

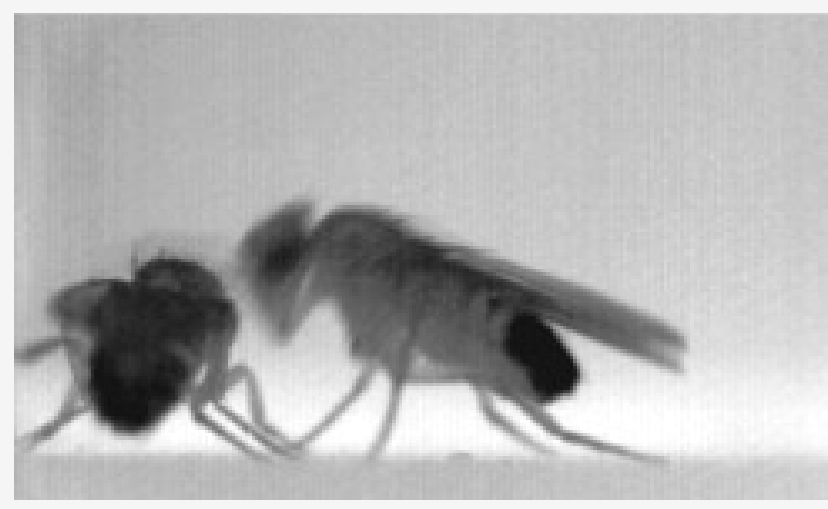
# Role of Octopamine Neurons in Heterospecific Aggressive Behaviors in *Drosophila Melanogaster*: Understanding Discriminatory Responses

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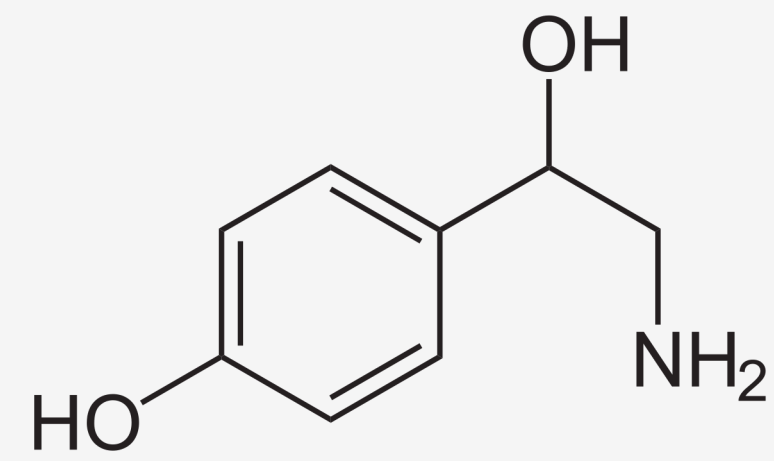
## Introduction

Discriminatory behaviors play a key role in informing ecological relationships and establishing social hierarchies. Aggression is an easily observable behavior in *Drosophila*. Current research confirms flies express increased aggressive responses toward heterospecific opponent males

