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Behavior Prioritization in *Drosophila*

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1) Introduction

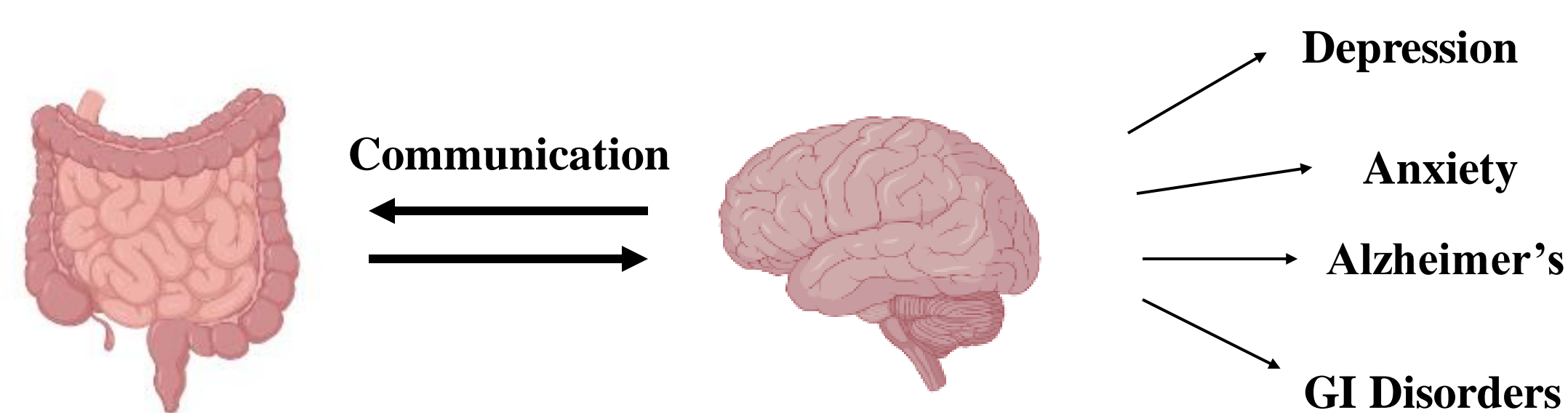
Animals must prioritize their needs to decide how to act in a way that meets their physiological needs and matches their environmental context. Our goal is to understand how internal states, such as hunger, are signaled from the intestines to the brain.

Previous work in the Certel Lab, using the model organism *Drosophila melanogaster*, has determined that the octopamine (OA) adrenergic receptor $OA\alpha 2R$ is expressed in the cells in the intestinal system.

We hypothesize that the binding of OA to $OA\alpha 2R$ inhibits the release of neuropeptides, such as hunger-signaling neuropeptide F, in EE cells. Here, we examine the gut-brain OA circuitry in *Drosophila* and predict the following:

