Dop2R* will be upregulated in females compared to males.

### *Drosophila melanogaster*

- Exhibit sexual dimorphism
- Share 60% of genes with *Homo sapiens*
- Rich in database resources
- Short life cycle



**Figure 1. Adult *D. melanogaster* by sex.** Adult female is on the left, adult male is on the right.

### GenX

- Chemical in the PFAS chemical family
- Used for non-stick coatings
- Inconclusive evidence for health risks
- Unregulated by the EPA

### Dopamine 2-like receptor (*Dop2R*)

- protein-coding gene located on Chromosome X
- regulates aggression, feeding, lifespan, nocturnal stress response, sleep, & learning

## Materials & Methodology

**Fly culture and maintenance:**  
*D. melanogaster* raised at 20-25°C and fed cornmeal based food. Flies were separated by sex  $\leq 4$  h post-eclosion.



**Control Condition:**  
Flies were fed cornmeal food for 7 days.

**Experimental condition:**  
Flies were fed cornmeal food infused with 1000 mg/kg of GenX for 7 days.

**Sample collection:**  
Flies were sacrificed after 7 days via freezing at 80°C and brains were dissected. 100 brains per pooled per sample and RNA was extracted using the RNeasy kit (Qiagen).

**Bioinformatics:**  
STRING protein interactions and gene homology analysis was done to learn about the gene function.

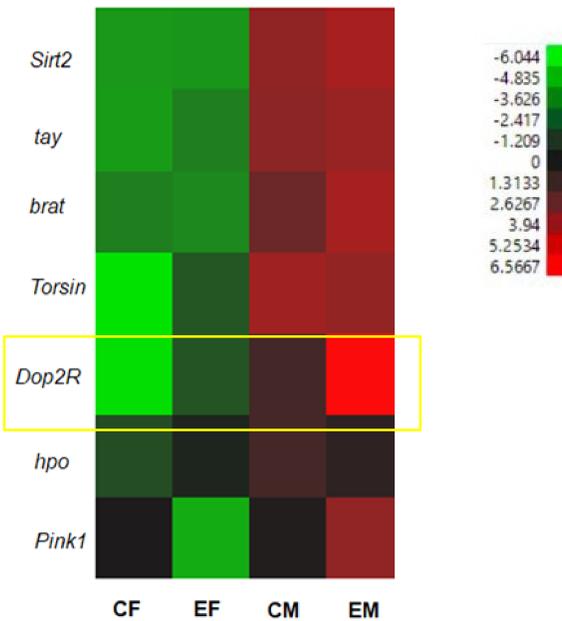
**qRT-PCR**  
*Dop2R* primers  
Forward: 5'-GGAGACGGGCAAATTCACGA-3'  
Reverse: 5'-GCCAGCAGAACAGGAAGACA-3'

**Data Analysis**

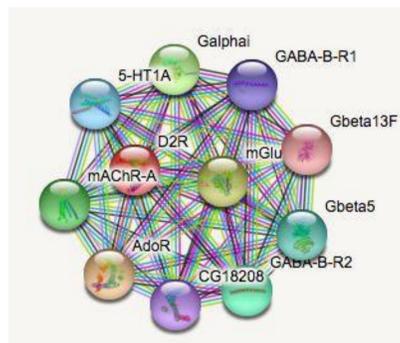
- *Dop2R* was normalized to *Actin*.
- Fold difference in gene expression was calculated using  $2^{-\Delta\Delta CT}$
- $\Delta\Delta CT$  was found for the different conditions that were compared by taking the  $\Delta CT$  difference between control female and control males, as well as between experimental condition and control condition for both males and females.
- A Student's t-test was conducted using the  $\Delta CT$  values, where a p-value of less than 0.05 was considered significant.

**Figure 2. Experimental outline**

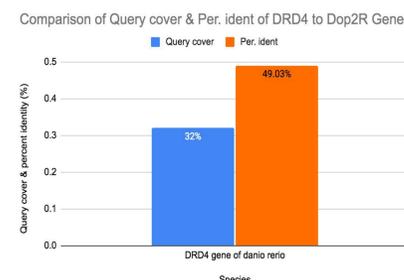
## Results



**Figure 3. Heatmap of gene expression in *Drosophila melanogaster*.** Red areas indicate higher *Dop2R* expression, whereas green areas indicate low gene expression. CF indicates control female, EF indicates experimental female, CM indicated control male, and EM indicates experimental male. *Dop2R* was upregulated 61.57 fold in experimental females compared to control females ( $p$ -value = 0.301), upregulated 27.34 fold in experimental males compared to control males and upregulated 209.3 fold in control males compared to control females.



**Figure 4. Predicted protein interaction.** STRING bioinformatics resource depicts relationships between the *Dop2R* protein and others. This figure was generated using <http://string-db.org/>



**Figure 5. Gene Homology with *D. rerio*.** Comparison of the quantity of percent identity and query cover (%) values shared by both the *Dop2R* gene in *drosophila melanogaster* and the *DRD4* gene in *danio rerio*. *DRD4* shares 49.03% of its percent identity/sequence with *Dop2R* and is 32% similar to *Dop2R* in terms of length.

## Discussion & Conclusion

- *Dop2R* encodes for a dopamine receptor, and is involved in processes such as movement, lifespan regulation and aggression.
- GenX chemicals were shown to upregulate *Dop2R* gene expression for *D. melanogaster* in both sexes. However, the data was not statistically significant ( $p$ -value = 0.301), when comparing experimental females to control females. This is likely due to the large variation across the replicates.
- Although we report a large fold change between male and female control conditions, we cannot compare these groups to perform statistical tests because we lack replicates of the male control condition.
- If these results were verified through the use of further replicates and statistical analysis, the upregulation of *Dop2R* in male flies suggests that they are more sensitive to dopamine induced responses.

## Study Limitations & Future Directions

- Insufficient replicates of control male samples resulted in not being able to perform statistical testing. In the future, we can increase the number of replicates per sample to analyze the data more thoroughly and to observe the variation in each condition.
- While *D. Melanogaster* shares several genes with humans, it may not be the model organism most similar to humans. To ensure that these effects are consistent, we can conduct this experiment using other model organisms.
- A single GenX exposure was tested. In the future, we could use higher or lower concentrations of GenX to examine whether varying GenX dosage yields different gene expression results.
- *D. Melanogaster* was administered GenX through food. For future experiments, we could change the method of exposure and determine if exposing the same amount of GenX in different forms yields similar gene expression levels.

## References