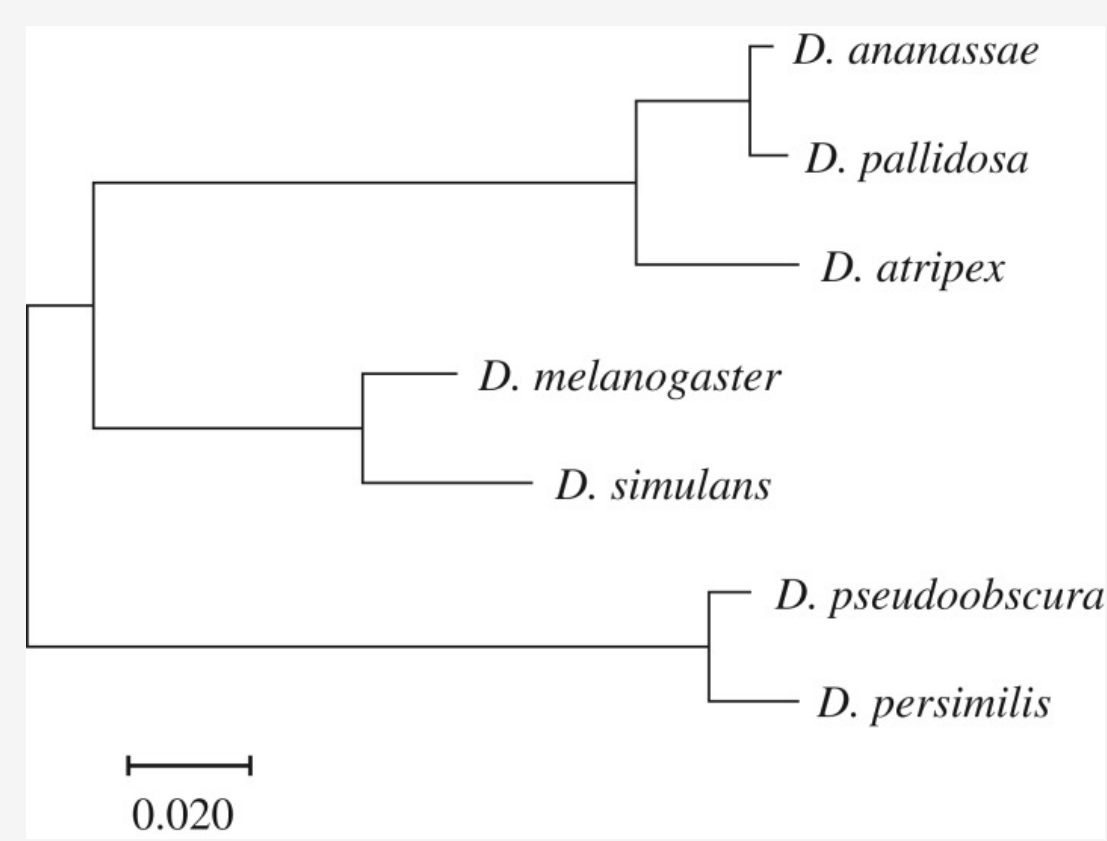


<sup>3</sup> Observable behaviors: approaching, lunging (see image to left), and boxing (raising of hind legs)

Octopamine is a neurohormone homolog of norepinephrine, a chemical that modulates the human "fight or flight" response



However, the molecular basis for discrimination among distantly related species remains under-researched. This study focuses on the effects of octopamine inhibition on heterospecific aggression, in comparison to the tested conspecific responses<sup>1</sup>



By inhibiting octopamine activity, the model predicts that there will be generally decreased aggressive behaviors, in addition to a proportional decrease with genetic distance