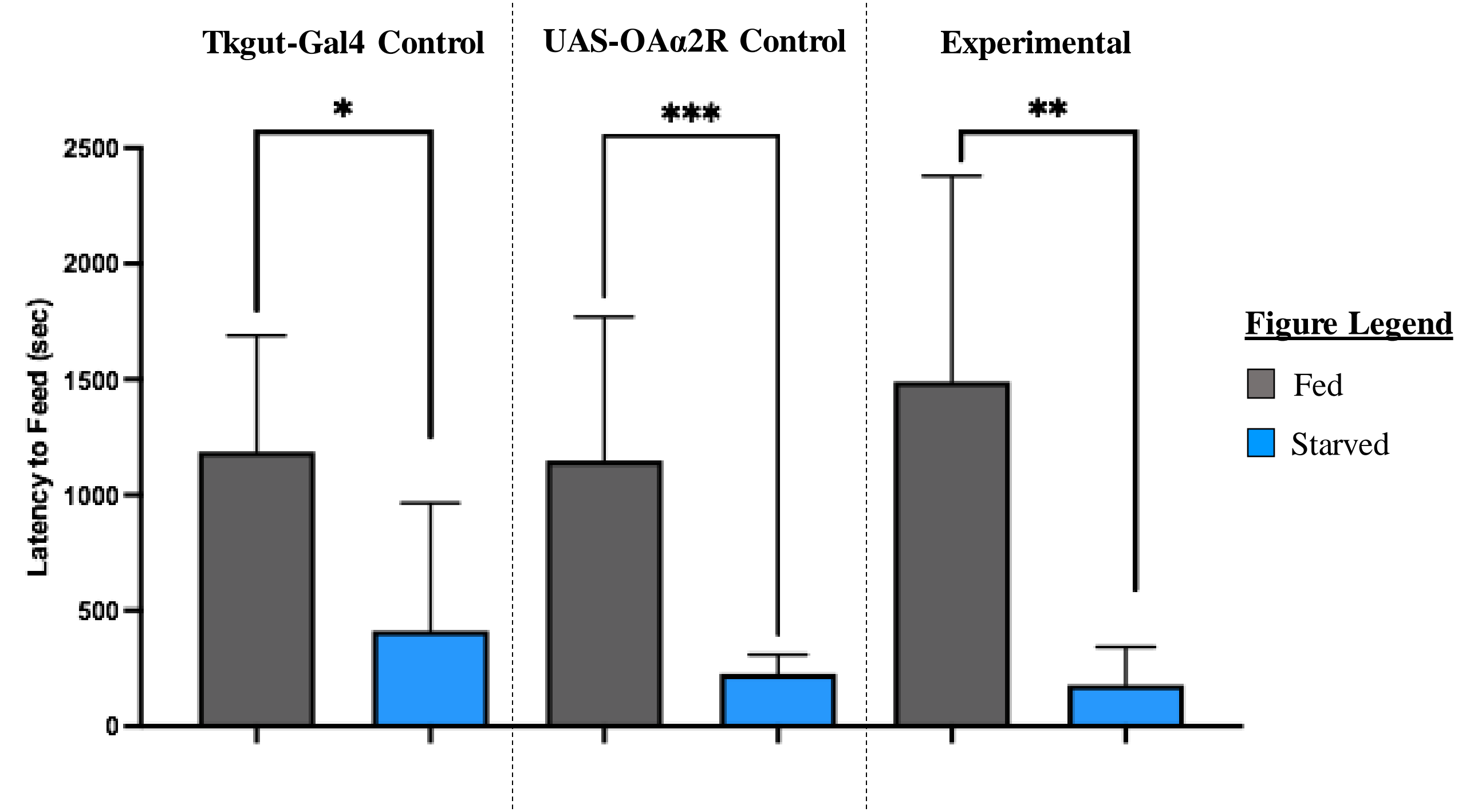
- **$OA\alpha 2R$ -mediated inhibition sequentially inhibits feeding behavior and promotes aggression**
- A reduction in $OA\alpha 2R$ expression in EE cells will result in increased feeding and reduced aggression.

A greater understanding of decision prioritization within the **gut-brain axis** will help map the internal codes of social behavior as well as reveal therapeutic targets [3,4].



4) Starved Flies Eat Earlier

Does an **$OA\alpha 2R$ Knockdown** impact the feeding behavior of starved and fed male flies?

