Effect of Ethanol on the *Hangover* Gene and Synaptic Growth in *Drosophila melanogaster*

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Introduction

- The common fruit fly, *Drosophila melanogaster (D. melanogaster*), is a popular model organism for biology.
- *Hangover* (*HANG*) is a gene encoding a nuclear protein that negatively regulates the development of axonal terminals at the neuromuscular junction of larvae. *HANG* also regulates a cellular stress pathway related to heat and ethanol exposure. (Schwenkert I, et al. 2008)
- **Hypothesis:** If *D. melanogaster* larvae are exposed to alcohol, then the *Hangover* gene expression will increase, and the larvae will experience hindered mobility during a crawling assay. It was hypothesized that the adult flies would not experience increased expression of *HANG* or changed mobility.

Methods

- **Primer synthesis:** Primers for *HANG* were designed using IDT Oligo-analyzer software.
- **Culturing:** *D. melanogaster* cultures were maintained in potato flake media with water (control) or 6.5% ethanol (treatment) for either 2 or 4 weeks at 22.5℃.
- **Larvae mobility assay:** The distance (cm) that larvae traveled over agar gel was measured after 20 seconds.
- **Adult mobility assay:** The distance (cm) that adults flies crawled up the side of a culture tube was measured after 12 seconds.
- **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 *HANG* and *GAPDH*.

Results

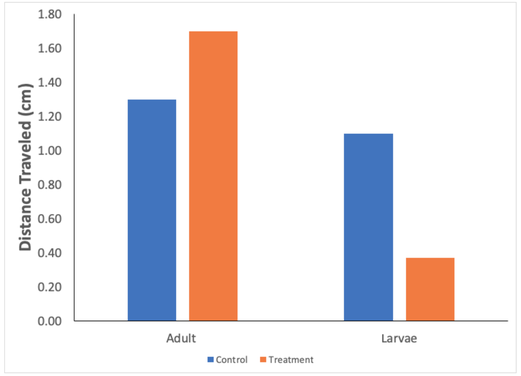

Figure 1: Mobility assays of control and four weeks of treatment with 6.5% ethanol. Larvae p-value: 0.00935212. Adult p-value: 0.55690868.

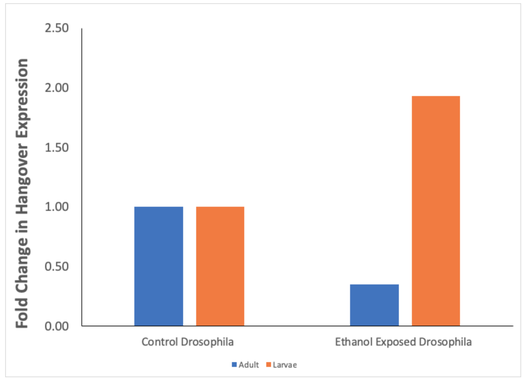

Figure 2: Fold change in the expression of the *Hangover* gene in both adult *D. melanogaster* and larvae.

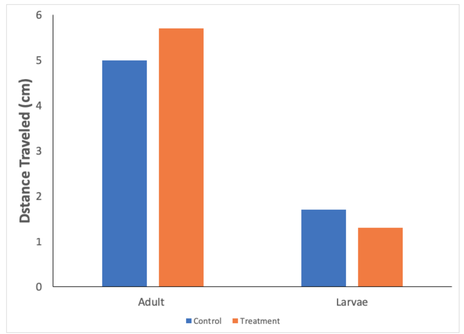

Figure 3: Mobility assays of control and two weeks of treatment with 6.5% ethanol.

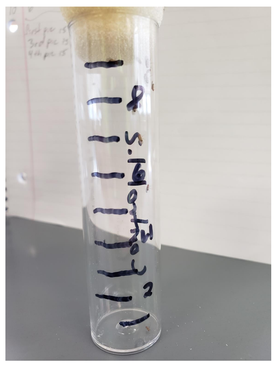

Figure 5: The adult *D.melanogaster* are put at the bottom of a cylindrical tube to perform in the adult mobility assay.

Figure 4: A larvae performing in the Larvae mobility assay.

Figure 6: Ben McFarland demonstrating how the *D. melanogaster* were anesthetized in order to remove them from their cultures.

Conclusion

- The Larvae Crawling assay indicated that ethanol exposure hindered larvae mobility, as seen in Figures 1 and 2.
- The larvae qPCR rounds demonstrated that ethanol exposure resulted in increased expression of *HANG,* as seen in Figure 2.
- The Adult Crawling assay indicated that ethanol exposure did not hinder the mobility of adult flies, as seen in Figures 1 and 3.
- The adult qPCR rounds demonstrated that ethanol exposure resulted in decreased expression of *HANG*, as seen in Figure 2.
- The results agreed with our hypothesis on gene expression and with our hypothesis for mobility, both in adult and larvae *D. melanogaster*. However, more studies need to be performed to increase sample sizes.
- Future applications of this study are to perform mobility assays and run qPCR rounds on the same group of flies every two weeks for six or more weeks to track the long term effects of ethanol on the expression of *HANG* and its respective effects on adult and larvae mobility.

References

Schwenkert I, Eltrop R, Funk N, Steinert JR, Schuster CM, Scholz H. 2008. Mech Dev. 125(8):700-11

Acknowledgements

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