Video Article

Drosophila Larval NMJ Immunohistochemistry

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Abstract

The *Drosophila* neuromuscular junction (NMJ) is an established model system used for the study of synaptic development and plasticity. The widespread use of the *Drosophila* motor system is due to its high accessibility. It can be analyzed with single-cell resolution. There are 30 muscles per hemisegment whose arrangement within the peripheral body wall are known. A total of 31 motor neurons attach to these muscles in a pattern that has high fidelity. Using molecular biology and genetics, one can create transgenic animals or mutants. Then, one can study the developmental consequences on the morphology and function of the NMJ. Immunohistochemistry can be used to clearly image the components of the NMJ. In this article, we demonstrate how to use antibody staining to visualize the *Drosophila* larval NMJ.

Video Link

The video component of this article can be found at https://www.jove.com/video/1108/

Protocol

Before you start

1. Prepare the following solutions: PBT (0.1 % Triton X100 in 1X PBS), PBTB (0.2% BSA in PBT), and PBTN (2% NGS in PBTB).
2. Dissect Drosophila larvae. Please see ***Drosophila* Larval NMJ Dissection.**

Immunohistochemistry

1. Move the larvae to a 1.5 ml tube containing PBT. Wash the larvae twice for 15 minutes in the PBT. Note: to wash, place the 1.5 ml tube on a nutator mixer. Remove the liquid with a pippetor and replace it with fresh liquid.
2. Remove the PBT. Wash in PBTB twice for 30 minutes.
3. Remove the PBTB. Wash in PBTN twice for 15 minutes.
4. Incubate in primary antibody diluted in PBTN at room temp for 1 hour or at 4º C overnight.
5. Rinse twice with PBTB. Note: to rinse add solution and remove quickly.
6. Wash twice for 15 minutes in PBTB.
7. Wash in PBTN for 30 minutes.
8. Incubate in secondary antibody (and conjugated primary antibody if you are using one) diluted in PBTN.
9. Cover in tinfoil and incubate for 1.5 hours at room temperature.
10. Rinse twice with PBTB.
11. Wash twice for 15 minutes with PBTB.
12. Proceed to mounting.

Mounting

Note : Mount in glycerol if samples will be imaged immediately. Mount in Prolong Gold if samples will be stored before imaging (for more than a week) or if samples must be imaged multiple times. To use, place a bottle of prolong in 65ºC water bath for 2-3 minutes. Take an aliquot of Prolong Gold and keep in on a heat-block at 45-50º C.

1. Pour stained preps in PBT in to a watch glass.
2. Put some glycerol on a clean glass slide for processing.
3. Move the animals to processing slide by picking them out of the watch glass by a corner using forceps. Ensure they are cuticle side down.
4. Remove the head and tail with a fresh razor blade on the processing glass slide. An exacto-knife with blade #16 works well for this.
5. On another slide (mounting slide), put a small drop of glycerol/prolong and spread it with clean forceps.
6. Move the dissected animals to the mounting slide by their edge, taking care not to invert them. Try to mount them in rows in the same orientation. Mount 6-8 animals per slide.
7. Drop a cover slip on by placing an edge into the glycerol/Prolong and slowly releasing it.

8. Seal the slide with nail varnish. Note [Do not image until the varnish is dry. This usually takes ten minutes. For samples mounted in prolong gold, let the slides dry for at least three hours before sealing or imaging.]

Discussion

Immunohistochemistry (IHC) is vital for the study of NMJ biology because it enables visualization of the NMJ. This is accomplished by using antibodies that recognize the neuronal membrane (e.g., HRP), the presynapse (e.g., CSP, SYT), and/or the postsynapse (e.g., DLG). Signaling molecules, structural proteins, or novel proteins of interest can also be stained. Then, genes can be mutated or missexpressed, and IHC can detect perturbation of synaptic structure and/or neuronal signaling.

The NMJ of *Drosophila* has gained great popularity since the early studies that illuminated its basic structure and function.[1-4] Many of the molecules that have been identified in studies of the *Drosophila* NMJ are conserved in vertebrates.[4] Therefore, the insights learned through studies of the Drosophila NMJ may be applicable to synaptic biology in many systems. Some possible applications include the study of molecules involved in synaptic development, plasticity, and neurological disease.

References

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