# No significant change in expression of *tay* in *Drosophila melanogaster* after exposure to GenX

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## Summary

- Exposed male and female *D. melanogaster* to the toxic chemical GenX
- Measured changes in *tay* expression in *D. melanogaster* brains as a result of exposure to GenX and baseline expression of *tay* between males and females using qRT-PCR
- *tay* expression has no significant changes between any of the conditions

## Abstract

Male and female organisms exhibit sexually dimorphic gene expression, resulting in potentially different responses to stressors. Most scientific studies are done with male organisms, ignoring this variance. Here we investigate the expression of *tay* in the male and female brains of the *D. melanogaster* at baseline and after exposure to GenX, a potentially toxic chemical, using qRT-PCR. Male *D. melanogaster* upregulate *tay* 1.24-fold after exposure to GenX (p=0.866) while females upregulate *tay* by 1.68-fold (p=0.548). Female *D. melanogaster* downregulate *tay* 333-fold compared to males at control conditions (p=0.057). Our conclusion is that there is no difference in gene expression.

## Introduction

**Hypothesis:**

- After exposure to GenX, *tay* will be upregulated in *D. melanogaster.*
- *tay* will be upregulated in female *D. melanogaster* compared to male *D.melanogaster*

**Figure 1. Diagram of phenotypic differences between male and female *D. melanogaster*.**

***D. melanogaster***

- Common model organism
  - Chosen for short lifespans, low maintenance, evolutionary similarities with human disease genes[4], and sexually dimorphic phenotypes

**GenX**

- Member of per- and polyfluoroalkyl substance (PFAS) group, man-made chemical compounds
- GenX is used commercially in food packaging, nonstick coatings, and firefighting foam.
- While information about its health effects is limited, GenX is said to negatively impact animals as a toxicant.[5]

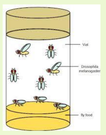
***tay***

- Associated with the organization of disc-derived wing veins (morphogenesis), the reduction of EGFR pathway activity, and visual pattern orientation [1]
- Functionally similar to GM22516 (*D. sechellia)* and serine-rich adhesin for platelets (*D. mauritiana)* [1]
- *tay* has proposed interactions with the Extracellular Regulated Kinase [2].
- *tay* is found on the X-chromosome

## Materials and Methods

Maintenance of *drosophila melanogaster*

Flies were maintained between 22-25°C and separated by sex ≤4h post- eclosion



| Control: | Experimental: |
|---|---|
| Flies fed cornmeal based food [7] for 7 days. | Flies exposed to GenX through cornmeal food, at 1000 mg/kg per day for 7 days. |

Flies were sacrificed by freezing at -80°C, and brains were dissected and pooled, with a 100 brains per sample

RNA was isolated using RNeasy kit (Qiagen).

qRT-PCR

*tay* Primers
Forward: 5'-AGCACAGGTCGCAAAGAAGTC-3'
Reverse: 5'-GGATTGCGCCGAAACATTGG-3'

Data Analysis

- STRING protein interactions and gene homology analysis was done to learn more about the gene function and interactions
- *tay* was normalized to *Actin*
- Fold difference in gene expression was calculated using $2^{-\Delta\Delta CT}$
- ΔΔCT was found for the different conditions that were compared by taking the ΔCT difference between experimental condition and control condition for both males and females, as well as taking the ΔCT difference between control female and control males.
- A Student's t-test was conducted using the ΔCT values. p value <0.05 was considered significant.

## Results

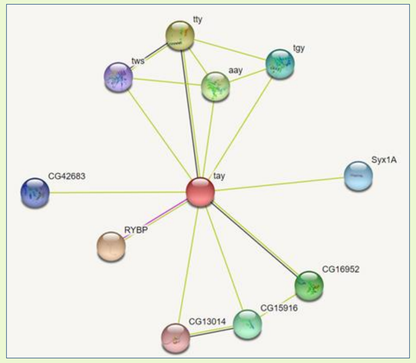



**Figure 3. Diagram showing the proteins that interact with tay.** Proteins connected to tay are generally regulatory proteins related to neurons. This figure was generated in STRING (https://string-db.org/).

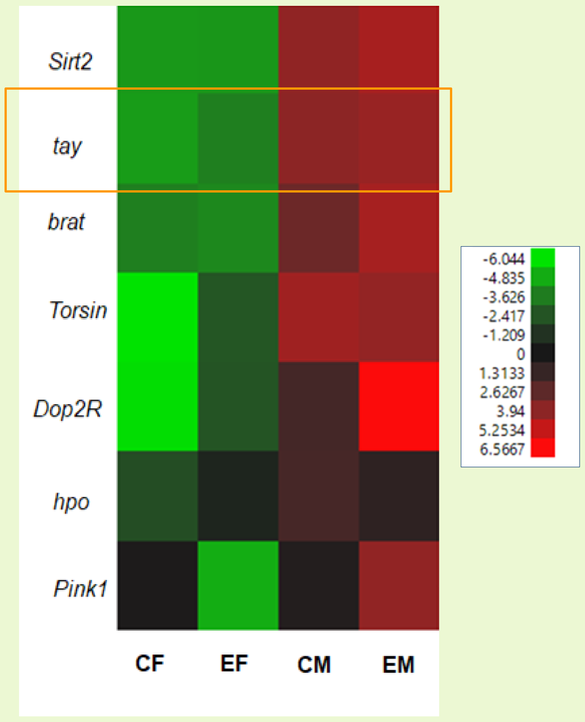



**Figure 4. Heatmap showing the change in gene expression in both female and male *D. melanogaster* in groups with and without exposure to GenX.** Red indicates a higher expression of *Tay* while green indicates a lower expression level. In females, *tay* (highlighted) expression was already low to begin with, while in males it was relatively higher. Female *D. melanogaster* had a fold change of 1.68 (p=0.548), while males had a fold change of 1.24 (p=0.866). Females express 333-fold less *tay* than males at baseline (p=0.057). There are no statistically significant changes between any of the conditions. CF represents Control Female, EF represents Experimental Female, CM represents Control Male and EM represents Experimental Male.

## Discussion and Conclusion

➢Heatmap and fold change show (Figure 4):

- Lower expression of *tay* in female *D. melanogaster* compared to male ones in both control and experimental conditions
- Upregulation of *tay* in both male and female *D. melanogaster* in the experimental condition compared to the control condition
- However, *p*-values for all comparisons are larger than the 0.05 threshold, so none of the observed differences is of statistical significance, and the null hypotheses were accepted

➢Upregulation of *tay* in *D. melanogaster* could result in changes to:

- Adult locomotive capabilities
- Changes to proper wing disk and other tissue development as a result of *tay* being an agonist for the EGFR signaling in *D. melanogaster*

➢ No significant difference in *tay* levels across the different fly groups indicate the above functions and processes are likely not affected by sex or GenX exposure.

## Limitations and Future Inquiry

- Fruit flies were exposed to only one concentration of GenX. Further experiments that expose fruit flies to different concentrations of GenX should be conducted.
- This study only looks at gene expression in pooled fly brains. We can look at individual fly brain gene expression patterns in the future.
- Expression of individual genes were analyzed. Further experiments can look at how GenX affects a group of genes involved in the same pathway, so we can better understand how GenX interacts with certain pathways
- *D. melanogaster* is evolutionarily distant from *H. sapiens*. Therefore, other model organisms that are more similar to *H. sapiens* should be used in experiments where they are exposed to GenX to better understand how GenX may impact humans.

## Sources