

Apr 25th, 9:00 AM - 10:00 AM

Effects of E-Cigarette Vapor on Dalpha7 Gene Expression in Adult *Drosophila melanogaster*

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Seal, Roma and Maag, Bella, "Effects of E-Cigarette Vapor on Dalpha7 Gene Expression in Adult *Drosophila melanogaster*" (2019). *Carroll College Student Undergraduate Research Festival*. 66.
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Effects of E-Cigarette Vapor on *Dalpha7* Gene Expression and Motor Function in Adult *Drosophila melanogaster*



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Introduction

- The species *Drosophila melanogaster* (*D. Melanogaster*) is a popular model organism in Neuroscience.
- *Dalpha7* is a gene that codes for the Nicotinic Acetylcholine Receptor in the nervous system in both humans and *D. melanogaster*.
- The goal of this project was to determine whether nicotine from E-cigarette vapor has a deleterious effect of the motor function in *D. melanogaster*.
- **Hypothesis:** If E-cigarette vapor is given to *D. melanogaster*, then there will be more expression of *Dalpha7*; resulting in the inhibition of motor function.

Methods

- **Primer synthesis:** Primers for *Dalpha7* were designed using IDT Oligoanalyzer software.
- **Culturing:** *Drosophila melanogaster* cultures were maintained in a medium containing potato flakes, yeast, and deionized water. The cultures were stored in a 22.5 ° C incubator.
- **Exposure:** Control flies exposed to CO₂ and experimental flies exposed to CO₂ and 1 syringe full (10ml) of E-Cigarette vapor containing 1.2% Nicotine, once a day, for four days.
- **RNA extraction:** RNA was extracted using Trizol and purified using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** cDNA was synthesized using RevertAid.
- **qPCR** was performed using PowerUp SyBr Master Mix and primers targeting *GAPDH* and *Dalpha7*. The cycle threshold values were used to generate fold changes in *Dalpha7* expression.
- **RING Assay:** A Negative Geotaxis assay was used to compare motor function between control and experimental *D. Melanogaster*.

Results

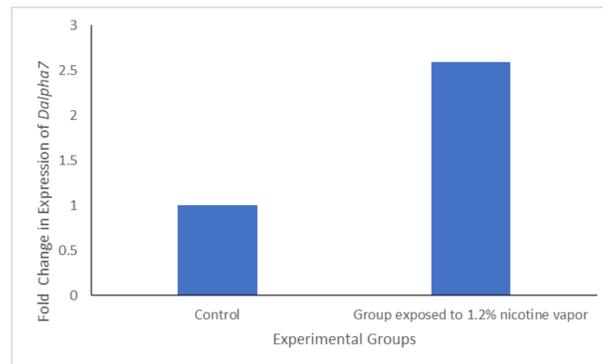


Figure 1: Comparison in fold change between control flies and flies exposed to E-Cigarette vapor containing 1.2% Nicotine. (P-value = .6016)

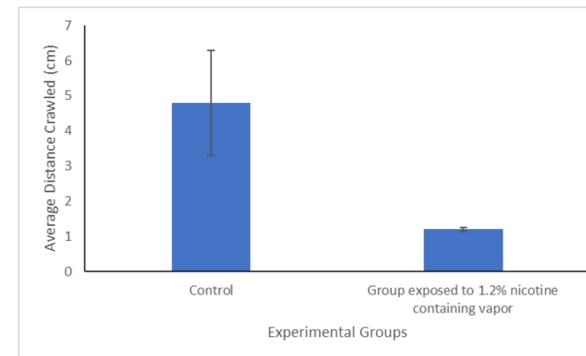


Figure 2: Comparison between motor function in control flies and flies exposed to E-cigarette vapor containing 1.2% Nicotine. Error bars show the standard error for each average. (P-value = 4.26 E-10)

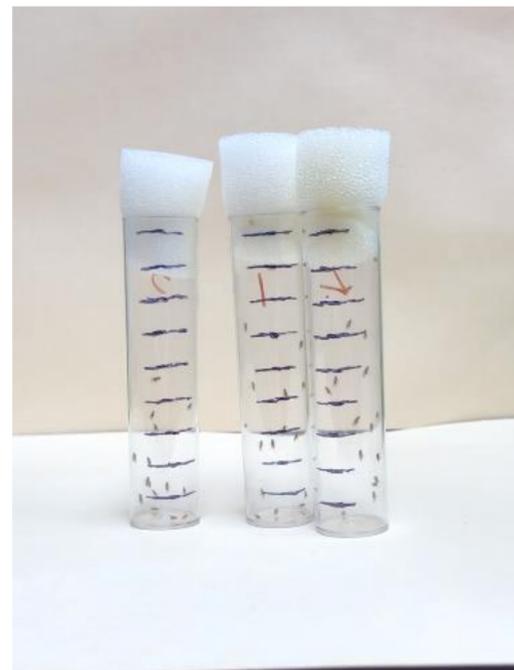


Figure 3: Control group 3 seconds after sharply tapping the vials on the table.

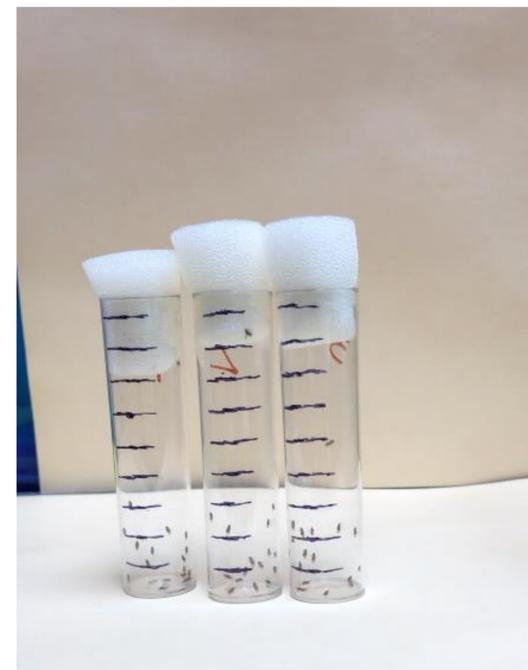


Figure 4: Experimental group 3 seconds after sharply tapping the vials on the table.

Conclusion

- The RING assay showed a slower reaction time in *D. Melanogaster* that were exposed to E-Cigarette vapor.
- The t-test p-value proved the difference to be significant for motor function (Fig. 2).
- The fold change for gene expression of *Dalpha7* between the control group and the group exposed E-cigarette vapor was 2.59.
- The p-value showed the difference in *Dalpha7* expression was insignificant (Fig. 1).
- The results disagreed with our hypothesis on *Dalpha 7* expression and agreed with our hypothesis regarding motor function.

Future Direction

Using the same experimental set-up, we would focus on the connection between E-cigarette vapor and motor function in *D. Melanogaster*, while testing the expression of a different gene.

References

Nichols, C. D., Becnel, J., & Pandey, U. B. (2012). Methods to Assay *Drosophila* Behavior. *Journal of Visualized Experiments : JoVE*, (61). <https://doi.org/10.3791/3795>

Acknowledgements

We would like to thank Dr. Stefanie Otto Hitt for her support and guidance with this project.