Video Article

Dissection of Larval CNS in *Drosophila Melanogaster*

Nathaniel Hafer, Paul Schedl
Department of Molecular Biology, Princeton University

URL: <http://www.jove.com/index/Details.stp?ID=85>

DOI: 10.3791/85

Citation: Hafer N., Schedl P. (2006). Dissection of Larval CNS in *Drosophila Melanogaster*. JoVE. 1. <http://www.jove.com/index/Details.stp?ID=85>, doi: 10.3791/85

Abstract

The central nervous system (CNS) of *Drosophila* larvae is complex and poorly understood. One way to investigate the CNS is to use immunohistochemistry to examine the expression of various novel and marker proteins. Staining of whole larvae is impractical because the tough cuticle prevents antibodies from penetrating inside the body cavity. In order to stain these tissues it is necessary to dissect the animal prior to fixing and staining. In this article we demonstrate how to dissect *Drosophila* larvae without damaging the CNS. Begin by tearing the larva in half with a pair of fine forceps, and then turn the cuticle "inside-out" to expose the CNS. If the dissection is performed carefully the CNS will remain attached to the cuticle. We usually keep the CNS attached to the cuticle throughout the fixation and staining steps, and only completely remove the CNS from the cuticle just prior to mounting the samples on glass slides. We also show some representative images of a larval CNS stained with Eve, a transcription factor expressed in a subset of neurons in the CNS. The article concludes with a discussion of some of the practical uses of this technique and the potential difficulties that may arise.

Protocol

1. Dissect 3rd instar larvae, leaving CNS attached to the cuticle. Place in a 1.5 ml tube.
2. Fix in PBS (1x) containing 2% formaldehyde for 40 min with rocking. ; Use 250ul for every 5-10 dissected CNS.
3. Remove formaldehyde and briefly wash 3 times with PBS + 0.1% Triton X-100 (PBST). Use a finely pulled Pasteur pipette to avoid loss of tissue.
4. Block non-specific protein binding sites and permeabilize cell membranes by incubating tissue 1-8 hrs in PBST + 5% BSA, rocking at 4°C.
5. Incubate in primary antibody diluted in PBST + 5% BSA rocking at 4°C overnight. Optimal antibody dilution will vary for different antibodies.
6. Rinse 3x in PBST followed by two 30min washes at 4°C.
7. Incubate in secondary antibody diluted in PBST + 5% BSA for 1-3 hrs rocking at 4°C. Rinse 3x in PBST followed by two 30min washes at 4°C. If the secondary is conjugated to a light sensitive compound, wrap the tube in aluminum foil.
8. Dissect tissue further (if needed) and mount on slides.

Discussion

This video demonstrates how to dissect the CNS from third instar *Drosophila* larvae. After dissection, the CNS can be stained using a standard immunohistochemistry protocol. Currently, much is still unknown about how the adult nervous system is generated as well as how it stores and transmits information. Studies of the developing *Drosophila* larval CNS should enhance our knowledge of nervous system wiring and function.

Drosophila larvae exhibit a number of stereotyped behaviors, such as a response to environmental cues. How are these behaviors learned and stored in the nervous system? Studies of the nervous system during larval development will help to answer this question.

At this stage in *Drosophila* development, the organism is preparing to undergo the vast morphological changes that occur during pupation. During this time, massive remodeling of the neuromuscular system occurs as the organism transitions from a crawling maggot into an adult fly. It will be interesting to determine how neurons change during this stage of development and if the same type of behaviors can be observed in the neurons of higher animals.

References

1. Lin, D.M. & Goodman, C.S. Ectopic and increased expression of fasciclin II alters motoneuron growth cone guidance. *Neuron* 13, 507-523 (1994)
2. Park, Y., Fujioka, M., Kobayashi, M., Jaynes, J., and Datta, S. even-skipped is required to produce a trans-acting signal for larval neuroblast proliferation that can be mimicked by ecdysone. *Development* 128, 1899-1909 (2001)