**Behavioral Assay-** Adopted from the Kravitz Laboratory at Harvard University<sup>4</sup>

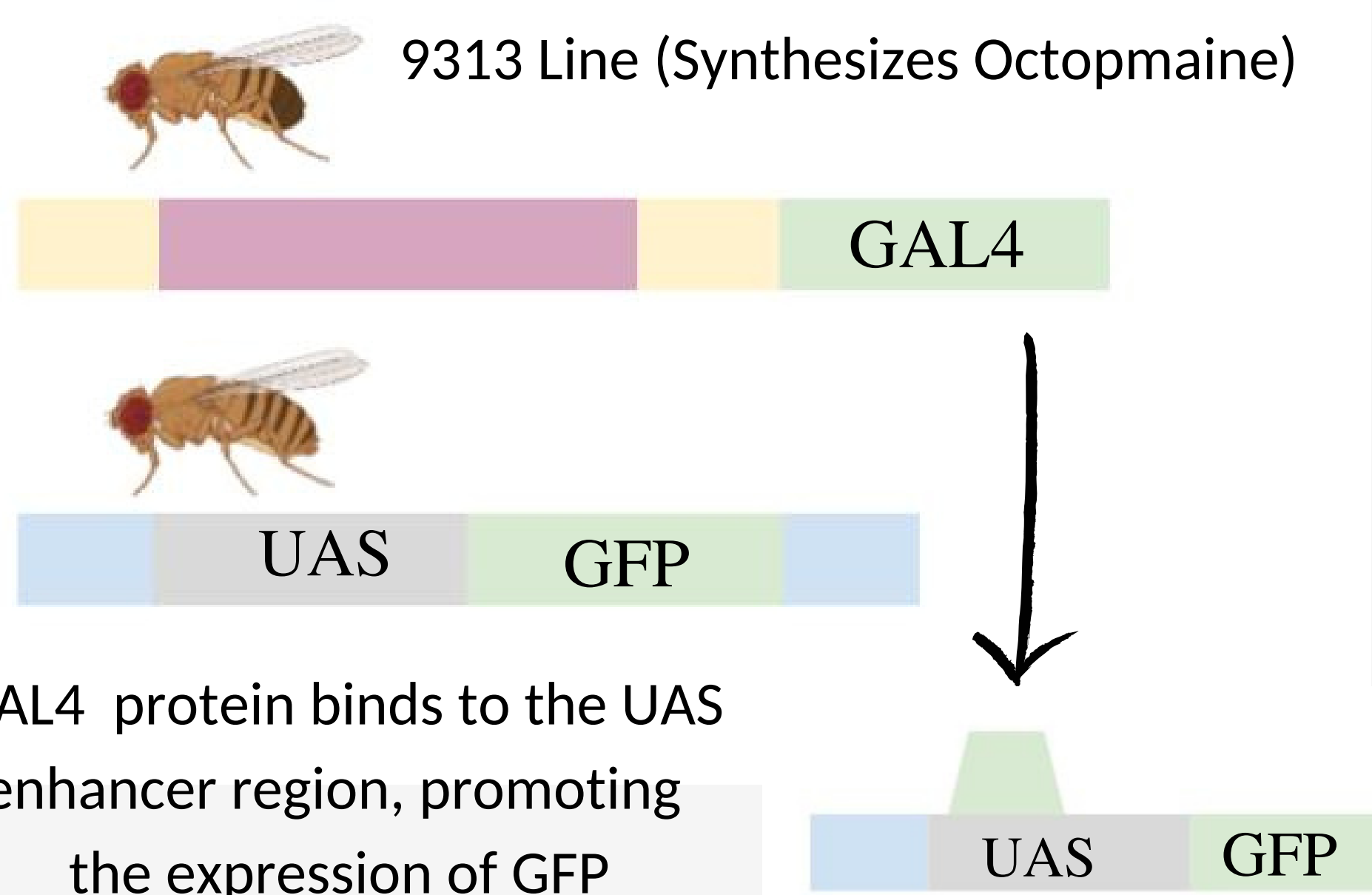
Procedure:

- Using a diamond glass cutter, cut three 1.5-inch rectangles. Hot glue the pieces in a triangle formation in the middle of a petrie dish.
- wipe down the base of the dish and chamber walls with 10% ethanol
- Prepare 50 ml of 2% agarose to fill the bottom of the chamber. wait 30 min to set
- Fill an eppendorf cap with melted fly food. Place a drop of apple juice. Rest a decapitated female fly in the center.
- Place the cap in the middle of the triangle chamber.
- Place two males in the chamber and record results over a 60 min period

