

Expression of D2 dopamine receptors, *Dop2R*, is higher in male *Drosophila melanogaster* by 4.15-fold

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Summary

- Are male and female brains functionally differently regulated?
- qRT-PCR was performed to determine differences in expression of a dopamine receptor, *Dop2R*, between male and female fruit flies.
- Males were observed to have 4.15-fold higher expression of *Dop2R* than females.

Abstract

Males and females are fundamentally different in gene expression and hormone regulation. This experiment quantifies the difference in *Dop2R* mRNA expression between male and female *Drosophila melanogaster* (fruit fly) brains. *Dop2R* encodes a dopamine receptor that is correlated to neurological disorders. qRT-PCR reveals sex-specific gene expression with 4.5-fold higher expression of *Dop2R* mRNA in male fruit flies compared to females, indicating sexual dimorphism in brain function.

Introduction

Hypothesis:

- Since males are more likely to develop dopamine-related diseases (e.g. Parkinson's disease, schizophrenia, and ADHD), *Dop2R* will have higher expression in male relative to female fruit flies.

Drosophila melanogaster (fruit flies):

- Fast generation turnover, easy to maintain, produces many offspring.
- Genome is about 60% similar to that of humans.¹

Dop2R:

- Gene that codes for D2 dopamine receptors in the central nervous system.
 - These receptors are essential for normal brain function in both organisms, controlling locomotion, learning, memory, and sleep.^{2,3}
- Misregulation of these receptors and associated structures lead to diseases like Parkinson's, schizophrenia, and ADHD. In Parkinson's disease, dopaminergic neurons are lost in the midbrain, and dopamine levels decrease by 93-99%, while D1 and D2 receptor type densities increase.⁴
- Males are more likely to develop Parkinson's disease, develop schizophrenia at an early age, and more likely to be diagnosed with ADHD.⁵

Methodology

Fly Keeping/Separation by Sex

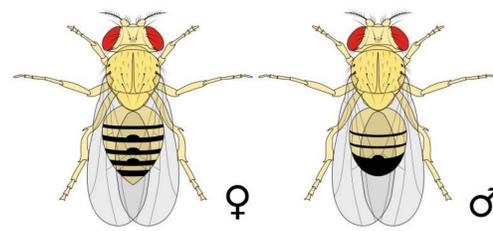


Figure 1. Anatomical differences in male and female *D. melanogaster*.

Virgin Separation

- Collected virgins ≥ 4 post eclosion
- Flies of both sexes were fed 5% sucrose for 72h (control)

Isolation of RNA from *D. melanogaster* Brains

- Homogenized fly brains to extract RNA solution
- Removed unneeded cellular materials

DNase Treatment of Extracted RNA

- DNase used to treat RNA from each pooled sample

qRT-PCR

Dop2R primers

- Forward Primer: 5' CGACGATTCGCCTTGTAGT 3'
- Reverse Primer: 5' CTCCTAGTGCCTGCAACTATTC 3'
- Performed with iTaq Universal SYBR Green kit

Data Analysis

- Calculated the $\Delta\Delta Ct$ – the difference between ΔCt females and ΔCt males

Results

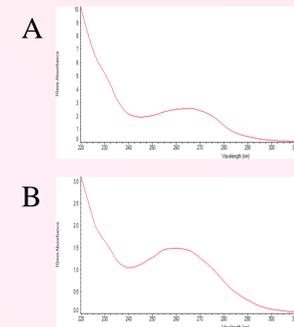


Figure 2. Total RNA yield of female and male brain samples.

(A) Total RNA yield from female brain samples, (B) total RNA yield isolated from male fly brains, and (C) total RNA concentrations for male and female pooled samples. Readings taken from Nanodrop 2000.

Sex	[RNA]	A260/A280
Male	97.6 ng/uL	1.97
Female	58.9 ng/uL	2.04

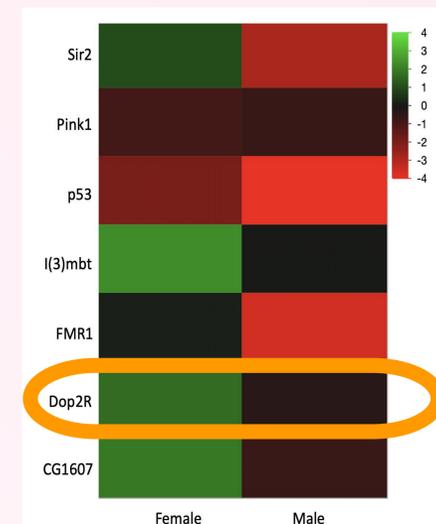


Figure 3. *Dop2R* mRNA expression is 4.5-fold higher in males relative to female fruit flies.

Red indicates higher expression while green indicates lower expression. All genes normalized to *gapdh1*.

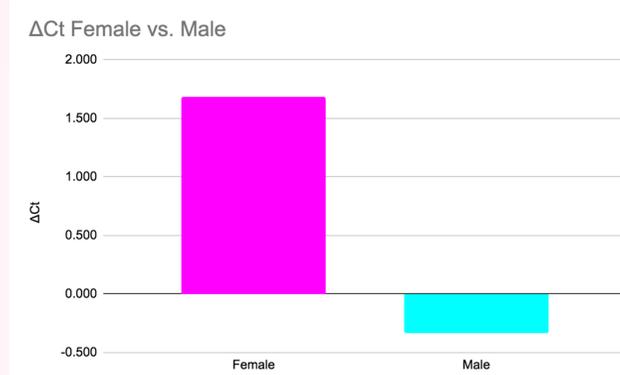


Figure 4. ΔCt values of *Dop2R* are higher in females relative to male fruit flies. $\Delta Ct = -0.366$ for males and $\Delta Ct = 1.686$ for females. $\Delta\Delta Ct = 2.052$. mRNA expression normalized to *gapdh1*.

Discussion & Conclusion

Dop2R is differentially expressed between female and male fruit flies.

- Males have 4.5-fold higher expression of *Dop2R* mRNA relative to females.
- This result agrees with the hypothesis and supports previous research on increased development of neurological disease in males.
- Higher baseline levels of *Dop2R* mRNA could be indicative of a male-specific risk for Parkinson's disease.⁴

The difference between male and female fruit fly expression of *Dop2R* provides a baseline for studying sexual dimorphic expression of fruit flies under stress.

Study Limitations & Future Directions

- Fruit fly brains were pooled, future studies can explore individual genetic differences between sexes.
- One life-stage (post-eclosion) was studied in this experiment, future studies could analyze more development stages.
- Study maternal and paternal effects of this gene on offspring.
- Investigate the baseline expression of *Dop2R* in females and males in higher order model organisms.
- Experiment on isolated neurons from different parts of the brain and on the other dopamine receptor types.

References

