

# Effect of Ethanol Withdrawal on *Dunce* Gene Expression and Learning in *Drosophila melanogaster*

## Introduction

- Drosophila melanogaster* (*D. melanogaster*) is a eukaryotic organism with genetic similarities to humans. This factor, along with ease of care, makes it an excellent model organism.
  - The *Dunce* gene encodes a cAMP Phosphodiesterase that functions in the formation of new memories by degrading cAMP.
  - Prior research has shown that *D. melanogaster* experiencing symptoms of withdrawal from alcohol have difficulty learning to pair heat shock with a scent.
- Hypothesis:** Ethanol withdrawal will inhibit short-term memory formation due to a decrease in expression of *Dunce*.

## Methods

- Primer Synthesis:** Primers for *Dunce* were designed using IDT Oligoanalyzer software.
- Culturing:** *D. melanogaster* cultures were maintained in potato media with 5% ethanol for a week. The experimental group was removed from 5% ethanol for 24 hours prior to testing.
- Behavioral Assay:** Larvae were subjected to agar plates made with Ethyl Acetate (EA) soaking in a 43°C water bath. Larvae were then tested for aversion to EA by placing them onto an agar plate with an EA scent zone.
- RNA Extraction:** RNA was isolated with Trizol and purified using Qiagen's RNeasy Mini Kit.
- Reverse Transcription:** cDNA was synthesized using RevertAid.
- qPCR:** Powerup SYBR was used for qPCR. *Gapdh* gene expression was used to normalize *Dunce* gene expression.

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**Figure 1:** Fly larvae were extracted from culture tubes after the end of treatment. The flies were then placed on an agar plate with scent to test for learning.



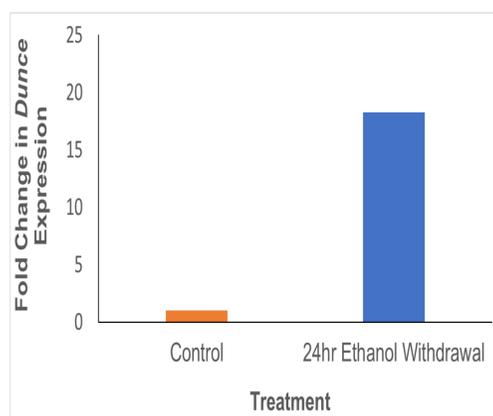
**Figure 2:** Bottom of an agar plate with full markings used for the behavioral assay.



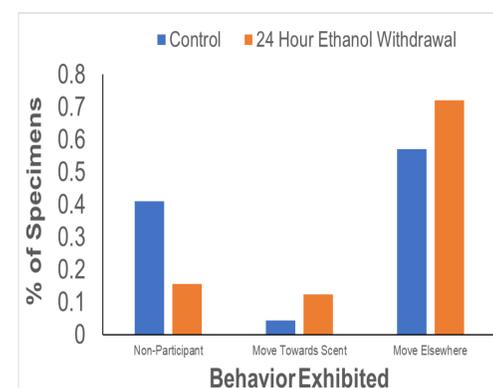
## Conclusion

- The behavioral assay was inconclusive, likely due to the usage of 2<sup>nd</sup> instar larva rather than third instar larva. The 2<sup>nd</sup> instar were too small and went missing during the assay, they were also less durable and likely experienced too much strain during the heat shock treatment, leaving them sluggish during the assay.
- qPCR showed that ethanol withdrawal had no significant impact in expression of the *Dunce* gene (Fig. 3).
- The results showed an increase in *Dunce* expression which conflicted with our original hypothesis. Although the difference was not significant ( $p=0.39$ ) we believe future experiments would show an impairment in learning due to increased cAMP Phosphodiesterase activity, leaving too little cAMP left for normal memory formation.

## Results



**Figure 3:** Fold change in expression of *Dunce* between the *D. melanogaster* control group and the group withdrawn from ethanol for 24 hours. ( $p=0.39$ ,  $t=-1.09$ ,  $df=2$ )



**Figure 4:** Results of the behavioral assay. *D. melanogaster* are classified based on the behavior exhibited; the individuals either remained in the center of the dish (non-participants), moved towards the scent, or moved out of the center but not towards the scent.

## References

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- Lin C, Rankin C. 2021. Alcohol addiction: chronic ethanol leads to cognitive dependence in *drosophila*. *Dispatch*. 22(24):PR1043-PR1044.

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