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Behavioral Impacts of the gut microbiome on *Drosophila Melanogaster*

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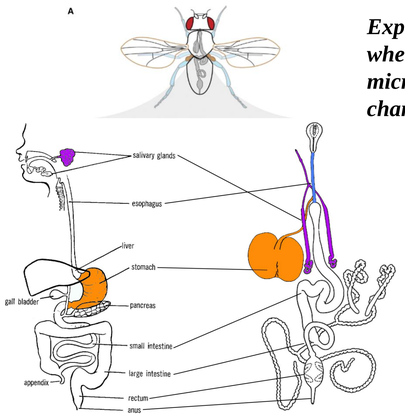
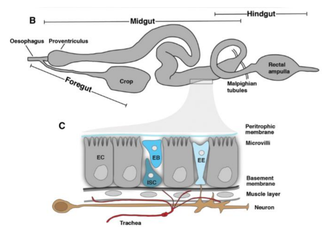
1) Introduction

The gut of an organism is home to a complex community of bacteria, fungi and viruses that collectively make up the gut microbiome. The prevailing hypothesis is that changes in the microbiome are communicated to the brain and subsequently impact behavior. Work in the Certel lab focuses on the role of octopamine in the model system *Drosophila melanogaster*. Octopamine (OA) is a neurotransmitter and neuromodulator expressed in the nervous system of invertebrates. We recently determined that a subset of OA neurons also innervate the gut and the crop (a food storage sack similar to the stomach). We have started experiments to examine the brain-gut octopamine circuitry and determine how the activity of this circuit is altered by the bacteria that compromise the insect microbiome.



Experiments in this project tested whether or not eliminating the gut microbiome will lead to a quantifiable change in aggressive behaviors.



I. Miguel-Aliaga, H. Jasper, and B. Lemaitre, 2018 [1].

Figure 1. Adult Intestine (A) Digestive tract orientation in the fly. **(B)** Anatomy of the digestive tract. **(C)** General cellular composition of the digestive tract.

2) Protocol for generating germ-free flies

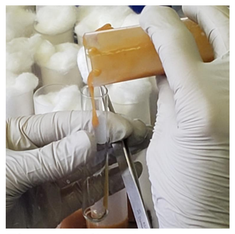


Figure 2. Aliquoting axenic media into sterile isolation vials.

Preparation of sterile (axenic) media

1. Autoclave food, tools, vials, isolation vials, cotton and kim wipes.
2. Propionic acid and tegosept (fungicides) and tetracylcine (antimicrobial/antibiotic) were added.
3. Food was poured into vials and isolation vials and plugged with sterile cotton.

Embryo collection, dechorionation & isolation [2]

1. Flies were anesthetized using CO_2 and transferred into an embryo collection cage.
2. Dechorionation was carried out in sterile conditions with autoclaved tools. Embryos were rinsed with bleach and mili-Q water, then transferred into vials of axenic media.
3. Male pupae were identified in the vial, then transferred to isolation vials of axenic media.

Embryos were collected, bleached, rinsed and placed into axenic media to develop into adult flies.

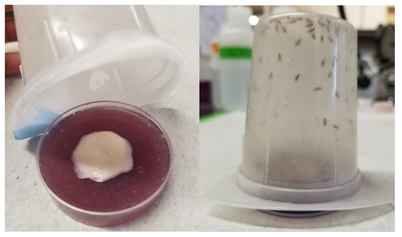


Figure 3. Embryo collection apparatus

3) Aggression Assay Protocol

Aggression Protocol [1]:

1. Group-housed males were transferred to 15 X 85 mm glass vials after eclosure.
2. Two size and age-matched (3-6 days) males of same genotype were allowed to walk into the sliding chamber.
3. Aggression was scored starting 30 mins after the first lunge.
4. The motor patterns measured were:

   a. latency to lunge
   b. lunge number
   c. wing threats
   d. male-male courtship

Aggression Chamber

Figure 4. Aggression chamber where behavioral assays were performed. A dot of yeast paste was added to the center of each food cup, so the flies had territory to defend.

Behavioral Patterns:

A

Still images of a lunge, an aggressive behavior pattern.

