Abstract

The Drosophila larval neuromuscular preparation has proven to be a useful tool for studying synaptic physiology. Currently, the only means available to evoke excitatory junctional potentials (EJPs) in this preparation involves the use of suction electrodes. In both research and teaching labs, students often have difficulty maneuvering and manipulating this type of stimulating electrode. In the present work, we show how to remotely stimulate synaptic potentials at the larval NMJ without the use of suction electrodes. By expressing channelrhodopsin2 (ChR2) in Drosophila motor neurons using the GAL4-UAS system, and making minor changes to a basic electrophysiology rig, we were able to reliably evoke EJPs with pulses of blue light. This technique could be of particular use in neurophysiology teaching labs where student rig practice time and resources are limited.

Protocol

Part 1: Animal care and genetic crosses

1. Maintain UAS-ChR2 and OK371-GAL4 fly lines in separate bottles containing standard fly media.
2. Collect virgins of OK371-GAL4 fly lines and males of UAS-ChR2 lines.
3. Put both males and females in a vial containing standard fly media mixed with 1 mM all-trans retinal (ATR). ATR food should be made by first melting regular fly media in a microwave for ~1 minute. Once melted, allow to cool for about 30 seconds to one minute and then add 100 μL of 100 mM ATR in 100% ETOH for every 10 mL of fly media. Place vials on ice, then store in a dark area at 4°C.
4. Let flies mate and lay eggs in a dark area at 22-25°C.
5. Wait 3-4 days until 3rd instar larvae are visible; at that point, dispose of adult flies.

Part 2: Rig setup

2. Place magnetic base with light source on air table of electrophysiology rig.
4. Give 1-5 V pulses to control circuit to activate blue light.
5. Adjust magnetic base and light source until blue light beam is focused on area to be occupied by larval dissection.

Part 3: Dissection

1. Place six 0.1 mm insect pins into the floor of a sylgard-lined dish.
2. Remove a 3rd instar larva from food media and place into any plastic Petri-dish.
3. Rinse larvae with saline to remove food.
4. Place larvae in dissecting dish near the pulled pins and add saline to ½ level.
5. Orient the larvae so that you can see two silvery tubes (trachea) running along the animal’s dorsal surface.
6. Insert a large pin directly into the tail in between the tracheal tubes. Hold the larvae down and place a second large pin into its head, being sure to stretch the body out lengthwise.
7. Make a small incision near the tail, and continue it up the length of the body. Make sure that the tips of the scissors are raised so as not to accidentally cut ventral nerves and/or body wall muscles.
8. Place four pins on the four corners of the animal’s now open midsection. Set them into the dissecting dish so as to fillet the animal. Pin preparation out taught.
9. Using forceps and scissors, remove the animal’s guts, trachea and fat bodies.
10. Rinse the prep with saline.
11. Locate the frontal lobes (as seen in Fig A) and ventral ganglion of the prep.
12. Using micro-scissors, carefully cut through the ventral ganglion just behind the frontal lobes.

Part 4: Muscle recording and blue light stimulation

1. Pull a 10-20 μm electrode using an electronic electrode puller (Sutter Instruments).
Representative Results:

Figure 1A shows a schematic of the recording setup and filleted preparation. Figure 1B shows typical EJP evoked by short light pulses. EJP amplitude shown is summed amplitude from two motor neurons known to both innervate M6. Lower light intensities only activated one motor unit (data not shown).

Figure 1: A) General schematic of an intracellular recording rig and with blue LED. Brain (Br) is removed to inhibit rhythmic activity in the ventral ganglion (VG). ChR2 is expressed in motor neurons using the GAL4-UAS system. B) Intracellular recording from a M6 muscle. 40 ms blue light pulses (at 127 µW/mm²) reliably evoke large synaptic potentials (asterisks) in M6.

Disclosures

We have no conflicts of interest to disclose.

Discussion

Critical steps involve both the initial dissection and the entering of muscle cells. If nerves are cut or muscle is damaged during the initial dissection it is difficult to continue the rest of the experiment. During dissection, one must be very careful to angle dissecting scissors upwards as much as possible during dorsal incision. During the second crucial step, entering a muscle cell, one must watch for a hyperpolarization past ~30 mV. Values above -30 mV indicate that the electrode is either not properly within a muscle cell or in an unhealthy cell.

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References