

tay does not have a sexually dimorphic expression in female and male *D. melanogaster*

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Summary

- Exposed male and female *D. melanogaster* to the potentially dangerous chemical GenX.
- Evaluated variations in gene expression of *tay* gene in both fly sexes using qRT-PCR.
- Found there was no statistically significant changes in gene expression between any of the conditions

Abstract

GenX is a chemical adopted to manufacture non-stick cookware, waterproof coatings, and more, but its effects on organisms are largely unknown. To better understand the effects of this chemical, we exposed *D. melanogaster* to GenX and analyzed expression of *tay*, a gene involved in the behavior and development of the organism, in both males and females. We also compared baseline expression of *tay* between males and females to assess sexually dimorphic expression of the gene. qRT-PCR data shows *tay* was upregulated 1.24-fold in GenX-exposed females (p=0.548) and 1.68-fold in GenX-exposed males (p=0.866), and downregulated 333-fold in control females compared to control males (p=0.057). We did not find any statistically significant variance in expression of *tay* in any condition.

Introduction

Hypothesis: Exposure to GenX changes the expression of the *tay* gene in *D. melanogaster*. Male *D. melanogaster* have a higher baseline expression of *tay* than female *D. melanogaster*. ***D. melanogaster*:** Model organism for the experiment. Chosen for its sexual dimorphism, short life cycle, high fertility rate, and easily observable developmental stages. Shares 75% of disease-associated genes with humans (1).



Figure 1. Picture of male *D. melanogaster*

GenX:

- GenX is used in the production of paints, non-stick products, food packages, etc.
- GenX is an alternative to PFOA with relatively unknown health effects
- Perfluorooctanoic acid, or PFOA, is a man-made chemical with diverse uses and is responsible for health and environmental issues.

Gene of interest:

- Tay bridge (*tay*) - responsible for adult walking behavior, vein morphogenesis, downregulation of epidermal growth, and behavioral response to pattern orientation.
- Activator of transcription and developmental regulator (*AUTS2*) is responsible for mental retardation and intellectual disability. *AUTS2* is an orthologue of *tay* in humans.

Methodology

Fly Maintenance: Flies were raised on BDSC cornmeal food at 25°C. Male and female virgin flies were separated <4 hours post-eclosion.

Treatments: Control flies were fed BDSC cornmeal food for 7 days and GenX-exposed flies were fed BDSC cornmeal food dosed with 1000 mg/kg per day for 7 days

Sample collection: After 7 days, flies were sacrificed via freezing at -80°C. 100 fly brains were dissected and pooled by sex and frozen at -80°C. RNA was extracted using the RNeasy kit (Qiagen) and stored at -80°C

qRT-PCR: *Tay* was targeted by the following primers
Forward primer:
3' CACGTAACAAAACCGCTGCC 5'
Reverse Primer:
3' AAACCCATCGACTTCTTTGCG5'

Data Analysis

- STRING protein and gene homology was done to learn more about the protein and its interactions
- tay* was normalized to *Actin*
- Fold difference in gene expression was calculated using $2^{-\Delta\Delta CT}$
- Afterwards $\Delta\Delta CT$ 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 the ΔCT values. (p value <0.05 is considered significant).

Results

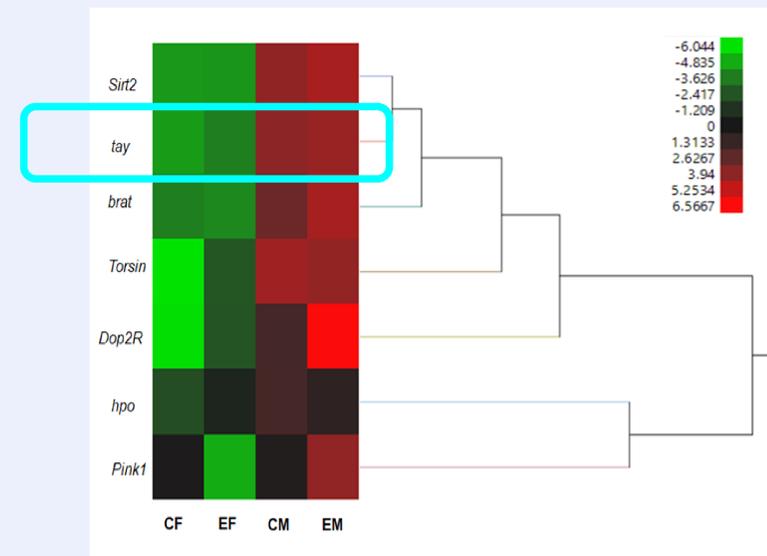


Figure 2. Heatmap showing the differential gene expression in control and GenX-exposed *D. melanogaster*. Gene expression downregulation is shown by green and upregulation is shown by red. GenX-exposed females had 1.681-fold upregulation compared to control females (p = 0.548), GenX-exposed males had 1.243-fold upregulation compared to control males (p = 0.866), and control females had 333-fold downregulation compared to control males (p = 0.057). CF indicates control female, EF indicates experimental female, CM indicated control male, and EM indicates experimental male.

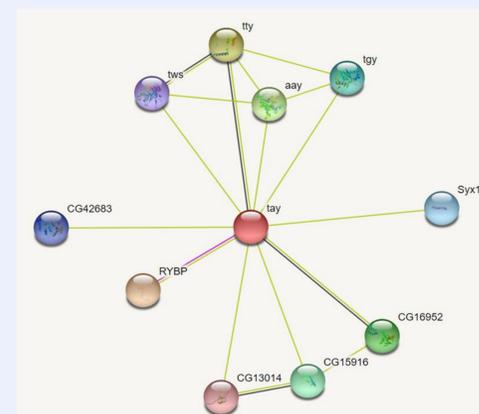


Figure 3. Diagram showing the proteins that interact with *tay*. Proteins connected to *tay* are generally regulatory proteins related to neurons. This figure was generated in STRING (<https://string-db.org/>).

Discussion and Conclusion

- There is no statistically significant difference between *tay* expression levels in female and male flies.
 - The heatmap (Fig. 2) demonstrates the difference between the average *tay* expression levels of female and male flies, although the p-value is close to, but not below the 0.05 threshold (p=0.057).
- After exposure to GenX, *tay* has a slight and not statistically significant upregulation for both males (1.681-fold, p=0.548) and females (1.243-fold, p=0.866).
 - Upregulation of *tay* in *D. melanogaster* can cause the enhancement of the inhibition of Extracellular regulated kinase (2).
 - This process is required for the normal functions of motoneurons and neural channels (2).
- As *tay* expression is not significantly affected by GenX exposure, we infer that *AUTS2*, an ortholog of *tay* in humans, is also not likely to be affected by GenX exposure.
 - AUTS2*, if affected, could possibly lead to neurodevelopmental disorders as well intellectual disability and developmental delay.

Study limitations/ Future directions

- Drosophila melanogaster* is an invertebrate system, thus having some biological processes differ than those of vertebrates
 - Should test with additional organisms that are evolutionarily similar to humans
- Cannot account for individual variation in gene expression; pooled fly brains were used
 - Should perform experiment with individual flies in the future
- Only exposed in one concentration of Gen X
 - Future studies should test multiple concentrations

References

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