Fly Away From Addiction: Testing Dibenzazepine's Effect on Ethanol Preference in *Drosophila melanogaster*

Question

Does the administration of 0.11mg/ml and 0.14mg/ml of Dibenzazepine, a γ-secretase inhibitor, affect ethanol preference (measured via average Normalized Food Consumption in µl/mg) in *Drosophila melanogaster* with induced addiction from 15% ethanol exposure over 3 days in their food source?

Importance

- According to the World Health Organization, 400 million people worldwide suffer from alcohol addiction.
- If this experiment finds that Dibenzazepine successfully lowers ethanol preference in *Drosophila*, it could be the beginning of finding a solution for those affected by alcohol addiction.

Background Info

CAFE Assay
- A feeding assay that is used to measure *Drosophila's* behavioral feeding patterns.
- Built for this research based on Oramas *et al.* and Diegalmann *et al.*

Notch Signaling Pathway
- When ethanol is consumed, the Notch Pathway is activated, causing positive alcohol related memories to be created.

Dibenzazepine (γ-Secretase Inhibitor)
- Dibenzazepine, a γ-secretase inhibitor, is a substance that blocks the activity of the γ-secretase enzyme in the Notch Pathway.
- When Dibenzazepine is introduced to the Notch Pathway, genes related to memory formation "turn off," which leads to neutral associations with ethanol.
- By stopping the activation of the Notch Pathway, alcohol-related memories are not created.
- In a study done by Oramas *et al.*, it was found that the concentrations 0.14mg/ml and 0.11mg/ml of Dibenzazepine lowered ethanol preference.

Drosophila melanogaster
- *Drosophila* will be used as a model organism because they find ethanol rewarding, have memory systems, and have the Notch Pathway.
- In a study done by Kaun *et al.*, it was found that *Drosophila* find ethanol rewarding and prefer it over a neutral scent.

Ethanol
- Ethanol, the active ingredient in most alcoholic beverages, works by modulating the central nervous system to make the brain feel pleasure or rewarded.
- The 15% ethanol mixture used in this experiment will be distributed through capillaries in a CAFE Assay.

Hypothesis

If addiction is induced in *Drosophila melanogaster* by feeding them 15% ethanol for 2 days and then they are then fed either 0.11mg/ml, 0.14mg/ml, or 0.0mg/ml of Dibenzazepine on day 3 (all other conditions kept the same), then on day 4 the *Drosophila* given 0.14mg/ml of Dibenzazepine will consume the least ethanol in a choice test between regular food and food containing 15% ethanol.

Justification

- When ethanol is consumed the Notch Pathway is activated, causing positive alcohol related memories to be created.
- Dibenzazepine, a γ-secretase inhibitor, blocks the activity of the γ-secretase enzyme, which then prevents it from activating other proteins involved in memory formation.
- Based on the properties of γ-secretase inhibitors, the administration of Dibenzazepine at a certain concentration should cause ethanol consumption to decrease in *Drosophila* with induced addiction because memories of the substance are being blocked.
- Oramas *et al.* showed that Dibenzazepine significantly decreases ethanol consumption in *Drosophila* with induced addiction. Based on these findings, it is believed that similar results will occur when using the same concentrations.

Abstract

Objectives

According to the W.H.O., 400 million people worldwide suffer from alcohol addiction. In response to this fact, this experiment aimed to find a treatment for alcohol addiction through a *Drosophila* model by inducing ethanol addiction and measuring if ethanol preference would be reduced when given Dibenzazepine, a γ-secretase inhibitor.

Methods

To induce addiction, *Drosophila* were given 15% ethanol through capillaries in a CAFE Assay for two days. After the two days, *Drosophila* were separated into groups and treated with either 0.00mg/ml, 0.11mg/ml, or 0.14mg/ml Dibenzazepine. Post treatment, a choice test was conducted in which *Drosophila* were given 15% ethanol and normal food. After the choice test, the distance the food moved in the capillary was measured to quantify Normalized Food Consumption.

Results

The results were based on the Normalized Food Consumption of both food types. It was found that the group given 0.11mg/ml of Dibenzazepine consumed the least amount of ethanol, 0.38µl/mg, instead of the hypothesized 0.14mg/ml, which consumed 0.42ml/mg, compared to the control that consumed 0.52ml/mg of ethanol. In addition, *Drosophila* given 0.14mg/ml of Dibenzazepine had the most equalized consumption with a 0.10µl/mg difference between food types.

Conclusion

This research has shown that Dibenzazepine is a slightly effective alcohol addiction treatment. The *Drosophila* given Dibenzazepine consumed a decreased amount of 15% ethanol compared to *Drosophila* not given any. However, the response was not proportional to the amount given.

Data Tables

Dibenzazepine (mg/ml)	Average Normalized Food Consumption: Regular (ml/mg)	Average Normalized Food Consumption: Ethanol (ml/mg)
0.00 mg/ml	0.27	0.52
0.11mg/ml	0.25	0.38
0.14mg/ml	0.32	0.42

Figures 1-4: Data Tables. Tables created by Finalist using Google Docs, 2026.

Graphs

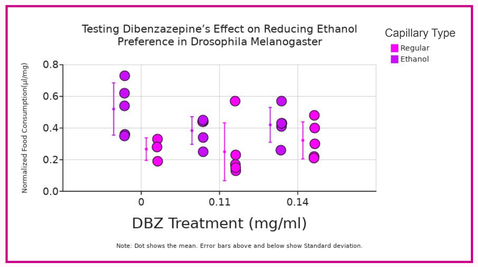

Figure 6: Normalized Food Consumption (µl/mg). Graph created by finalist using Data Classroom, 2026.