B

Wing Extension

Male-male Courtship

Figure 5. Images depicting **(A)** aggressive behaviors and **(B)** courting behaviors.

4) Increased Aggression in Germ-free Flies

The behavior of the following males were quantified

- *Experimental: Canton S wildtype – Germ Free*
- *Control: Canton S wildtype*

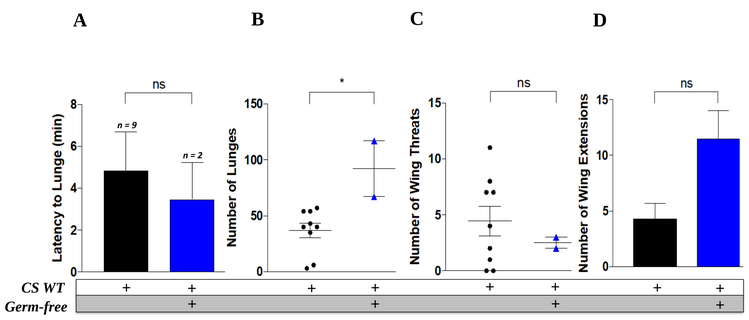


Figure 6. (A-C) Aggressive behaviors of experimental flies compared to controls. **(D)** Male-male courtship. Mann-Whitney tests were conducted in **(A-D)** to determine statistical significance. Error bars denote S.E.M. * equals p≤0.05, ** equals p≤0.01, *** equals p≤0.001

We are at the beginning stages of collecting enough data for interpretation. However, at this point in our experiments, there was a significant increase observed in the number of lunges between germfree and control males.

5) Future Directions and Parallel Project

Future Directions

1) Verify the flies are germfree
- Brain Heart Infusion agar
- PCR and 16S rRNA Sequencing

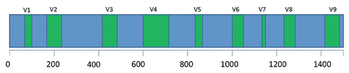


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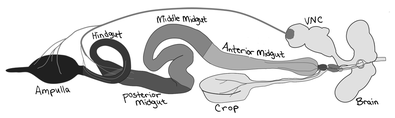
Figure 7. Hypervariable regions within the 16S rRNA gene.

2) Increase *n* of behavior assays
- Control: CS WT
- Experimental: CS WT – germfree

Parallel Project:

OA neurons in the brain send axons to innervate the gut (red) and the crop. We know that OA neurons are required to promote aggression. OA neurons could be a key group of neurons that either signals to or receives signals from the gut or crop. Such information would be key for an organism to integrate information regarding its internal state and external cues to decide whether to start fighting.



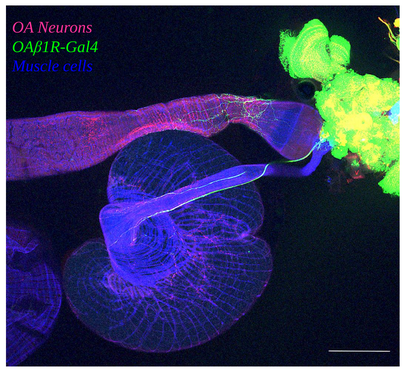


Figure 8. Confocal image of brain, midgut and crop.

6) Conclusions and Acknowledgements

Conclusions:

- My current preliminary results suggests there may be differences in the key aggression pattern of lunge number between wild type and germfree wildtype flies.
- Further assays will be recorded and scored to determine if additional parameters including latency to lunge, wing threats or unilateral wing extensions are different.

References

[1] I. Miguel-Aliaga, H. Jasper, and B. Lemaitre, 2018. Anatomy and Physiology of the Digestive Tract of Drosophila melanogaster in *Genetics* vol 210, 357 - 396

[2] Certel SJ., 2012. Scoring and Analyzing Aggression in Drosophila in *Cold Spring Harbor Protoc.*

[3] Sabat et al., 2015. A Protocol to Generate Germ Free Drosophila for Microbial Interaction in *Adv Tech Biol Med*

[4]Kietz, C., Pollari, V., & Meinander, A. (2018). Generating germ-free Drosophilato study gut-microbe interactions: Protocol to rear Drosophila under axenic conditions in *Current Protocols in Toxicology, e52.*

Acknowledgements:

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