Video Article

Whole Cell Recordings from Brain of Adult Drosophila

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Abstract

In this video, we demonstrate the procedure for isolating whole brains from adult Drosophila in preparation for recording from single neurons. We begin by describing the dissecting solution and capture of the adult females used in our studies. The procedure for removing the whole brain intact, including both optic lobes, is illustrated. Dissection of the overlying trachea is also shown. The isolated brain is not only small but needs special care in handling at this stage to prevent damage to the neurons, many of which are close to the outer surface of the tissue. We show how a special holder we developed is used to stabilize the brain in the recording chamber. A standard electrophysiology set up is used for recording from single neurons or pairs of neurons. A fluorescent image, viewed through the recording microscope, from a GAL4 line driving GFP expression (GH146) illustrates how projection neurons (PNs) are identified in the live brain. A high power Nomarski image shows a view of a single neuron that is being targeted for whole cell recording. When the brain is successfully removed without damage, the majority of the neurons are spontaneously active, firing action potentials and/or exhibiting spontaneous synaptic input. This in situ preparation, in which whole cell recording of identified neurons in the whole brain can be combined with genetic and pharmacological manipulations, is a useful model for exploring cellular physiology and plasticity in the adult CNS.

Protocol

I. Dissection of brains from adult fly

1. Place a small drop (~100 µl) of dissecting solution in center of a 35 mm Petri dish.
2. Catch adult female using aspirator. Under dissecting microscope, use two syringe needles to decapitate the fly.
3. Place head in a small drop of dissecting saline (with papain added) in a Petri dish.
4. Position head with with proboscis facing bottom of the dish.
5. Hold the cuticle covering the left compound eye with needle 1. Make a diagonal cut that extends from the dorsal to the ventral surface with needle 2, just medial to needle 1.
6. Hold the mouthparts with needle 1 and use needle 2 to make a horizontal cut that extends from the ventral surface of the left eye to the right eye, at the level of the base of proboscis.
7. Hold the cuticle covering the right compound eye with needle 1. Make a diagonal cut that extends from the dorsal to the ventral surface with needle 2, just medial of needle 1.
8. Rotate the head so that the rostral side is on the Petri dish surface. Insert needle 1 between the rostral cuticle and brain, and use needle 2 to follow the same path as the first needle. Ideally, the capsule will be peel away from the brain with both optic lobes attached since these are important in stabilizing the brain for recording.
9. The trachea, air sacs, and other connective tissues are carefully removed using two fine tip forceps.
10. Whole dissection should take 3-10 minutes

II. Mounting the CNS

1. The brain is transferred to the recording chamber using a yellow tip pipet.
2. In the recording chamber, the brain is stabilized by placing the platinum frame such that two fine cross hairs make contact with the tissue at the junction between the optic lobes and central brain region.
3. Each brain is allowed to rest in the recording chamber with continuous perfusion with oxygenated saline (95% oxygen and 5% carbon dioxide) for at least 10 minutes. Perfusion of the chamber with oxygenated saline is continued throughout the recording period. Preparation is visualized using an upright microscope(Axioskop 2FS; Zeiss, Oberkochen, Germany) with a fixed stage and a 40x water immersion objective (Achroplan; numerical aperture,0.8; Zeiss) and Nomarski optics. GFP was viewed with a BP 505-530 fluorescence filter.

III. Electrophysiology

1. Pipets of 8-14 Mohms are used for whole cell recordings
2. Current-clamp and voltage-clamp recordings are performed using a List EPC7 or an Axopatch 200B amplifier,a Digidata 1322A D-A converter (Molecular Devices, Foster City, CA), a Dell Dimension 8200 computer (Dell Computer, Round Rock, TX), and pClamp 9 software (Molecular Devices).

Discussion

The isolated whole brain preparation we illustrate in this video allows assessment of cellular mechanisms underlying excitability and synaptic transmission in identified neurons, including those in the olfactory processing pathways, in the adult Drosophila brain (Gu and O Dowd, 2006). This approach is complementary to study of neuronal activity in the brain of intact adult Drosophila (Wilson et all 2004), in much the same way recordings from neurons in a mammalian brain slice are complementary to recordings in awake behaving mammals. There are two major advantages of the in situ fly brain preparation when compared to the mammalian slices. First the entire fly brain fits easily into the recording chamber so it is not necessary to excise a small piece of tissue from a larger neural circuit that occurs when making mammalian brain slices. Therefore the neuronal circuits within the isolated Drospohila brain remain largely intact. Secondly, the cell bodies of most of the neurons are near

the brain surface, readily accessible for whole cell recording and they remain functionally active when continuously perfused with oxygenated saline for several hours.

Using a glass bottom recording chamber also allows identification of specific neurons on the basis of their location and/or GFP expression. In this preparation, recording during perfusion requires stabilization of the brain. This is not compatible with standard procedures, including strategically placed gold wire or nylon mesh, that are effective for tissues such as hippocampal slices, due to the small size of the whole brain (~1mm). Therefore we a designed holder with a platinum frame and nylon cross hairs positioned to contact the brain in only two locations to minimize damage to neurons located near the brain surface. This stabilizes the brain, with either the anterior or posterior surface facing up and the opposite surface just lightly resting on the floor of the recording chamber. Using this device we are able to routinely maintain stable whole cell recordings for up to an hour, even from small neurons including Kenyon cells, while doing pharmacological manipulations that require bath application of specific drugs. The holder should also be useful in securing other small tissue samples for electrophysiological studies such as spinal cord slices from neonatal rodents.

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References

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