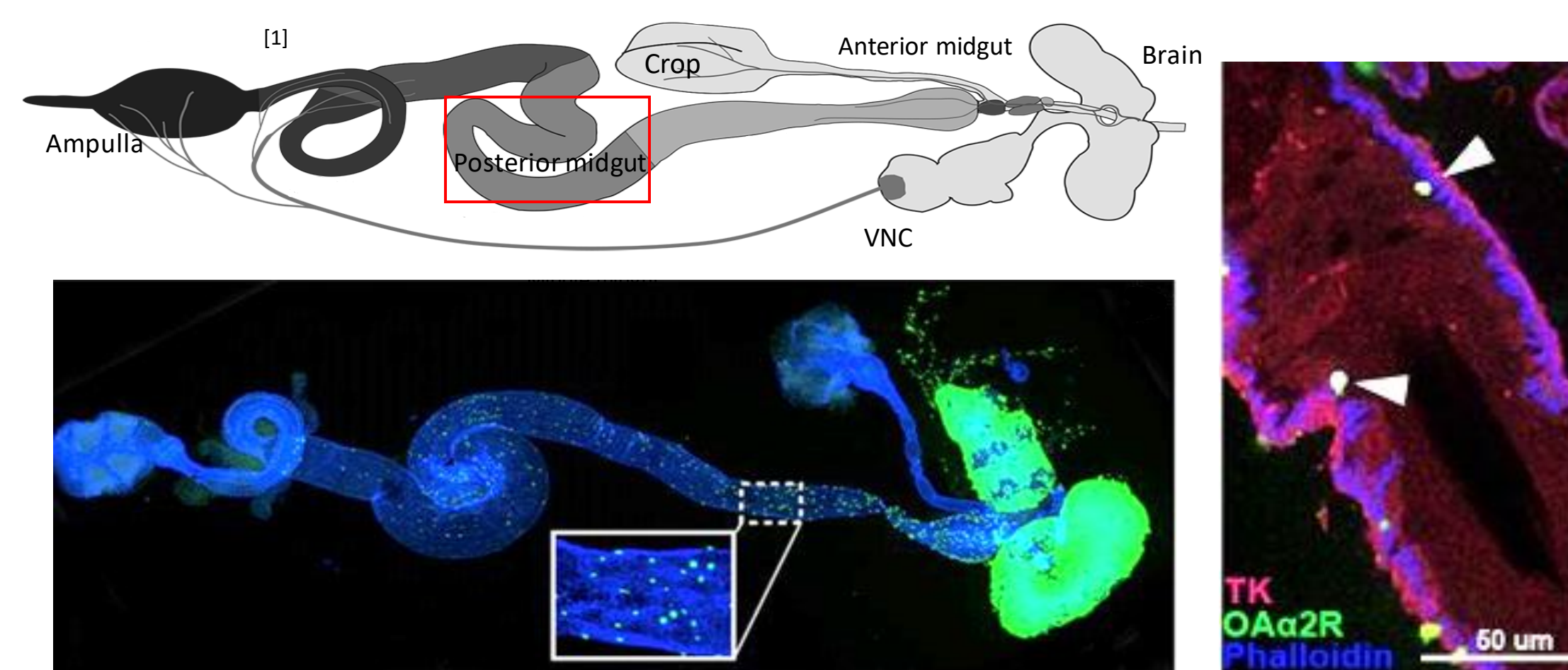


Results indicate that starved flies eat faster than fed flies, as expected. Knocking down $OA\alpha 2R$ does not change latency to feed. At this point, however, we are unable to determine if $OA\alpha 2R$ males feed more.

Mann-Whitney tests were conducted to determine statistical significance. Error bars denote S.E.M. * Denotes $p \leq 0.05$, ** Denotes $p \leq 0.01$, and *** Denotes $p \leq 0.001$.

2) $OA\alpha 2R$ Expression in the Gut

$OA\alpha 2R$ is required to mediate cell communication in many locations including the brain, ventral nerve cord, and numerous cells within the intestines.

