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Optogenetic manipulation of *Drosophila* larval motor circuits

Nicholas M. Devetter
College of Saint Benedict/Saint John's University

Optogenetic Manipulation of Drosophila Larva Motor Circuits

Nicholas DeVetter and Dr. Jennifer Schaefer

Department of Biology

College of St. Benedict | St. John's University

Rationale

Purpose:

Identify specific interneuron populations involved in muscle contraction in *Drosophila* larva through ontogenetic manipulation of motor neural circuits.

Previous Findings:

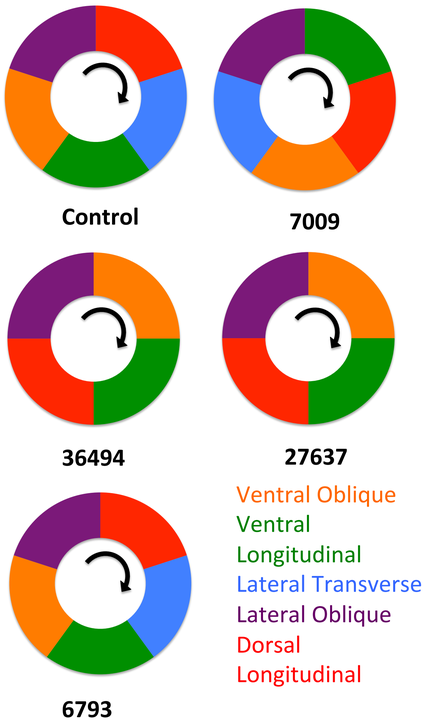


Figure 1: Muscle contraction order during forward crawling (Decker and Schaefer, 2014)

References:

Hoang B, Chiba A. 2001 Single cell analysis of Drosophila larval neuromuscular synapses. *Devel. Biol.* 229(1): 55-70.

Lee T, Luo L. 2001. Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. *Neurosci.* 24:251-254.

Jones R. 2013 What do singe cell green algae have to do with the state of the art of neuroscience? Retrieved from http://knowingneurons.com/2013/02/08/what-do-single-cell-green-algae-have-to-do-with-the-state-of-the-art-of-neuroscience/

Acknowledgements:

Thank you to MC Decker for the use of her data in Figure 1.

Methods

1. Use GAL4-UAS system to generate larvae with channelrhodopsin2 embedded in cell membranes of interneuron populations of interest.

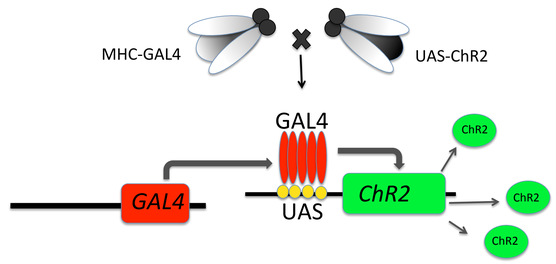
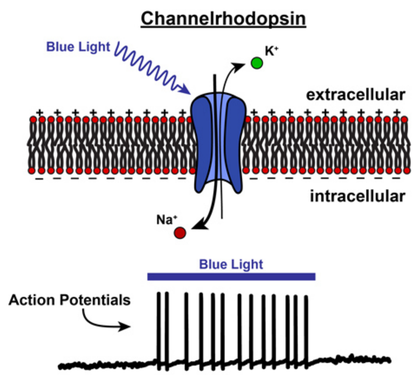


Figure 2: GAL4-UAS Tissue Specific Gene Expression

2. Collect extracellular recordings of muscles during contraction in the dissected Drosophila larva. ChR2-stimulated action potentials lead to depolarizing potentials and contraction in muscle cells if the interneuron population of interest is important for Drosophila crawling neural circuits.

Figure 3. Channelrhodopsin 2 is activated by blue light that opens the cation channel, allowing cations to enter the cell. Depolarization of the membrane leads to an increase in the frequency of action potentials generated by the neuron.

(Jones, 2013)



3. Compare recordings from negative control, positive control, and experimental populations to identify which interneuron populations are sufficient to generate muscle excitation. These interneuron populations are putative constituents of the Drosophila larval crawling neural circuitry.

Table 1. Drosophila stock expression patterns

OK371	Motor neurons (positive control)
6793	Acetylcholine
7009	Dopamine and serotonin
36494	Tyramine and octopamine
27637	Serotonin receptor 1B
24635	Glutamate

Results

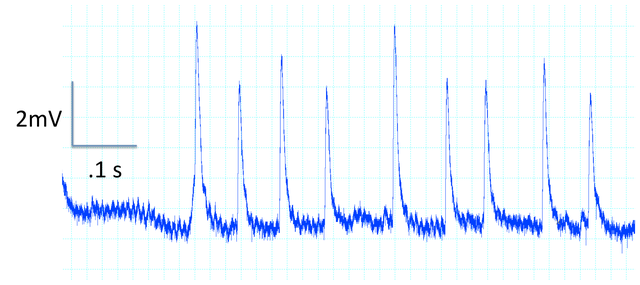


Figure 3: Extracellular recording of natural depolarizing potentials of Wild type muscle cells during contraction

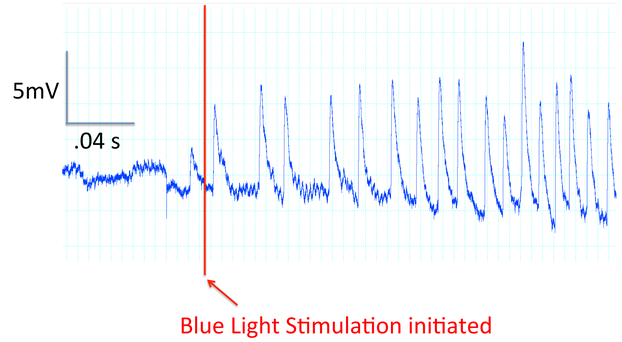


Figure 4: Extracellular recording of depolarizing potentials in in response to blue light activation of OK371 x ChR2 (motor neurons)

Future Directions

1. Extracellular recording from specific muscle groups to determine which interneuron populations are sufficient to generate muscle excitation in specific muscle groups.

2. Optogenetic activation of interneuron populations of interest with severed connections to the ganglia and brain lobes to determine if muscular excitation is dependent on communication with the brain, or if activation of interneuron populations is sufficient to generate muscle excitation.