

Modulation of central pattern generator output by peripheral sensory cells in *Drosophila* larvae

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Goals

- 1) Observe the behavioral effects of remotely activating different populations of peripheral sensory neurons in freely behaving larval *Drosophila*
- 2) Record rhythmic bursts of excitatory junctional potentials (EJPs) at the larval neuromuscular junction (NMJ) in semi-intact larvae as a correlate of motor pattern production
- 3) Record activity at the larval NMJs during activation of a specific set of peripheral sensory cells

Part I: Background

The peripheral nervous system of the *Drosophila* abdomen

Each abdominal hemi-segment in *Drosophila* larvae contains 43 identified peripheral sensory cells. Each cell has a characteristic cell body location underneath abdominal body wall muscles (Figure 1A,B). Sensory cells are divided into 3 general classes based on morphology: multi-dendritic neurons (sub-types I-IV), external sensory organs, and chordotonal organs (Figure 1C).

For many years, neurobiologists have studied how genes control the development and morphological features of type IV (and other) sensory cells. But it is only recently that researchers have begun to study the functional role of these neurons in the context of locomotion. What do we know so far? We know that silencing large populations of peripheral sensory cells drastically slows larval locomotion (Hughes and Thomas 2007). We also know that remotely activating large populations of sensory neurons also drastically slows larval locomotion (Pulver et al. 2009). But what happens when we activate specific subsets of sensory cells?

In lab today we will focus our attention on one particular type of sensory cell, the Type IV multi-dendritic neurons. These cells have segmentally organized axonal terminations in the central nervous system (Figure 1D) (Grueber et al. 2007) and dendrites that tile the larval ectoderm (Figure 1E-H) (Grueber et al. 2002). If we look in cross section at the larval ventral ganglion, we can see that Type IV

neurons project to the ventral most area of the ventral ganglion (Zlatic et al. 2009). Type IV sensory terminals do not overlap anatomically with motor neuron dendrites in the larval central nervous system.

Recent work has shown that activity in type IV sensory mediate escape behaviors in response to noxious stimuli: mechanical 'poking' (Zhong et al. 2010), high temperature(Tracey et al. 2003), and high intensity UV light (Xiang et al. 2010). Activating these neurons with ChR2 evokes lateral rolling in larvae. Genetically silencing these neurons prevents larvae from performing lateral rolls when they normally would. So these neurons are both necessary and sufficient for lateral rolling. Larvae move fastest when they are laterally rolling, and this behavior appears to be adaptive for escaping from parasitoid wasp attack (Hwang et al. 2007).

The gross behavioral results of activating type IV sensory cells have been characterized in previous publications, but researchers have yet to 1) quantitatively measure the sequence of larval behaviors elicited by Type IV activation or 2) measure the actual motor patterns underlying Type IV escape behaviors. This is what we want you to do in lab today---new experiments that have not been done by researchers!