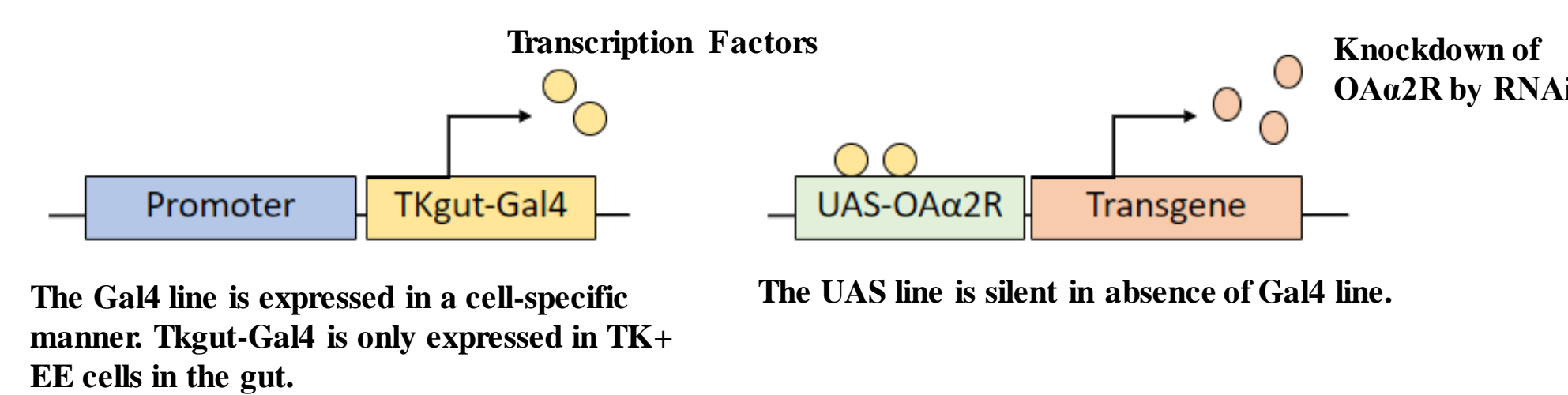


$OA\alpha 2R$ Expression in *Drosophila*. Image generated by Ashley Bielawski.

$OA\alpha 2R$ is expressed in specific neurons and cells to regulate the gut-brain signaling of hunger.

- $OA\alpha 2R$:**
- α_2 -adrenergic octopamine receptor (GPCR)
 - Expressed in enteroendocrine (EE) cells across the midgut

The Gal4-UAS System

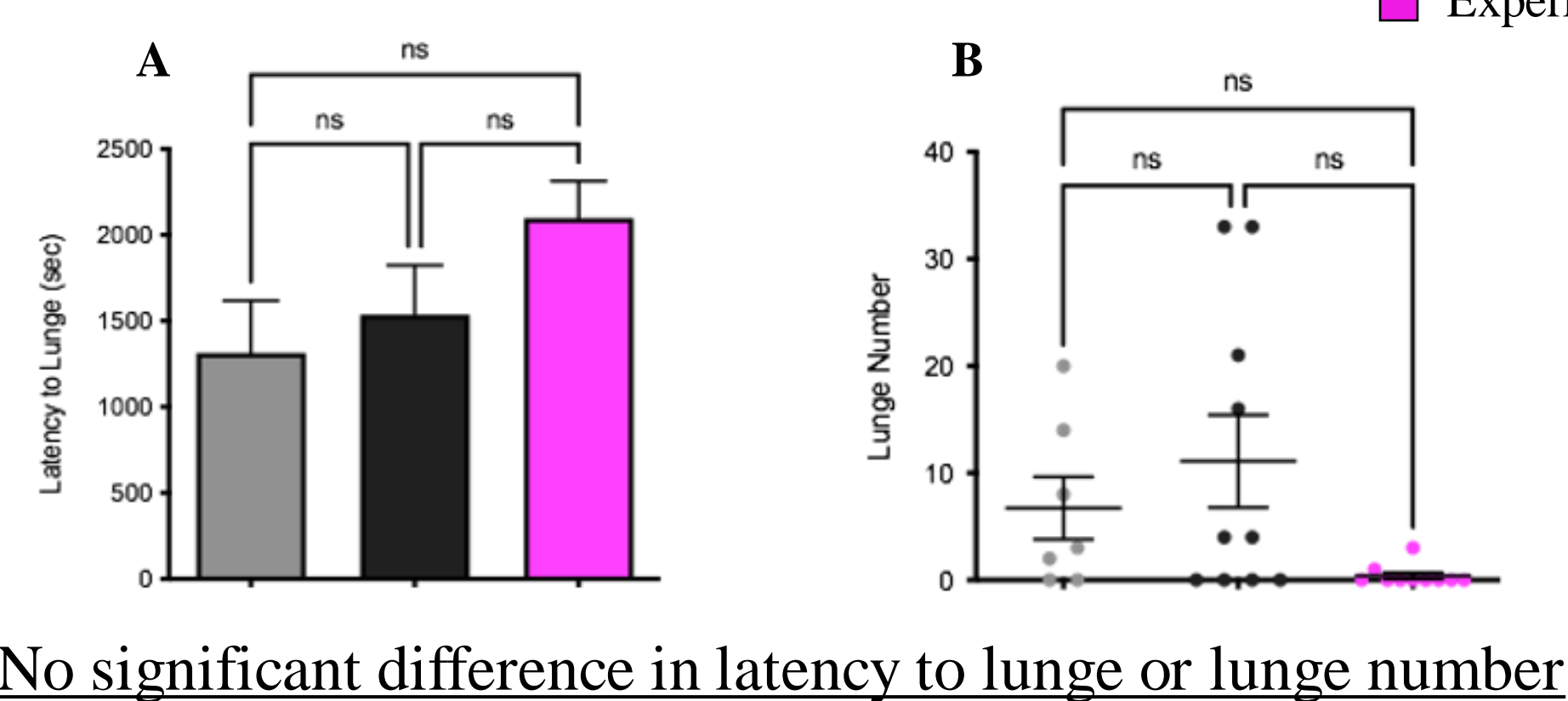


The Gal4 line is expressed in a cell-specific manner. Tkgut-Gal4 is only expressed in TK+ EE cells in the gut.