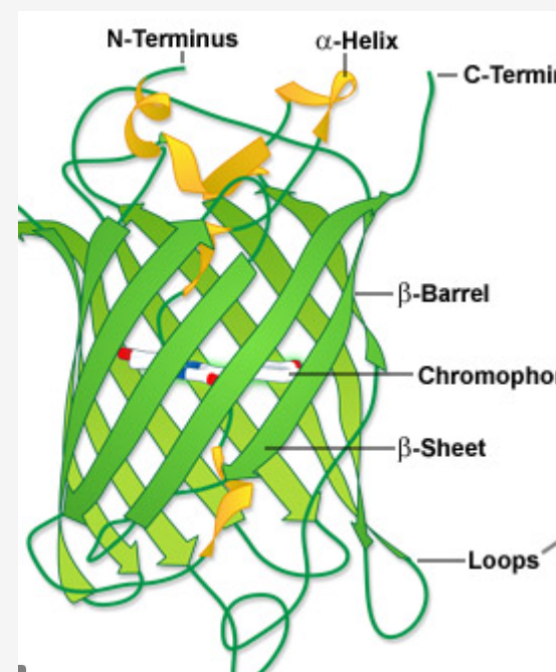
## Methods

GAL-4/UAS System: a versatile genetic tool used for enhancing/inhibiting specific genes or neuronal areas.

GAL4 is a specific activator that promotes transcription of the target gene by binding to the enhancer region, indicated by the UAS sequence.



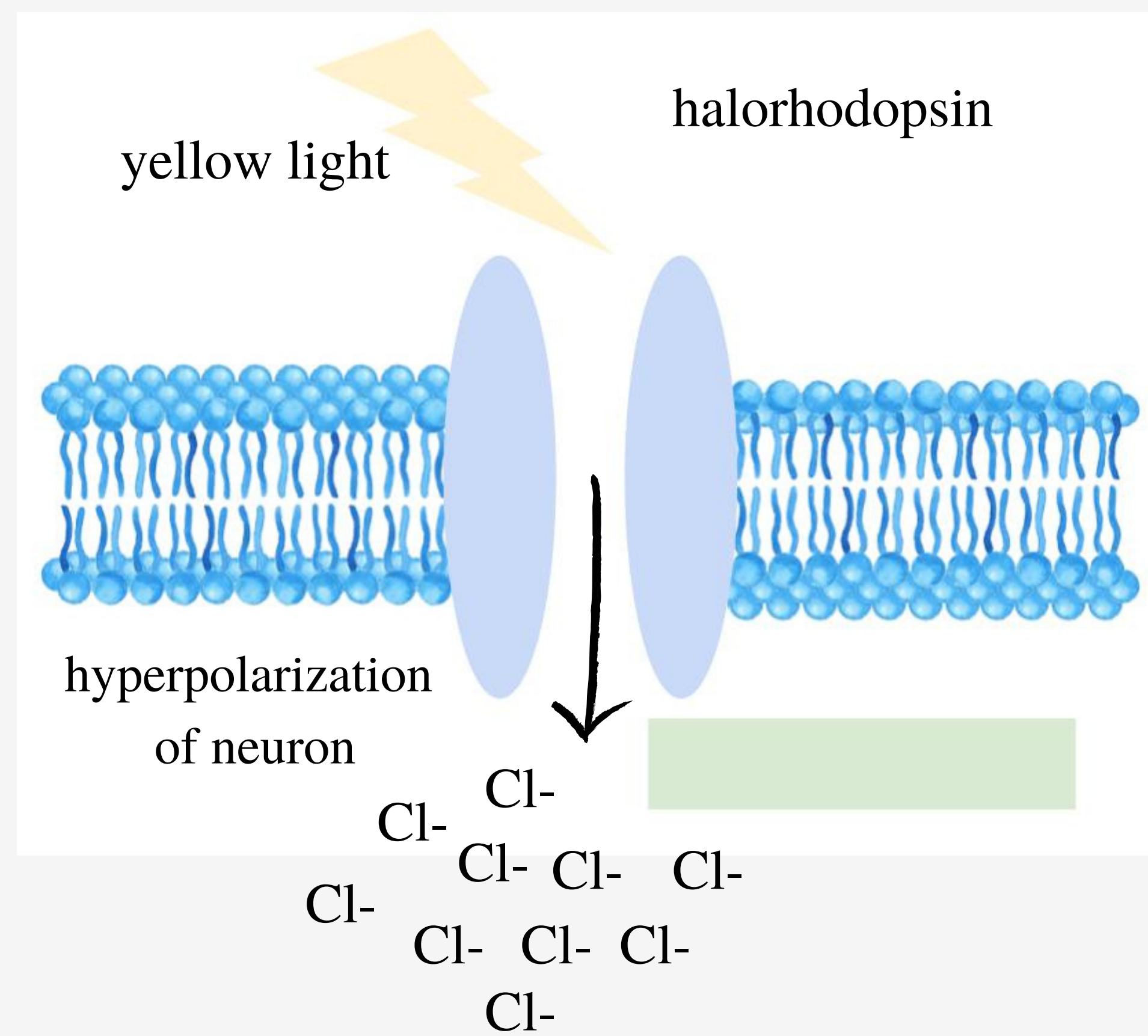
GAL4 protein binds to the UAS enhancer region, promoting the expression of GFP