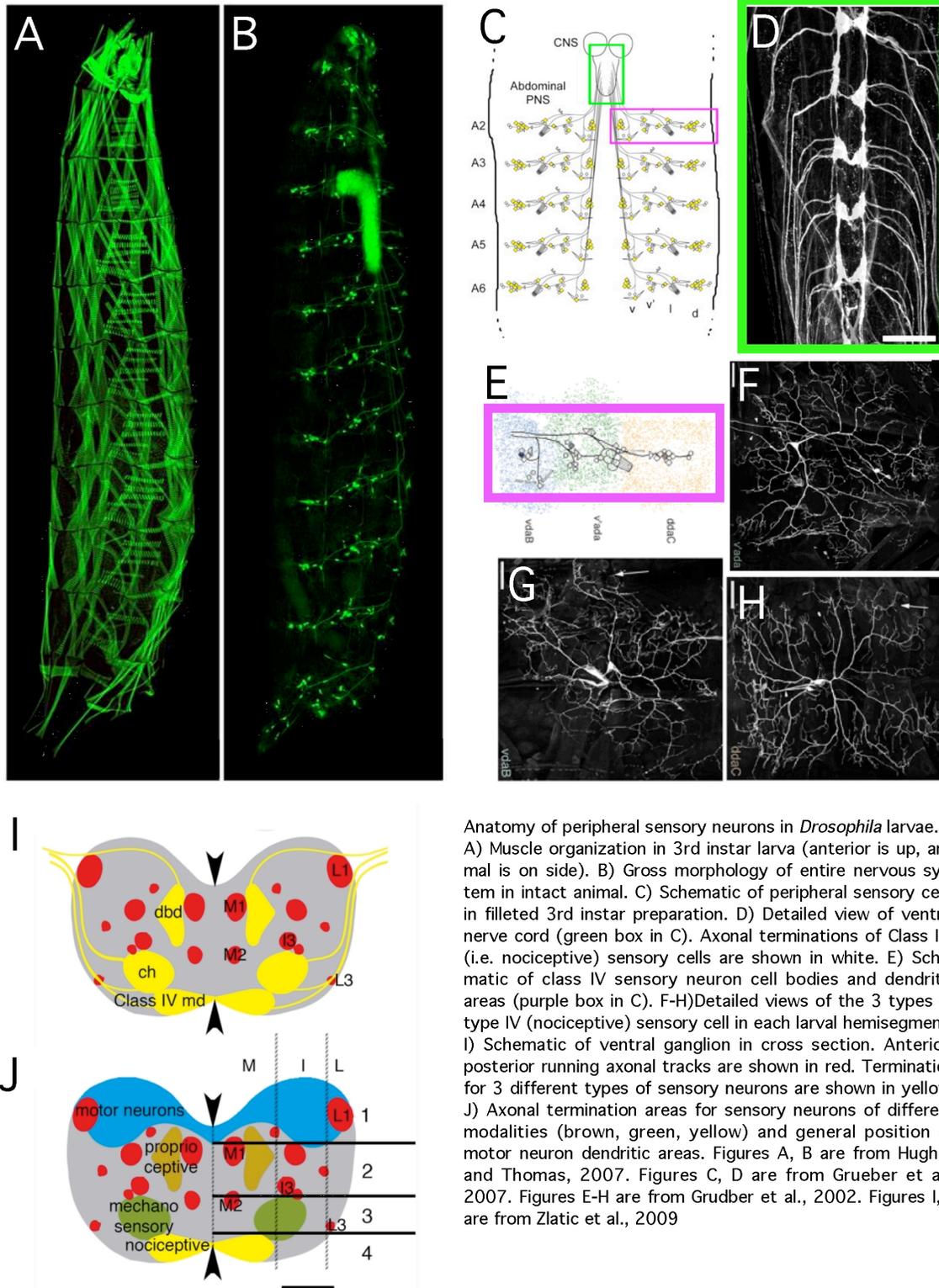


Figure 1

Part IV: Lab Exercises

BEHAVIOR

Methods

- 1) Obtain examples of 3 different genotypes of flies (one is wild type, one has ChR2 expressed in all sensory neurons, one has ChR2 expressed in nociceptive neurons)
- 2) Place larva of each type on separate Petri dishes
- 3) Observe and describe their natural movement
- 4) Focus blue light on individual larva and observe their behavior
- 5) Using the controller box, deliver short (~1 sec) blue light pulses to each larva and observe behavioral responses

VERY IMPORTANT: DO NOT STIMULATE LARVAE CONSTANTLY FOR LONG PERIODS OF TIME AT FIRST. GIVE THEM SHORT (~1S) BLUE LIGHT PULSES FOLLOWED BY SEVERAL SECONDS OF NORMAL LIGHT

Questions to think about

1. *How many different behaviors do larvae do during normal crawling?*
2. *Are there stereotyped sequences of behaviors (i.e. is one specific behavior always followed by another specific behavior)?*
3. *If so, how can you quantify sequences of behaviors?*
4. *How does a 1s pulse affect behavior in each genotype?*
5. *What happens when you give longer duration light pulses?*
6. *How do you quantify the behaviors you see during the light pulse?*
7. *Which flies have ChR2 expression in all sensory cells? Which have expression in nociceptive cells? Which flies are wild type?*

PHYSIOLOGY

NMJ Preparation:

1. Obtain pre-dissected larva from instructor with ChR2 expressed in nociceptive neurons (animal is filleted out, but entire CNS and PNS is intact)
2. Place preparation on rig and locate muscles
3. Load a 15-30 Mohm glass electrode with 3MKCl onto electrode holder
4. Adjust blue light so that it is focused on preparation.

Recording:

1. Insert the electrode into a muscle cell (same as any other intracellular recording!) and record endogenous activity. Note the position of the muscle cell in the larval wall. For bursting preps, measure cycle period of bursting, duty cycle of bursts and EJP frequency within bursts (for recordings with unstable baselines, high pass filter data by 10k before analyzing).
2. Stimulate the preparation with single light pulses of increasing duration (e.g. 10ms, 50ms, 100ms, 500ms, 1sec, 10sec, 1 min. Observe the effects of light

stimulation on muscle contraction patterns. Measure delay to first EJP and EJP frequencies in response to increasing duration light pulses.

3. If the muscles are spontaneously active when you stimulate with blue light, does the stimulation affect the timing of rhythmic EPSPs? Measure rhythmic EPSP responses as above.
4. If the preparation is not active before light stimulation, are coordinated rhythmic muscle patterns elicited? If so describe them. Measure rhythmic EPSP responses as above.

Questions to think about

1. Visually, what types of muscle activity do you see in the semi-intact prep in control conditions (no blue light)?
2. What kind of endogenous activity do you see at the NMJ in control conditions? How does endogenous activity compare to muscle contractions in intact animals? If it is different, what are some possible explanations for the difference?
3. Visually, what happens when nociceptive neurons are stimulated in the semi-intact prep? Is it similar to what you see in the intact animal?
4. What kind of activity do you see at the NMJ in response to light pulses? What happens when you increase the light pulse duration? Why do you see what you see?
5. How does the delay to first EJP in these experiments compare to the delays seen last week when you directly activated motor neurons? Is there a difference? If so, why? What does that difference suggest?

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