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Dissection and Staining of *Drosophila* Optic Lobes at Different Stages of Development

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INTRODUCTION

The *Drosophila* visual system is composed of the retina and the optic lobes, which are the ganglia where photoreceptors project and initial processing of visual inputs occur. This protocol outlines procedures for dissecting the optic lobes from *Drosophila* larvae, pupae, and adults. It also describes methods for visualizing the anatomy of brain neural circuits by staining with fluorescent secondary antibodies and primary antibodies specific for various neuronal populations and architectural features.

MATERIALS

RECIPES: Please see the end of this article for recipes indicated by <R>.

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

CO₂ (for anesthetizing adult flies)

Drosophila at stage of interest

Glycerol (50%)

Normal goat serum (Lampire Biological Laboratories) (5%, v/v in PBST)

Aliquot in very small volumes and store at –20°C.

Paraformaldehyde (16%) (Electron Microscopy Sciences)

PBST (PBS containing 0.3% Triton X-100)

<R>Phosphate-buffered saline (PBS) (1×, pH 7.4)

Primary antibodies (see Table 1)

Secondary antibodies (see Table 2)

Vectashield mounting medium (for preserving fluorescence) (Vector Laboratories)

Equipment

Cover glasses (18 × 18-mm and 24 × 50-mm) (Fisher Scientific)

Dissecting microscope

Dissection dishes (three-well, glass) (Fisher Scientific)

Forceps (two pairs; Dumont #55; Fine Science Tools)

Micropipettor and tips

Microscope slides (Fisher Scientific)
 Minutien pins (0.1-mm diameter) (Fine Science Tools 26002-10)
 Mounted pins (fine)
 Nail polish (clear)
 Orbital shaker (Bellco Biotechnology)
 Paintbrush (small)
 Petri dishes
 Pin holders (12-cm) (Fine Science Tools 26016-12)
 Slide holder (dark)
 Sylgard 184 Silicone Elastomer Kit (Dow Corning) (Stern 1999)
 Tubes, 1.5-mL

METHOD

Dissection

The dissection procedure should take ~10–25 min per experiment. It is critical to minimize dissection time to avoid tissue degradation.

- 1 Dissect *Drosophila* brains as described below according to the developmental stage desired.

Larval brains

- i Collect larvae at the stage of interest either from the walls of the vial using forceps or with a PBS-soaked paintbrush for younger larvae. Alternatively, fill the vial with 50% glycerol and larvae will come up to the surface.
- ii Place larvae in a Petri dish layered with Sylgard transparent resin containing drops of PBS.
- iii Remove the brain lobes by holding the larval body with one pair of forceps and pulling from the larval mouth hook with a second pair. Discard the larval body. Remove the excess tissue surrounding the brain lobes using fine mounted pins.
- iv Place the clean brain lobes in a glass dissection dish well containing 150 μ L of PBS. Keep the dish on ice.

A typical experiment requires 10–20 larval brains.

Pupal brains

- v Collect pupae at the appropriate developmental stage in a Petri dish layered with Sylgard transparent resin containing drops of PBS.

Pupae can be distinguished from slow-moving third-instar larvae because they stop crawling and remain attached to the wall of the vial. White pupae are considered the earliest stage in pupal development, whereas older pupae are dark brown. To study specific pupal development stages, wait the appropriate number of hours before collecting.

vi Submerge pupae in PBS and hold the pupal case through the abdominal part with one pair of forceps. With another pair, open the pupal case and pull the pupae out. Gently open the pupae and push the brain out. Remove excess tissue surrounding the brain lobes using fine mounted pins.

vii Using a pair of sharp forceps held closed, place the clean brain lobes in a glass dissection dish well containing 150 μ L of PBS. Keep the dish on ice.

A typical experiment requires six to 10 pupal brains.

viii Using fine pins, remove or gently separate the pupal eye to avoid interference with visualization of the pupal optic lobe.

This step is critical for visualizing the medulla neuropil.

Adult brains

ix Anesthetize adult flies of the appropriate genotype with CO₂. Decapitate and place the heads in a glass dissection dish well containing PBS. Keep the dish on ice.

x Perform the dissection in a Petri dish layered with Sylgard transparent resin containing drops of PBS, or alternatively, in the dissection dish well containing PBS.

xi Holding the head through the maxillary cavity, submerge it in PBS, and remove the maxillary palps with forceps. Hold both sides of the maxillary cavity and gently pull the two pairs of forceps away from each other to open the head cuticle. Remove the brain from the head cuticle and remove any excess surrounding tissue using fine mounted pins.

xii Using a pair of sharp forceps held closed, place the clean adult brain lobes in a glass dissection dish well containing 150 μ L of PBS. Keep the dish on ice.

A typical experiment requires six to 10 adult brains.

xiii Remove the retina and lamina to avoid interference with the visualization of the medulla/ lobula complex and poor antibody penetration.

This step is critical.

Fixation

The fixation and staining of brains should take ~1.5 d.

2 Add 50 μ L of 16% paraformaldehyde to the glass wells containing 150 μ L of PBS and the dissected tissue. Fix the larval, pupal, or adult brains by incubating on an orbital shaker with gentle agitation for 20 min at room temperature.