Effect	Degrees of Freedom (df)	F-statistic (MS / MS residual)	P-value	Interpretation of P
DBZ (mg/ml) (X)	2	0.79	0.46	Means: **little to no indication** that there are differences between groups
Capillary Type (Z)	1	10	<0.01	Means: **extremely strong confidence** that there are differences between groups
Interaction (X * Z)	2	0.78	0.47	Means: **little to no indication** that the effect of X depends on Z
Error or Residual	22			

Figure 7: Analysis of Data Collected. Table created by Finalist using Data Classroom, 2026.

Diagram of Design

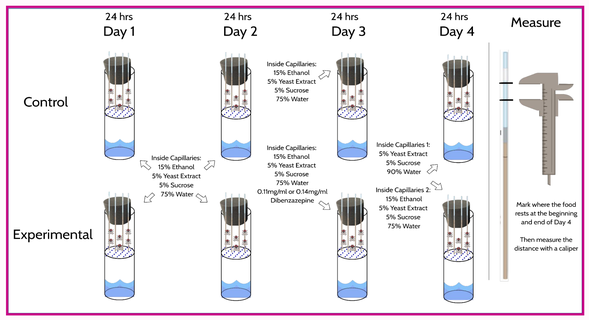

Figure 5: Diagram of Design. Diagram created by Finalist using Canva, 2025.

Photos

Figure 8: Finalist collecting data. Image taken of Finalist by Finalist's mentor, Jerilyn Neshek, 2025.

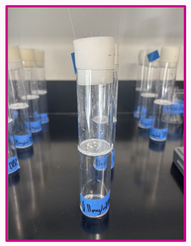

Figure 9 : CAFE Assay. Image taken by Finalist, Lilly Swigert, 2025.

Figure 10: Drilling CAFE Assay holes. Image taken by Finalist's mentor, Jerilyn Neshek, 2025.

Procedure

1. Construct CAFE Assays.
2. Make ethanol-laced food to be used throughout the experiment (3.21 mL 70% ethanol, 0.75 g sucrose, 0.75 g yeast extract, 0.75 g yeast extract).
3. Fill and place capillaries for Day 1-2: 15% ethanol-laced food.
4. Make food treatments by mixing Dibenzazepine with ethanol-laced food.
5. Fill and place capillaries for Day 3 with either:
   a. 15% Ethanol + 0.11mg/ml Dibenzazepine food.
   b. 15% Ethanol + 0.14mg/ml Dibenzazepine food.
6. Make regular food (0.25 g sucrose, 0.25 g yeast extract, 5 ml water).
7. Fill and place capillaries for Day 4: 15% ethanol-laced and regular food.
8. Record results from the choice test.
9. Calculate Normalized Food Consumption using formula (Diegalmenn *et al.*).

Discussion

Summary of Results
- *Drosophila* given Dibenzazepine consumed a decreased amount of 15% ethanol-laced food compared to *Drosophila* not given any.
  - The Normalized Food Consumption of 15% ethanol in *Drosophila* given 0.14 mg/ml of Dibenzazepine was 0.42µl/mg, whereas the group given 0.00 mg/ml of Dibenzazepine consumed 0.52µl/mg.
- *Drosophila* given Dibenzazepine consumed a more equalized diet between the two food types compared to *Drosophila* not given any.
  - *Drosophila* given 0.14mg/ml of Dibenzazepine consumed 0.10µl/mg more 15% ethanol food than regular food, whereas the group given 0.00mg/ml of Dibenzazepine consumed 0.25µl/mg more 15% ethanol food than regular food.

Data Interpretation
- Overall, the data shows that feeding *Drosophila* Dibenzazepine after inducing addiction to ethanol will slightly decrease preference to ethanol and equalize consumption between the two food types.
- This suggests that Dibenzazepine is a slightly effective pharmacotherapy for decreasing ethanol consumption and preference in *Drosophila* with induced addiction.
- However, the p-value contradicts this, suggesting that the consumption was random.

Data Justification
- It is logical that Dibenzazepine can reduce ethanol preference, because it is a γ-secretase inhibitor that blocks the activity of the γ-secretase enzyme in the Notch Signaling Pathway, causing positive alcohol related memories to not be formed, blocking addiction to the substance.

Conclusion

Predicted Hypothesis and Results
- It was hypothesized that on day 4 the *Drosophila* given 0.14mg/ml of Dibenzazepine would consume the least ethanol-laced food in a choice test between regular food and food containing 15% ethanol.
- The hypothesis was not supported by the data.
- The results showed that *Drosophila* given 0.14mg/ml of Dibenzazepine did not consume the least amount of ethanol compared to the other groups.
  - *Drosophila* given 0.11mg/ml consumed 0.38µl/mg ethanol food.
  - *Drosophila* given 0.14mg/ml consumed 0.42µg/ml ethanol food.
  - *Drosophila* given 0.00mg/ml consumed 0.52µl/mg ethanol food.

Inconsistent with Prior Research
- Oramas *et al.* found that *Drosophila* given 0.14mg/ml of Dibenzazepine consumed the least ethanol compared to other groups.
- The results of this experiment are not wholly consistent with Oramas *et al.*

Research Impact
- These results can be used to plan further studies that test Dibenzazepine on other organisms with the goal of one day having it be used in pharmacotherapy for people suffering from alcohol addiction.

References

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4. Petruccelli, E., Feyder, M., Ledru, N., Jaques, Y., Anderson, E., & Kaun, K. R. (2018a, December 5). *Alcohol activates scabrous-notch to influence Associated Memories.* Neuron.https://pmc.ncbi.nlm.nih.gov/articles/PMC6323638/#:~:text=Intriguingly%2C%20there%20is%20accumulating%20evidence,associated%20with%20Alcohol%20Use%20Disorder.

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