



Separate search terms with commas

Search

[Browse -](#)[Sign Out](#)

Whole Cell Recordings from Brain of Adult Drosophila

Huaiyu Gu (/author/Huaiyu_Gu), Diane K. O'Dowd (/author/Diane+K._O%27Dowd)

Department of Development and Cell Biology, Department of Anatomy and Neurobiology, **University of California, Irvine (UCI)** (/institutions/NA-north-america/US-united-states/CA-california/16242-university-of-california-irvine)

This work was supported by NIH grants DA14960 and NS27501 to DKOD. Additional support for this work was provided by a grant to UC Irvine, in support of DKOD, from the Howard Hughes Medical Institute through the HHMI Professors Program.

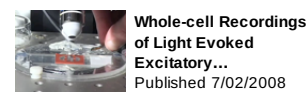
Video Article Chapters

- 0:04 Title
- 0:22 Introduction
- 0:53 Preparation of Dissection Solution
- 2:15 Adult Drosophila Selection
- 2:32 Sex Differentiation
- 3:04 Decapitation and Removal of Brain
- 4:19 Removal of Trachea and Enzyme Treatment
- 5:27 Stabilizing Brain in Recording Chamber
- 7:58 Conclusion

You have **full access** to this article through .

0

Related Videos



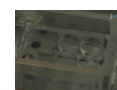
Whole-cell Recordings of Light Evoked Excitatory...
Published 7/02/2008



Single Sensillum Recordings in the Insects...
Published 2/17/2010



Postsynaptic Recordings at Afferent Dendrites...
Published 2/10/2011



Mapping Inhibitory Neuronal Circuits by Laser...
Published 10/06/2011

Cite this Article

Gu, H., O'Dowd, D. K. Whole Cell Recordings from Brain of Adult Drosophila. *J. Vis. Exp.* (6), e248, doi:10.3791/248 (2007).

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

Download

EndNote, RefWorks, Reference Manager, ProCite
(/legacy/GetRIS.php?id=248)

JoVE PDF Article (/pdf/248)

Materials List (/pdf-materials/248)

XML File (/pdf/default.aspx?ID=248)

Information

Date Published	7/29/2007
JoVE Section	General (/general)
JoVE Issue	6 (/archive/6)
Comments	3 Comments
View Count	30,740 (Details...)
DOI	doi:10.3791/248

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 μ l) 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 (Achromplan; 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).

Keywords

Neuroscience (*/keyword/neuroscience*), Issue 6 (*/archive/6*), neuron (*/keyword/neuron*), electrophysiology (*/keyword/electrophysiology*), insect CNS (*/keyword/insect+cns*), GFP (*/keyword/gfp*), *Drosophila* brain (*/keyword/drosophila+brain*), adult fly (*/keyword/adult+fly*), whole cell recording (*/keyword/whole+cell+recording*)

Translate this page to:

Choose Language...

Navigate this Article

[Cite this article](#)
[Abstract](#)
[Protocol](#)
[Discussion](#)
[Disclosures](#)
[Acknowledgements](#)
[Materials](#)
[References](#)
[Ask the author](#)
[Top of page](#)

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 al 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 *Drosophila* 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.

Disclosures

Acknowledgements

This work was supported by an NIH grant NS27501 to DKOD. Additional support for this work was provided by an HHMI Professor Program Grant to DKOD.

Materials

Name	Type	Company	Catalog Number	Comments
#5 Foceps	Tool	Fine Science Tools	No. 11251-10	Be careful, never damage the fine tips of forceps
Papain	Reagent	Worthington Biochemical	3126	20 U/ml activated by 1 mM L-cysteine
Dissecting microscope	SMZ-2B Model:C-PS	Nikon Instruments		Equipped with gooseneck fiber optic lights

				positioned a low angle
Needle & Syringe		PrecisionGlide™, Becton Dickinson & Co	305175 & 309602	Cheap and durable
Upright microscope	Axioskop2 FS plus	Carl Zeiss, Inc.		Equipped with a fixed stage, 40X water immersion objective, Nomarski optics, and fluorescent lamp
Patch clamp amplifier	200 B	Molecular Devices		
Digidata D-A converter	1322A	Molecular Devices		

References

1. Gu, H. and O Dowd, D.K. Cholinergic synaptic transmission in Adult *Drosophila* Kenyon cells in situ. *Journal of Neuroscience* 26: 265-272 (2006)
2. Wilson, RI, Turner, GC, and Laurent, G. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* 303: 366-370 (2004).

