

Dop2R does not have altered expression in female *D. melanogaster* exposed to GenX, a potential toxicant

Osumanu A.¹, Ramos A.², Martinez J.³, Blandy M.⁴, Sanchez R.⁵, Hirata, K.⁶

¹Edinaman Senior High School, ²International School of Beaverton, ³Oceanside High School, ⁴Cleveland High School, ⁵King Chavez Community High School, ⁶Boz Life Science Research and Teaching Institute

Summary

- *Drosophila melanogaster* (fruit flies) were dosed with 1000 mg/kg-day GenX for 7 days
- Quantified gene expression of *Dop2R* under various conditions using qRT-PCR.
- We conclude there was no change in expression of *Dop2R* after female flies were exposed to GenX.

Abstract

GenX is a man-made chemical used to make food packaging, fire fighting foam, and nonstick coatings. Its toxicity is unknown, so we assessed the effect of GenX chemicals on the expression of *Dop2r* in *Drosophila melanogaster* (fruit flies). *Dop2R* is a dopamine receptor involved in physiological functions such as memory, arousal, and mating. *Dop2R* was expressed 61.5-fold more in GenX-exposed females than in non-exposed females ($p = 0.301$) and 27.4-fold more in GenX-exposed males than non-exposed males. Additionally, there was a 209-fold downregulation in non-exposed female when compared to non-exposed males. There were no statistically significant differences in expression of *Dop2R* among the conditions.

Introduction

Fruit fly

60% of fruit fly genes have homologs in humans and 75% of known human genetic diseases have counterparts in the fruit fly genome (1). Fruit flies are low maintenance and sexually dimorphic.

Dop2R gene

In flies, *Dop2R* is a receptor for dopamine. The human ortholog of *Dop2R*, *DRD2*, is associated with diseases in the central nervous system, and mental health disorders. Downregulation of *Dop2R* is associated with schizophrenia and ADHD. Human males are three times more likely than females to be diagnosed with ADHD (2).

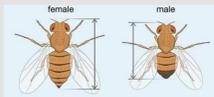


Figure 1. Adult male and female fruit flies.

GenX

Chemical replacement for PFOA in the production of nonstick chemicals. The adverse health effects of GenX are unknown, and it is not regulated by the EPA.

Hypothesis

Exposure to GenX chemicals increases the production of Dopamine 2 receptor in both male and female *Drosophila melanogaster*. Males have a higher baseline expression of *Dop2R* than females.

Materials and Methods

Fly maintenance: Flies were maintained at 22-25°C and fed cornmeal-based food. Flies were separated by sex ≤ 4 h post-eclosion.

Treatments: Control flies were fed cornmeal-based food for 7 days. GenX exposed flies were fed cornmeal-based food dosed with 1000 mg/kg of GenX per day, for 7 days.

Sample Collection: After 7 days, flies were sacrificed by freezing at -80°C. Their brains were dissected and pooled by sex, with each replicate containing 100 brains. RNA was extracted using the RNeasy kit (Qiagen).

qRT-PCR: Primers were designed to target *Dop2R*.
Forward primer
3' GGAGACGGGCAAATTCACGA 5'
Reverse primer
3' GCCAGCAGAACAGGAAGACA 5'

Bioinformatics: STRING protein interactions were analyzed to understand the gene function

Data Analysis: *Dop2R* expression 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 ΔCT 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 performed using ΔCT values. When the p-value was < 0.05 , the data was considered significant.

Figure 2. Overview of experimental methods

Results

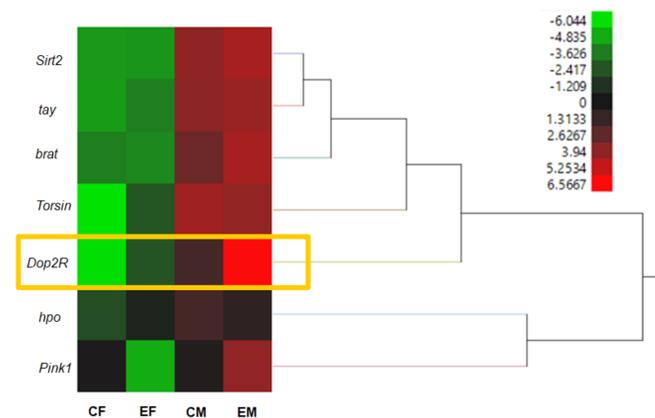


Figure 2. Hierarchical clustering of genes of male and female fruit flies exposed to different conditions. Red indicates gene upregulation, while green indicates gene downregulation. *Dop2R* was upregulated 61.57 fold in experimental females compared to control females (p -value = 0.301), upregulated 27.34 fold in experimental males compared to control males and upregulated 209.3 fold in control males compared to control females. CF indicates control female, EF indicates experimental female, CM indicated control male, and EM indicates experimental male.

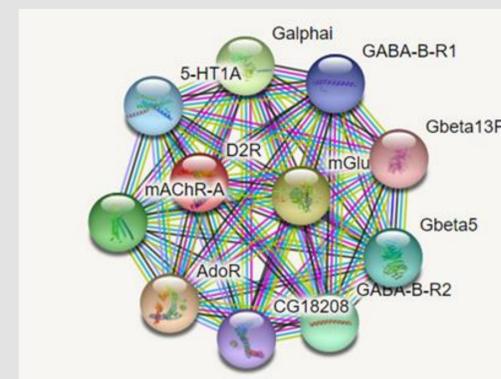


Figure 3. Proteins that interact with *Dop2R* (D2R). Most of the proteins connected to D2R are related to the G-protein signaling pathway. This figure was generated using STRING (<https://string-db.org/>).

Discussion

- Although we report relatively high fold-change values across experimental groups, none of the differences were of statistical significance:
 - Due to large variations across replicates, the 61.57 fold increase of *Dop2R* level was not statistically significant ($p = 0.301$)
 - We failed to obtain data for the experimental replicates of the control male group, so we were not able to perform statistical test for comparisons involving this group.
- *Dop2r* is a gene that encodes the receptor of dopamine
 - This receptor is vital in physiological functions such as memory, arousal, and mating.
 - If the higher expression of *Dop2R* in male flies is verified with more data, this pattern could suggest male flies might be more sensitive to dopamine-induced responses.

Limitations and Future Directions

- Did not have enough replicates of *Dop2R* for control male condition
 - Increase the number of replicates to perform statistical tests
 - increase number of replicates to visualize variability within the same condition
- Only tested one dose of GenX
 - Dose flies with a range of GenX concentrations

References

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