The UAS line is silent in absence of Gal4 line.

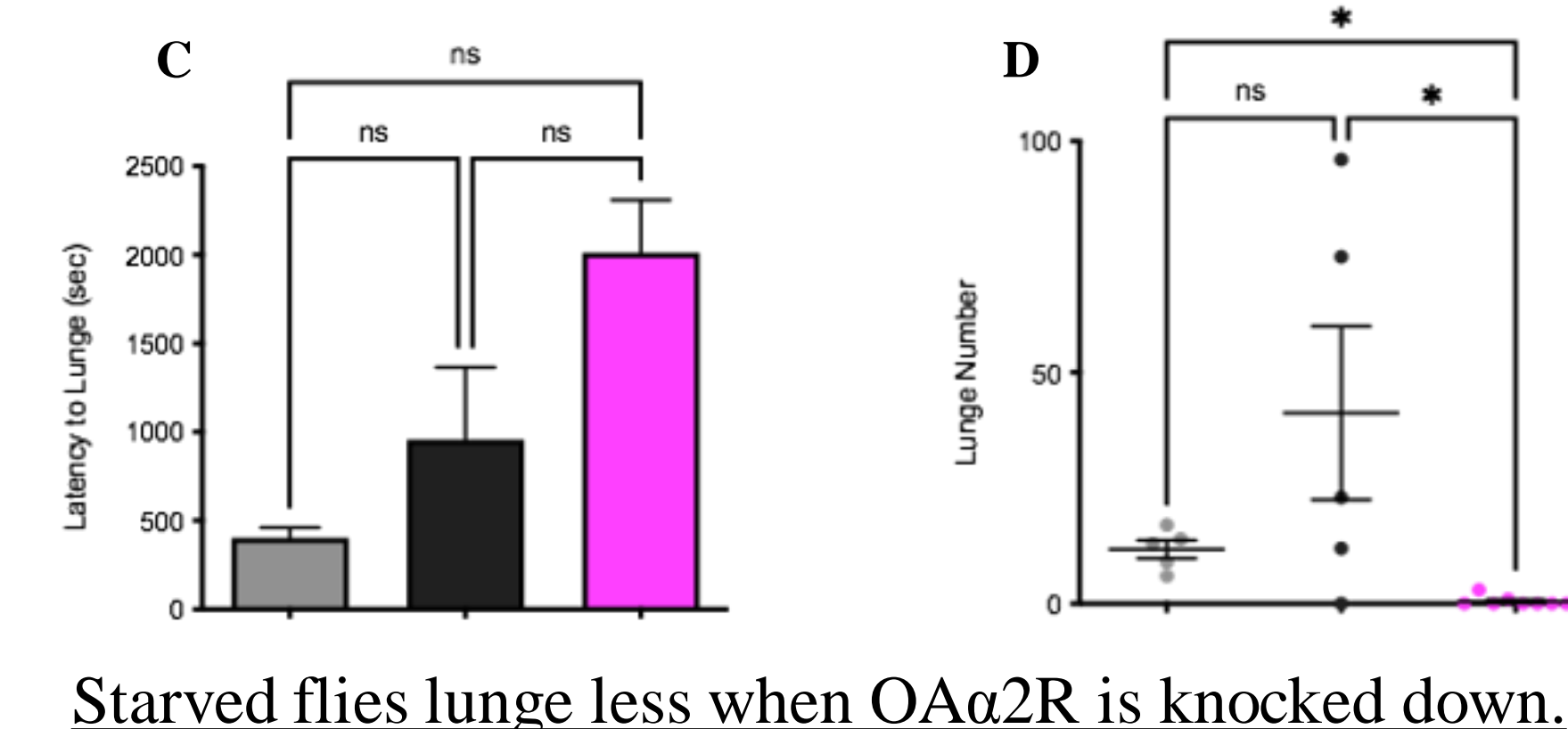
5) Starved $OA\alpha 2R$ -Knockdown Flies Fight Less

Does an **$OA\alpha 2R$ Knockdown** influence aggressive behavior in **fed** male flies?



