Identifying octopamine receptor expressing neurons in the adult Drosophila male

Terra MH Hanks

University of Montana - Missoula, terra.hanks@umontana.edu
Identifying octopamine receptor expressing neurons in the adult *Drosophila* male

Terra Hanks\(^1\), Jonathan Andrews\(^2\), Sarah Certel\(^{1,2}\)

\(^1\)Division of Biological Sciences, University of Montana, Missoula, MT
\(^2\)Center for Structural and Functional Neuroscience, University of Montana, Missoula, MT

1-What is Octopamine?

Octopamine (OA) is a biogenic amine equivalent to the human neuromodulator norepinephrine.

In insects OA acts functionally as a neuromodulator. It is important in many physiological processes.

Specifically in *Drosophila*, they are important for the generation of certain behaviors including:
- Aggression
- Arousal
- Learning
- Memory

*Drosophila* have approximately 100 OA-expressing neurons. OA signals subsequent neurons by binding to specific G-protein coupled receptors.

My focus is primarily on Octopamine β1 Adrenergic-like Receptors (OAβ1R).

2-Research Goals:

It is my aim to quantify the number of OAβ1R-expressing neurons and to map the morphology of said neurons within the male *Drosophila* nervous system.

3-Genetic tools to illustrate OA β1R-expressing neurons

**Gal4/UAS System** (Brand and Perrimon, 1983)

**Gal4 expressing line**

**UAS-target gene line**

\[\text{Gal4} \leftrightarrow \text{UAS} \]

Endogenous promoter/enhancer

\(\beta 1R\)

**Gal4**

\(\text{UAS}[\text{Gal4 binding sites}]\)

**GFP**

4-Dissection Methods

- Crossed genetically suitable strains
- Dissected progeny in PBS over ice
- Fixed brains and central nerve cord in 4% PFA
- Washed in a series of PBS, PBT, & blocking solution
- Incubated brains and central nerve cord in primary antibodies overnight
- Washed again
- Incubated in secondary antibodies for 2 hours
- Washed again
- Mounted on 25 x 77 mm slide
- Examined under confocal microscope
- Prepared images

5-Expression patterns of OA β1R-expressing neurons in the legs, ventral nerve cord, & brain

- **Legs**
  - The green fluorescent protein (GFP) in the forelimbs indicates the OAβ1R-expressing neurons. The pattern is congruent in multiple legs allowing me to count the number of such neurons.

- **Ventral Nerve Cord**
  - Fiber tracts and cell bodies are visible throughout the ventral nerve cord. By isolating the expression of a red fluorescent protein (RFP) to the nucleus of the OAβ1R-expressing neurons, the specific cell count can be observed. The majority of these neurons appear to be concentrated within the last segment.

- **Brain & Subesophageal Ganglion (SOG)**
  - Using the same genetic tools as in the ventral nerve cord and legs, cell count and fiber tracts can be established throughout the brain. Synaptic terminals can be identified by labeling the protein synaptotagmin. The SOG, a region that receives pheromone input, appears to have bilateral symmetry.

6-My research identified the following:

- **Legs**
  - Crossed genetically suitable strains
  - Dissected progeny in PBS over ice
  - Fixed brains and central nerve cord in 4% PFA
  - Washed in a series of PBS, PBT, & blocking solution
  - Incubated brains and central nerve cord in primary antibodies overnight
  - Washed again
  - Incubated in secondary antibodies for 2 hours
  - Washed again
  - Mounted on 25 x 77 mm slide
  - Examined under confocal microscope
  - Prepared images

7-Conclusions

- I observed between eighteen and twenty-one OAβ1R-expressing neurons in the forelimbs.
- I located synaptic terminals and thirty-four OAβ1R-Gal4 GFP-expressing neurons in the ventral nerve cord.
- I identified morphology and location of nine GFP-expressing neurons through the OAβ1R-Gal4 line in the SOG.

This research has provided a foundation for further analysis of octopamine neurons in *Drosophila melanogaster*.

Acknowledgements:

Funded by Montana Space Grant Consortium & Ridge Library Research Award. I would also like to thank Sarah Certel and Jonathan Andrews.