GFP (green fluorescent protein) is a marker protein that, in this study, marks octopamine with fluorescence, allowing it to be visualized<sup>2</sup>

The following cross was prepared to inhibit octopamine expression:

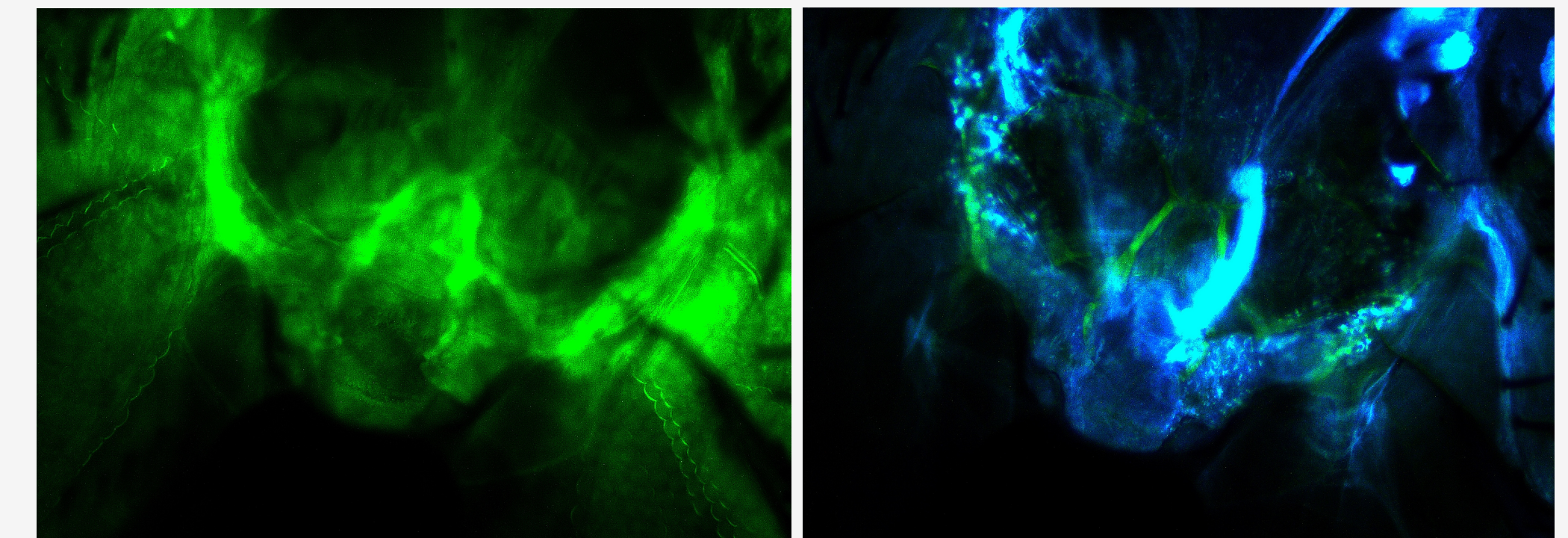
**9313 (GAL4-octopamine) X 8514 (UAS-halorhodopsin)**



Halorhodopsin is a light-gated ion pump that moves chloride ions into the cell, causing hyperpolarization and disruption of the membrane potential. Using an optogenetic approach, exposure to yellow light causes the neuron to cease firing.