No significant difference in latency to lunge or lunge number

Does an **$OA\alpha 2R$ Knockdown** influence aggressive behavior in **starved** male flies?

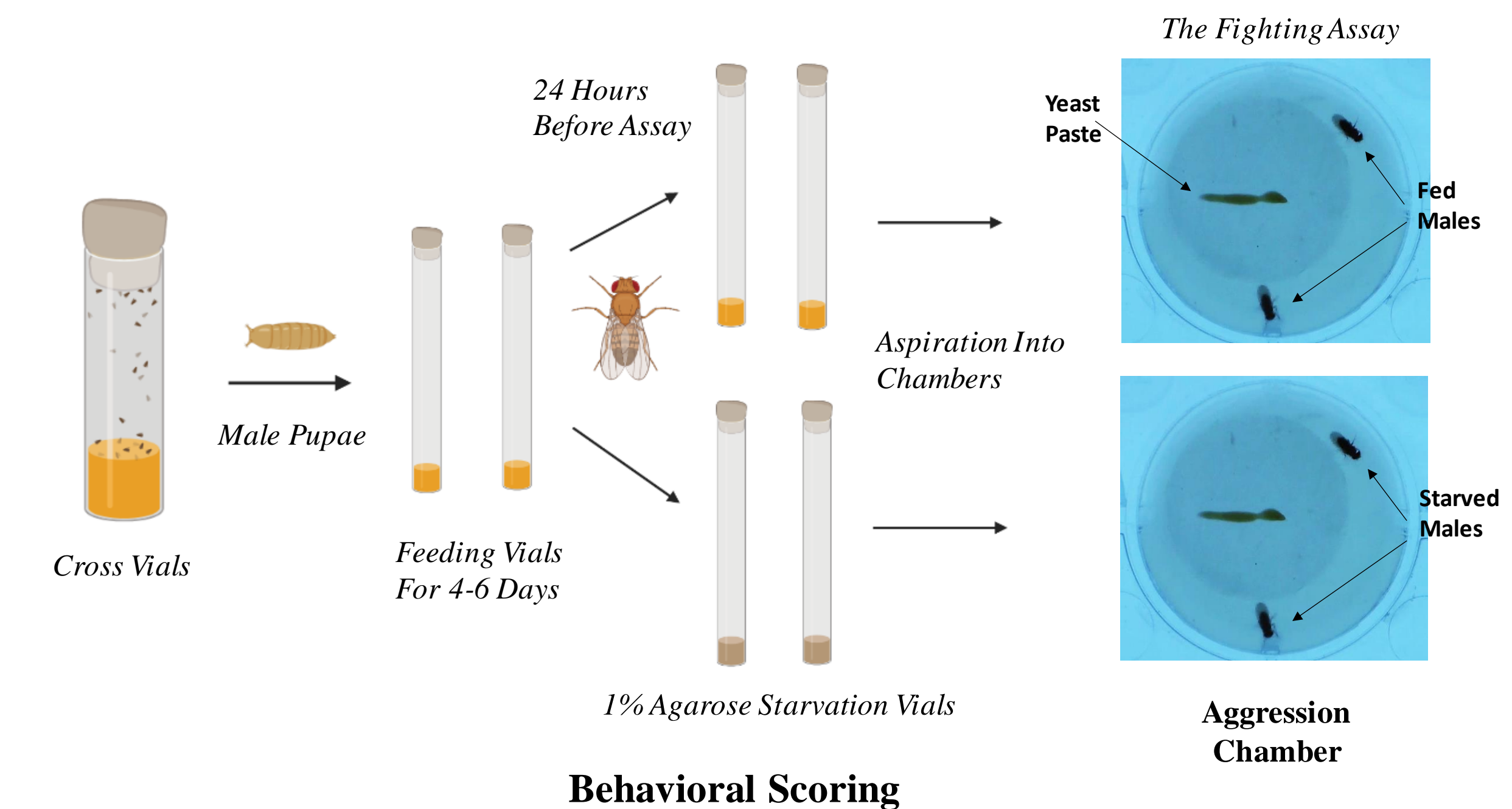


Starved flies lunge less when $OA\alpha 2R$ is knocked down.