3 Remove the paraformaldehyde and wash with agitation three times in fresh PBS for 5 min each.

Staining

Day 1

4 Add 200 μ L of blocking solution (5% normal goat serum in PBST) and incubate the fixed brains on an orbital shaker with gentle agitation for at least 30 min at room temperature in darkness.

5 Dilute primary antibodies in PBST in 1.5-mL tubes (you will need 200 μ L per well).

- 6 Remove the blocking solution and add primary antibody. Incubate with agitation overnight at room temperature in darkness.

For the majority of commercial antibodies, it is possible to omit the blocking step and incubate the dissected brains directly in primary antibodies overnight.

Day 2

- 7 Remove primary antibody and wash three times in PBS for 20 min each at room temperature.

Primary antibodies can be reused three times or more.

- 8 Dilute secondary antibodies in PBST in 1.5-mL tubes (you will need 200 μ L per well). Add secondary antibodies to brain preparations. Incubate on an orbital shaker with agitation for 3 h at room temperature in darkness.

Prepare the secondary antibodies fresh and discard after use.

- 9 Remove secondary antibodies and wash three times in PBS for 20 min each at room temperature.

Mounting

Use the bridge method illustrated in Figure 1 to mount *Drosophila* brains and preserve their three-dimensional configuration. This procedure should take ~5–10 min per experiment.

- 10 Add two 2- μ L drops of 50% glycerol to each end of a microscope slide. Cover these drops with 18 $\times$ 18-mm cover glasses.
- 11 Add a 5- μ L drop of Vectashield mounting medium in between the two cover glasses.
- 12 Using a pair of sharp forceps held closed, place the brains in the mounting medium. Using fine mounted pins, align all the brains in the same orientation for ease of imaging.
- 13 Place a 24 $\times$ 50-mm cover glass on top of the bridge to cover the brains.
- 14 Seal the edges of the cover glass with nail polish and store the samples at 4°C in a dark slide holder.

RECIPES

Phosphate-buffered saline (PBS)

Reagent	Amount to add (for 1X solution)	Final concentration (1X)	Amount to add (for 10X stock)	Final concentration (10X)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na ₂ HPO ₄	1.44 g	10 mM	14.4 g	100 mM
KH ₂ PO ₄	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
CaCl ₂ ·2H ₂ O	0.133 g	1 mM	1.33 g	10 mM
MgCl ₂ ·6H ₂ O	0.10 g	0.5 mM	1.0 g	5 mM

PBS can be made as a 1X solution or as a 10X stock. To prepare 1 L of either 1X or 10X PBS, dissolve the reagents listed above in 800 mL of H₂O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H₂O to 1 L. Dispense the

solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle or by filter sterilization. Store PBS at room temperature.

FIGURE 1.
Bridge method for mounting *Drosophila* optic lobes.

Table 1Primary antibodies used to label *Drosophila* brains

Primary antibody	Source	Dilution
To detect expression of green fluorescent protein(GFP)- or lacZ-based constructs:		
Rabbit anti-GFP	Molecular probes; A11122	1:1000
Sheep anti-GFP	Serotec; 4745-1051 or similar	1:1000
Rabbit anti- β -Gal	ICN/Cappel or similar	1:20,000
Mouse anti- β -Gal	Promega; Z3781	1:500
To label relevant architectural features:		
Mouse anti-nc82 (to label general larval, pupal, and adult neuropil)	Developmental studies Hybridoma bank	1:50
Rat anti-DE-cadherin (DCAD2) (to label the larval neuroepithelium and neuropil in larvae and early pupae)	Developmental studies Hybridoma bank	1:50
Rat anti-DN-cadherin (DN-EX 8) (to label general neuropil in larvae, pupae, and adult)	Developmental studies Hybridoma bank	1:50
To label neuronal populations in larvae:		
Mouse anti-24B10 (to label photoreceptors and their projections)	Developmental studies Hybridoma bank	1:50
Mouse anti-Dachshund (mAbdac 2-3) (to label lamina precursors and lobula cells)	Developmental studies Hybridoma bank	1:100
Mouse anti-Elav (9F8A9) (to label neurons)	Developmental studies Hybridoma bank	1:25
Rat anti-Elav (7E8A10) (to label photoreceptors and their projections)	Developmental studies Hybridoma bank	1:25

Table 2Secondary antibodies used to label *Drosophila* brains

Secondary antibody	Source	Dilution
Donkey anti-sheep Alexa488	Molecular probes; A-11015	1:1000
Donkey anti-mouse Alexa488	Molecular probes; A-21202	1:1000
Goat anti-rabbit Alexa488	Molecular probes; A-11034	1:1000
Donkey anti-mouse Alexa555	Molecular probes; A-31570	1:1000
Donkey anti-rabbit Alexa555	Molecular probes; A-31572	1:1000
Goat anti-guinea pig Alexa555	Molecular probes; A-21435	1:500
Goat anti-rat Alexa555	Molecular probes; A-21434	1:500
Donkey anti-mouse Alexa647	Molecular probes; A-31571	1:500
Donkey anti-rabbit Alexa647	Molecular probes; A-31573	1:500
Donkey anti-rat Cy5	Jackson ImmunoResearch Europe; 712-175-153	1:400
Goat anti-guinea pig Alexa647	Molecular probes; A-21450	1:500