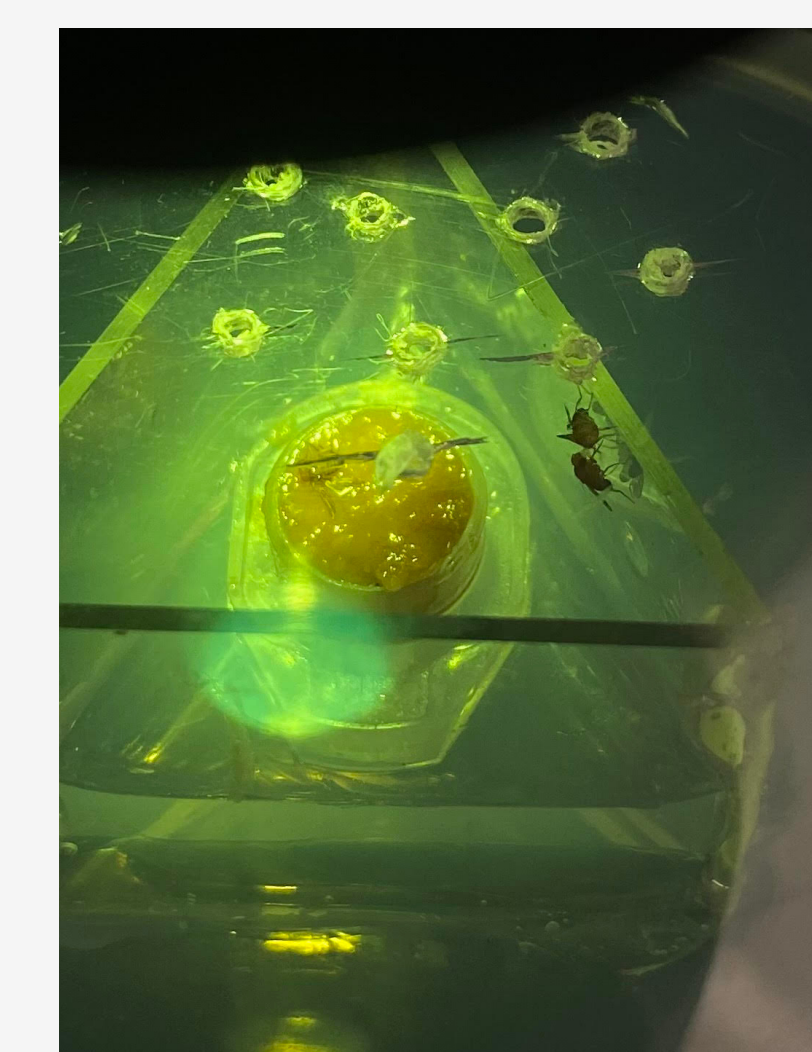
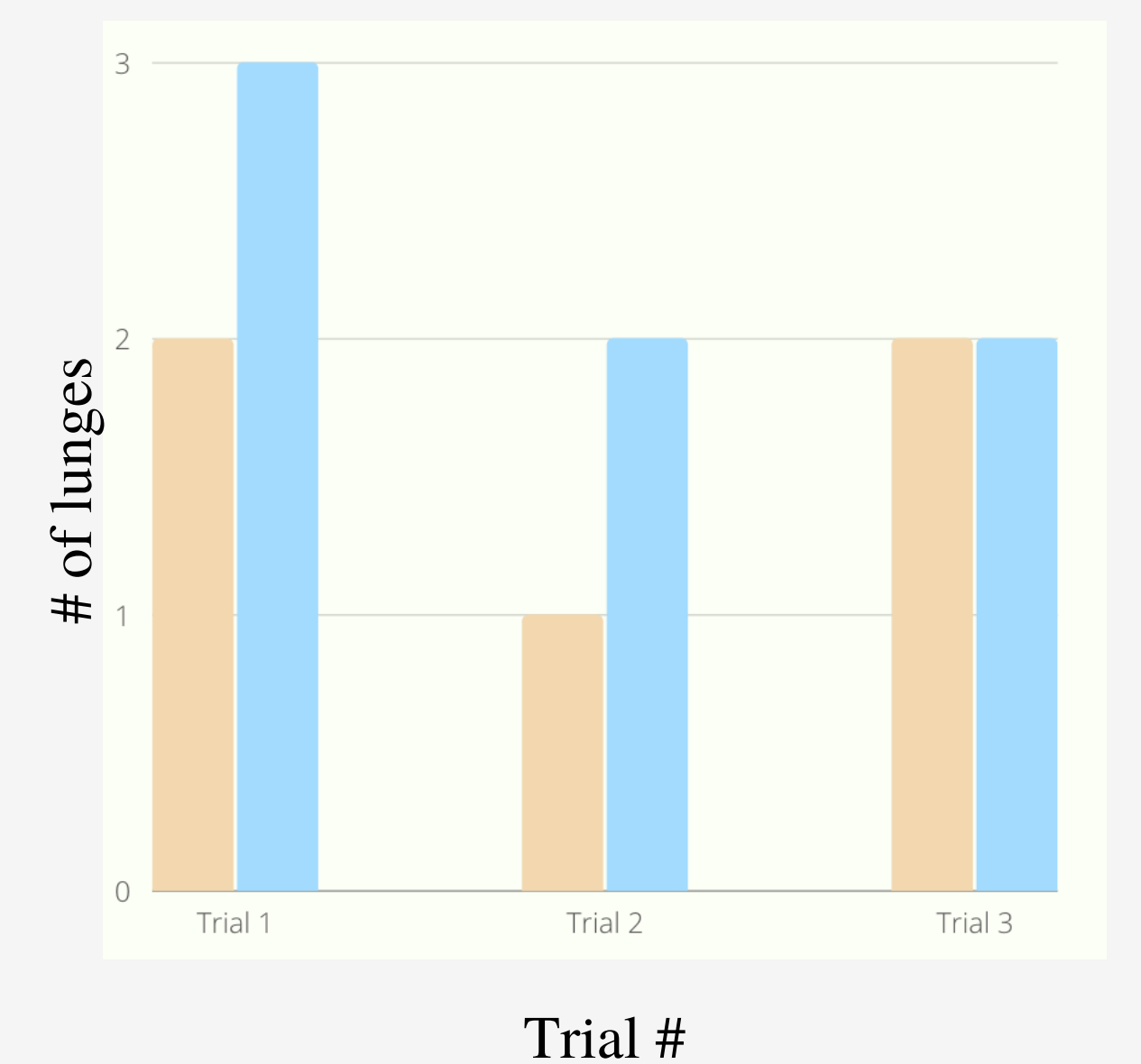
## Results

Imaging: GFP Fluorescence (left), DAPI (right)



Significance: GFPs mapping capabilities illuminate the octopamine neurons in the brain of the 9313 line

Minimal data was collected, but the trials performed indicate that the control had heightened aggression, compared to the experimental groups (inhibited octopamine)



**Behavioral Notes**  
acutely inhibited: lunges appeared more subtle, closely resembling an approach  
control: these lunges were far more aggressive and included physical contact of legs or heads

## Conclusions

Although limited results, this study provides a basis for future research in this relatively underresearched area of aggression. With a similar genome to humans, *Drosophila* research in discriminatory behaviors can hopefully offer insight into the innate mechanisms that perpetuate discrimination amongst humans.

## Acknowledgments

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