Caffeine as a Seizure-Modulating Agent in *Drosophila melanogaster*: A Behavioral and Optogenetic Approach

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Introduction

- Epilepsy is a neurological disorder that affects nearly 50 million people worldwide and is characterized by recurrent seizures caused by abnormal brain activity.
- Most seizure treatments are sedatives that suppress brain activity; however, these may cause side effects and fail in drug-resistant cases.
- Caffeine, a widely consumed stimulant, indirectly enhances dopamine signaling, which may modulate seizure dynamics through neuromodulatory pathways.
- Stimulants are rarely explored in epilepsy research despite their known effects on arousal and neural excitability.
- Drosophila melanogaster provides a genetically tractable model to study seizures due to its conserved neural circuitry and seizure-like behaviors (e.g., spasms, immobility, convulsions).
- This study uses optogenetics to activate and inhibit cholinergic neurons via Channelrhodopsin (ChR2) and Halorhodopsin (Hdo) in order to mimic hyperexcitable seizure states *in Drosophila melanogaster.*
- Caffeine is introduced through food to evaluate its potential to rescue seizure behavior in treated flies compared to controls.
- Behavioral assays measure seizure severity and "latency to stand" after stimulation; EGFP imaging is used to visualize brain structure post-assay.
- This research explores whether caffeine can serve as a neuromodulatory seizure rescue agent and contribute to future non-sedative therapeutic strategies.

Methods

Fly Line Generation

Used *UAS-GAL4* binary system to target cholinergic neurons

Crosses Performed

1. ACh-GAL4 × UAS-ChR2 (Channelrhodopsin for neuronal activation)

2. ACh-GAL4 × UAS-Hdo (Halorhodopsin for neuronal inhibition)

3. ACh-GAL4 x UAS-EGFP (Enhanced Green Fluorescent Protein for fluorescent imaging of cholinergic circuits)

Larvae were raised on food containing all-trans retinal to activate opsin expression in ChR2 and Hdo lines.

Treatment Groups (n=8 flies per cross)

Flies from the cross, ACh x ChR, were divided into two groups of 4 flies each by caffeine exposure.

Flies from the cross, ACh x Hdo, were divided into two groups of 1 fly each by caffeine exposure for a pilot study.

Groups

ACh x ChR with/without caffeine (4 flies/group)

ACh x Hdo with/without caffeine (1 fly/group in pilot experiment)

Behavioral Assay Setup

Flies were anesthetized and transferred to Petri dishes

8 dishes for ACh x ChR with 1 fly in each petri dish

2 dishes for ACh x Hdo with 1 fly in each petri dish

Optogenetic Stimulation

ACh × ChR2 (Activation):

Red light (625–630 nm) applied for 5 minutes.

Measured:

Time of seizure onset

Time of recovery (latency to stand)

ACh × Hdo (Inhibition):

Yellow light (590–595 nm) applied for 5 minutes.

Observed whether optogenetic inhibition altered seizure behaviors.

Behavioral Scoring

Seizure phenotypes identified via video playback:

Loss of posture

Wing buzzing

Repetitive proboscis extension

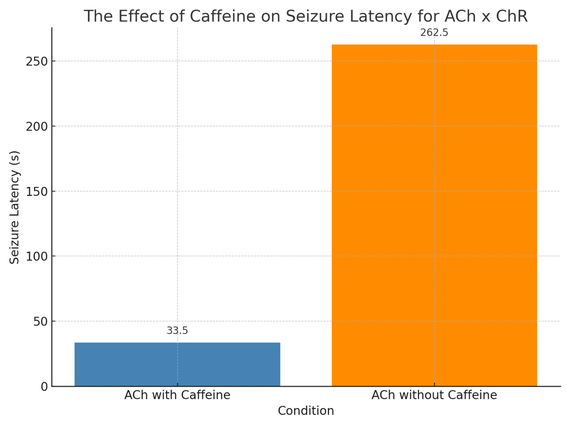
Recovery latency (seizure duration)

EGFP Brain Imaging

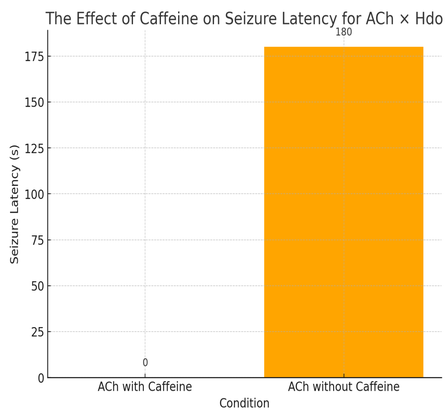
Used *ACh × UAS-EGFP* flies to visualize cholinergic neural architecture.

Fluorescence microscopy used post-assay to examine potential caffeine-related anatomical differences.