Kruskal-Wallis and Dunn's multiple comparison tests were conducted in (A-D) to determine statistical significance. Error bars denote S.E.M. * Denotes $p \leq 0.05$.

3) Methods and Assay

Is $OA\alpha 2R$ required to signal internal states and regulate aggression?



- Behavioral Scoring**
- Latencies (time in seconds between aspiration and initial behavior) and total lunge number were recorded
 - Number of lunges were scored for 10 minutes after an initial lunge, and observation was capped at 40 minutes if no lunge or feed occurred
 - Recording latency provides a baseline with which to compare initial fighting in fed vs. starved flies
 - Lunging is a key component of social hierarchy establishment and is a common metric of *Drosophila* aggression

Behavioral Patterns:

- latency to feed
- latency to lunge
- lunge number

Still Images of Lunge



A key aggressive pattern in males

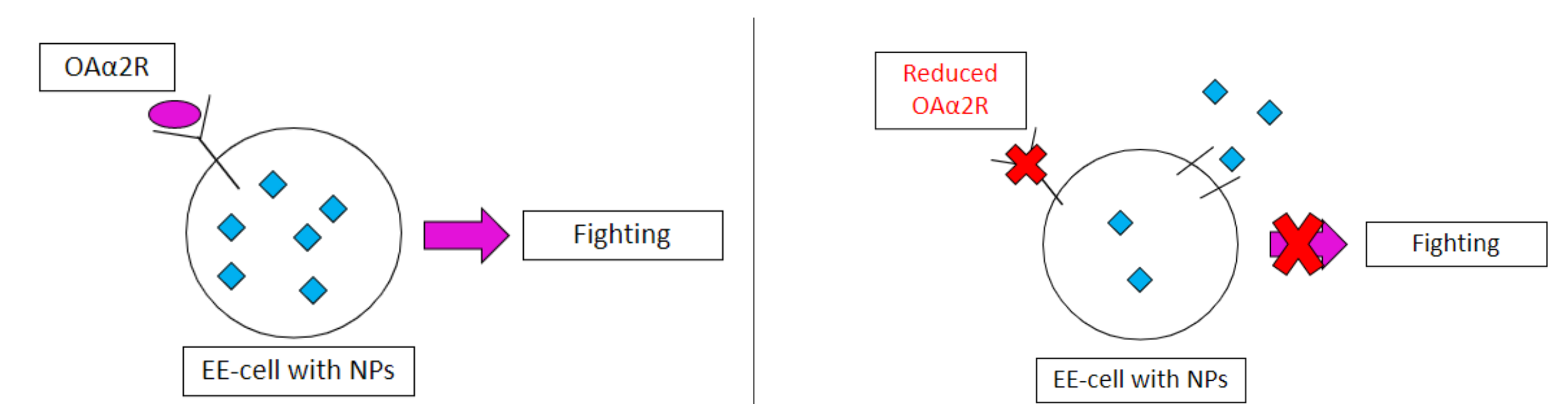
6) Conclusions, Future Directions, and Acknowledgements

Conclusions:

- Lunge number was decreased for $OA\alpha 2R$ knockdown in starved flies, supporting previous work
- $OA\alpha 2R$ knockdown data suggests that $OA\alpha 2R$ does not inhibit feeding behavior

Future Directions:

- Confirm that $OA\alpha 2R$ knockdown flies fight less
- Revised Hypothesis: **$OA\alpha 2R$ inhibits the release of neuropeptides, thus promoting the transition from feeding to fighting**



References

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- [4] J. Foster, and K.A. Neufeld, 2013. Gut-Brain Axis: How the Microbiome Influences Anxiety and Depression in *Trends in Neurosciences* vol 36, 305-312.

Acknowledgements:

Funding was provided to SJC from the US Army Research Office W911NF-23-1-0005, a University of Montana GRIP award, and support from the Neuroscience SURF program to AB.