Video Article

Loading Drosophila Nerve Terminals with Calcium Indicators

Adam J. Rossano[1], Gregory T. Macleod[1]

[1]Department of Physiology, University of Texas Health Science Center at San Antonio (UTHSCSA)

Correspondence to: Gregory T. Macleod at macleod@uthscsa.edu

URL: http://www.jove.com/video/250
DOI: doi:10.3791/250

Keywords: Neuroscience, Issue 6, Drosophila, neuron, imaging

Date Published: 7/30/2007

Citation: Rossano, A.J., Macleod, G.T. Loading Drosophila Nerve Terminals with Calcium Indicators. *J. Vis. Exp.* (6), e250, doi:10.3791/250 (2007).

Abstract

Calcium plays many roles in the nervous system but none more impressive than as the trigger for neurotransmitter release, and none more profound than as the messenger essential for the synaptic plasticity that supports learning and memory. To further elucidate the molecular underpinnings of Ca^{2+}-dependent synaptic mechanisms, a model system is required that is both genetically malleable and physiologically accessible. Drosophila melanogaster provides such a model. In this system, genetically-encoded fluorescent indicators are available to detect Ca^{2+} changes in nerve terminals. However, these indicators have limited sensitivity to Ca^{2+} and often show a non-linear response. Synthetic fluorescent indicators are better suited for measuring the rapid Ca^{2+} changes associated with nerve activity. Here we demonstrate a technique for loading dextran-conjugated synthetic Ca^{2+} indicators into live nerve terminals in Drosophila larvae. Particular emphasis is placed on those aspects of the protocol most critical to the technique's success, such as how to avoid static electricity discharges along the isolated nerves, maintaining the health of the preparation during extended loading periods, and ensuring axon survival by providing Ca^{2+} to promote sealing of severed axon endings. Low affinity dextran-conjugated Ca^{2+}-indicators, such as fluo-4 and rhod, are available which show a high signal-to-noise ratio while minimally disrupting presynaptic Ca^{2+} dynamics. Dextran-conjugation helps prevent Ca^{2+} indicators being sequestered into organelles such as mitochondria. The loading technique can be applied equally to larvae, embryos and adults.

Video Link

The video component of this article can be found at http://www.jove.com/video/250/

Protocol

1. Select a clean dissection dish that has not been exposed to any fixatives.
2. Dissect a wandering 3rd instar Drosophila larva in Schneider's Drosophila Medium containing Ca^{2+} and L-glutamine, (do not cut any nerves or damage muscle fibers Nos. 7, 6, 13 or 12).
3. Select a glass filling-pipette with a 12 micron tip (internal diameter).
4. Using a syringe and tubing (to apply negative pressure to the pipette) ensure that the pipette tip is not obstructed.
5. Select a fine plastic filling-filament that can be inserted down the length of the glass pipette.
6. Draw ~ 1 cm of 5 mM dextran-conjugated Ca^{2+}-indicator into the plastic filament.
7. Cut all segment nerves.
8. Support the pipette on a ramp that will allow the pipette tip to approach the ventral midline of the dissected larva.
9. Draw the cut end of a nerve to segment No.4, without pinching the nerve, into the end of the pipette (include a small amount of Schneider's medium).

Copyright © 2007 Journal of Visualized Experiments

10. Remove the tubing and insert the plastic filament into the pipette until the end of the filament is within 50 microns of the cut end of the nerve (avoid touching the nerve).

11. Eject sufficient Ca^{2+}-indicator onto the nerve ending to increase the volume of the Schneider's medium by about 33% (final concentration should be < 2mM). Important - This must be completed within 5 minutes of cutting the nerve.

12. Place the preparation in the dark at room temperature while the nerve loads.

13. After 40 minutes remove the Ca^{2+}-indicator using the filament.

14. Leave the pipette in place and fill it completely with fresh Schneider's medium, as this will be used to apply stimulating pulses to the nerve.

15. Allow the Ca^{2+}-indicator to equilibrate in the nerve for at least 60 minutes, but no more than 4 hours, before commencing Ca^{2+}-imaging.

16. Rinse the preparation with fresh Schneider's medium every 30 minutes while it is equilibrating.

17. 20 minutes before imaging replace Schneider's medium with Hemolymph-Like No.6 solution (HL6; Macleod et al. 2002; 2003).

18. L-glutamic acid or glutamate can be added to HL6 solution at 7mM to desensitize postsynaptic glutamate receptors to prevent nerve-evoked muscle contraction (Macleod et al. 2004; Reiff et al. 2002; 2005).

Disclosures

The authors have nothing to disclose.

References

1. Macleod, G.T., Hegstrom-Wojtowicz, M., Charlton, M.P. & Atwood, H.L. Fast calcium signals in Drosophila motor neuron terminals. J. Neurophysiol. 88, 2659-2663 (2002).

2. Macleod, G.T., Suster, M.L., Charlton, M.P., Atwood, H.L. Single neuron activity in the Drosophila larval CNS detected with calcium indicators. J. Neurosci. Methods. 127, 167-178 (2003).

3. Macleod, G.T., Marin, L., Charlton, M.P., Atwood, H.L. Synaptic vesicles: test for a role in presynaptic calcium regulation. J. Neurosci. 24, 2496-2505 (2004).

4. Reiff, D.F., Thiel, P.R., Schuster, C.M. Differential regulation of active zone density during long-term strengthening of Drosophila neuromuscular junctions. J. Neurosci. 22, 9399-9409 (2002).

5. Reiff, D.F., Ihring, A., Guerrero, G., Isacoff, E.Y., Joesch, M., Nakai, J., Borst, A. In vivo performance of genetically encoded indicators of neural activity in flies. J. Neurosci. 25, 4766-4778 (2005).

Copyright © 2007 Journal of Visualized Experiments