Ask the Author

3 Comments

As a fly researcher with no prior experience in electrophysiology, I intend to set-up the necessary facility, gain skill and subsequently implement, in our ongoing *Drosophila* neuropharmacogenomic modeling work, recording from brains of intact organism as well as from single neurons from isolated brain as described in the present paper. In this backdrop, I would like to know if the materials and devices mentioned in this paper would be sufficient for recording from brains of intact organism. If not, what else will be required? Where from one can obtain them? Kindly also suggest alternative suppliers, if any, for material and devices that have already been successfully used in intact organism and single neuron recordings.

1 (/video/248/whole-cell-recordings-from-brain-of-adult-

We have not recorded from brains in intact *Drosophila* with our equipment but I believe the set up we describe in this video is very similar to that used by Rachel Wilson and her colleagues at Harvard who are recording from intact organisms. In our lab we use both the Axon Instruments and List patch clamp amplifiers and they both work well. We have only used the Zeiss upright microscope.

2 (/video/248/whole-cell-recordings-from-brain-of-adult-

Is it possible to obtain or buy the designed holder (platinum frame and nylon cross hairs)?

3 (/video/248/whole-cell-recordings-from-brain-of-adult-

We make these in the lab. You just need a short piece of platinum wire. Bend to shape you want. Use nylon hairs glued to wire with super glue. Nylon fibers are from a fine nylon mesh. We make under microscope and the nylon fibers are stretched over frame, taped on either side so they are tight, and then super glue applied to frame over the fiber. Each fiber glued in place allowed to dry before next one put on. Then trim all excess at end.

3.1 (/video/248/whole-cell-recordings-from-brain-of-adult-

Post a Question / Comment / Request

Your comment (in English, please):

Allowed HTML tags: *i*, *b*, *u*, *sup*, *sub*

You are signed in as jzacka@worthington-biochem.com

[Post Comment](#)

About JoVE

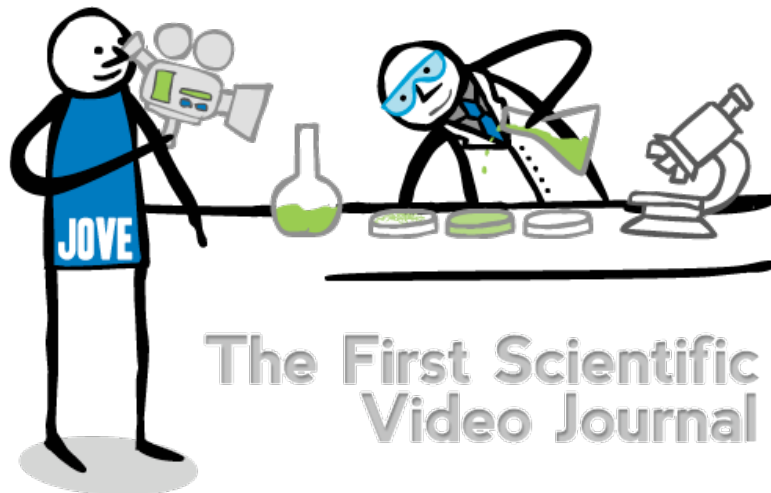
The Journal of Visualized Experiments (JoVE) is a PubMed-indexed video journal. Our mission is to increase the productivity of scientific research.

Follow JoVE

[blog \(http://blog.jove.com\)](http://blog.jove.com) [rss \(/rss\)](#)

[facebook \(http://www.facebook.com/JoVEjournal\)](http://www.facebook.com/JoVEjournal)

[twitter \(http://twitter.com/JoVEjournal\)](http://twitter.com/JoVEjournal) [newsletter](#)



[Subscribe \(/subscribe\)](#) [About \(/about\)](#) [Editorial Boards \(/editorial-boards\)](#) [Publish \(/publish\)](#) [Press \(/about/press-releases\)](#)
[Advertise \(http://industry.jove.com\)](http://industry.jove.com) [JoVE Shop \(http://shop.jove.com\)](http://shop.jove.com) [Contact \(/about/contact#contact\)](#) [Troubleshooting \(/about/contact\)](#)