Results

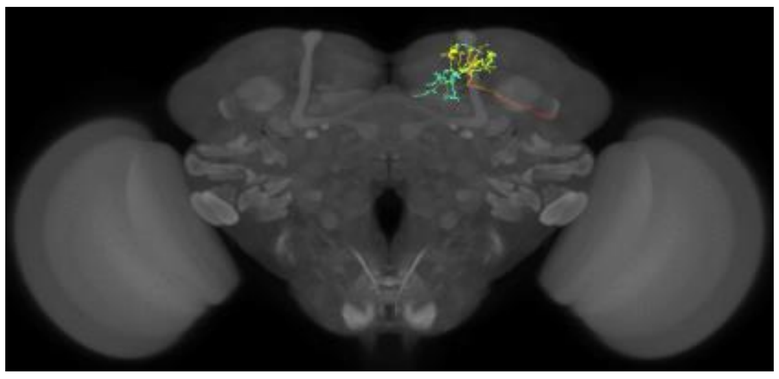


- An independent t-test analysis was conducted for the ACh x ChR cross. The sample size was n = 8 flies, and degrees of freedom (df) = 6.
  - The null hypothesis for the ACh x ChR cross is that there is no statistically significant difference between the ACh with caffeine and ACh without caffeine groups.
  - The alternative hypothesis for the ACh x ChR cross is that there is a statistically significant difference between the ACh with caffeine and ACh without caffeine groups.
  - The t-statistic calculated was 4.12. The critical value, taken with a degree of freedom of 6 and an alpha value of 0.005, is 3.707. Because the t-statistic is greater than the critical value, I reject the null hypothesis and come to the conclusion there is a statistically significant difference in ACh with caffeine and ACh without caffeine.

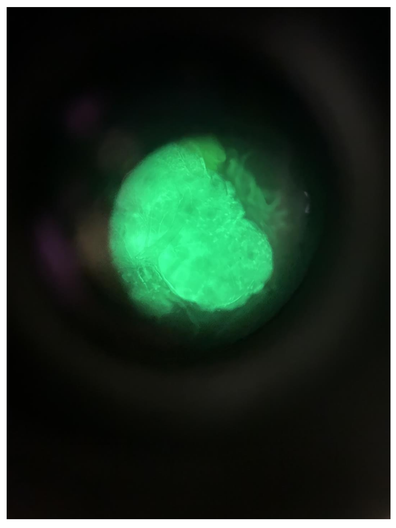


- A pilot study was done with the ACh x Hdo cross with 2 ACh flies: 1 exposed to caffeine and the other not exposed to caffeine.
- The ACh with caffeine group had a seizure latency of 0 seconds.
- The ACh without caffeine group had a seizure latency of 180 seconds.

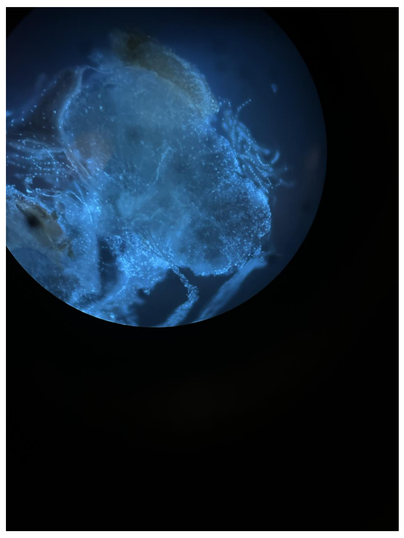
Visualization



Panel A. Representative ACh-ergic wiring diagram illustrating the circuit within the central complex (CC), comprised of unipolar neurons localized and targeted by the GAL4-driver line utilized in this study. The 3-D rendering was generated using the open-source Virtual Fly Brain Database.



Panel B. Enhanced Green Fluorescent Protein (EGFP) Stain of Acetylcholine (ACh) Neurons in Brain of *Drosophila Melanogaster*



Panel C. DAPI Counterstain of Acetylcholine (ACh) Neurons in Brain of *Drosophila Melanogaster*

Discussion/ Conclusions

- Both of the groups from the ACh x ChR & ACh x Hdo crosses, ACh with caffeine & ACh without caffeine, experienced recurrent seizures with frequent wing buzzing, repetitive leg movements, repetitive proboscis extension, and loss of posture.
- The time per seizure (seizure latency) for the ACh with caffeine group for ACh x ChR cross was much lower, at around 33.5 seconds, compared to the time per seizure for the ACh without caffeine group for ACh x ChR cross at around 262.5 seconds.
  - The ACh x Hdo cross in the pilot experiment followed this same trend as well, with the exception of much lower time values for the group. The ACh with caffeine group did not seize at all, while the time per seizure for the ACh without caffeine group was 180 seconds
  - These findings lead me to conclude that halorhodopsin (deactivation of acetylcholine neurons) is more effective in combination with caffeine in reducing seizure severity compared to channelrhodopsin.
- The severity of seizure-like activity (SLA) for the ACh with caffeine group was also much lower compared to SLA severity for the ACh without caffeine group of the ACh x ChR & ACh x Hdo crosses.
- This research study would be best complemented with a future study that includes a larger sample size as well as a longer testing duration.

References

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