Protocol

Dissection of *Drosophila* Adult Brains for Patch-Clamping Neurons

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The brain of adult flies (*Drosophila melanogaster*) has been studied in detail from several perspectives, including the anatomical and molecular characterization of hundreds of neuronal types. However, information regarding the electrophysiological properties of most neuronal types is lacking. This protocol provides detailed information on how to dissect the brain of adult flies to produce an ex vivo preparation in which central neurons can be patch-clamped. Immobilizing fresh and tiny tissues, such as fly brains, to perform successful patch-clamp recordings is a critical step; here, we explain how this can be achieved using cyanoacrylate glue.

MATERIALS

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.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Adult external saline <R>

This is different than the classical HL salines (Feng et al. 2004). The solution can be prepared in advance if glucose and calcium chloride are omitted (add before use).

Drosophila adults of the desired genotype carrying an element to fluorescently label the lateral ventral neurons (LNvs), such as *Pdf*-RFP (or *Pdf*-Gal4>UAS-GFP/RFP)

Ice

Vetbond 3M Tissue Adhesive (*N*-butyl cyanoacrylate)

Equipment

Dissecting microscope (e.g., Leica S6E)

Dissection pins (0.1-mm in diameter; e.g., Living Systems Instrumentation)

Glue pipettes (any electrode pipette)

Microcentrifuge tubes (0.5-mL)

Pasteur pipettes

Pipette tips (1-mL, blue)

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Plastic tubing (fine, flexible; e.g., Tygon, T3601-13)

Sharpened forceps <R> (two; see Steps 3, 6, and 8)

The fine forceps must be in perfect shape for dissections to be completed quickly and with minimal effort. Unfortunately, no matter how careful you are with your favorite pair of forceps, they will bend at some point. Subtle damage to forceps is fixable by sharpening, and it is always better to invest some time sharpening your bent forceps than to spend a whole day attempting to dissect with defective tools. Sharpening forceps is an art; becoming good at it requires practice, but is worthwhile, as the forceps you sharpen may even be better than a new pair.

Sylgard-coated coverslips and Petri dishes <R>

Before the day when you will patch-clamp, prepare many Sylgard-covered coverslips (square, 18 × 18-mm or round, 10-mm in diameter); you can prepare many at once so that you have to do this only once in a while. Sylgard-covered coverslips can be used only once; therefore, during a day of patch clamping, many coverslips will be used.

See Discussion.

METHOD

1. Transfer flies by flipping them (inverting their container) into an empty vial. Anesthetize flies by placing the vial on ice for a few minutes until the flies stop moving.
2. Record the time when you start the dissection.

   *The activity of LNvs depends both on the time of day and on the time after dissection, as discussed in Introduction: **Patch-Clamping Fly Brain Neurons** (Fernandez-Chiappe and Muraro 2022a); therefore, recording the time at the start of the dissection is crucial for interpreting results later.*
3. Take a fly and hold it down ventral side up using a pair of fine forceps. Use another pair of fine forceps to manipulate an insect pin through the fly's thorax (Fig. 1A), immobilizing it on a

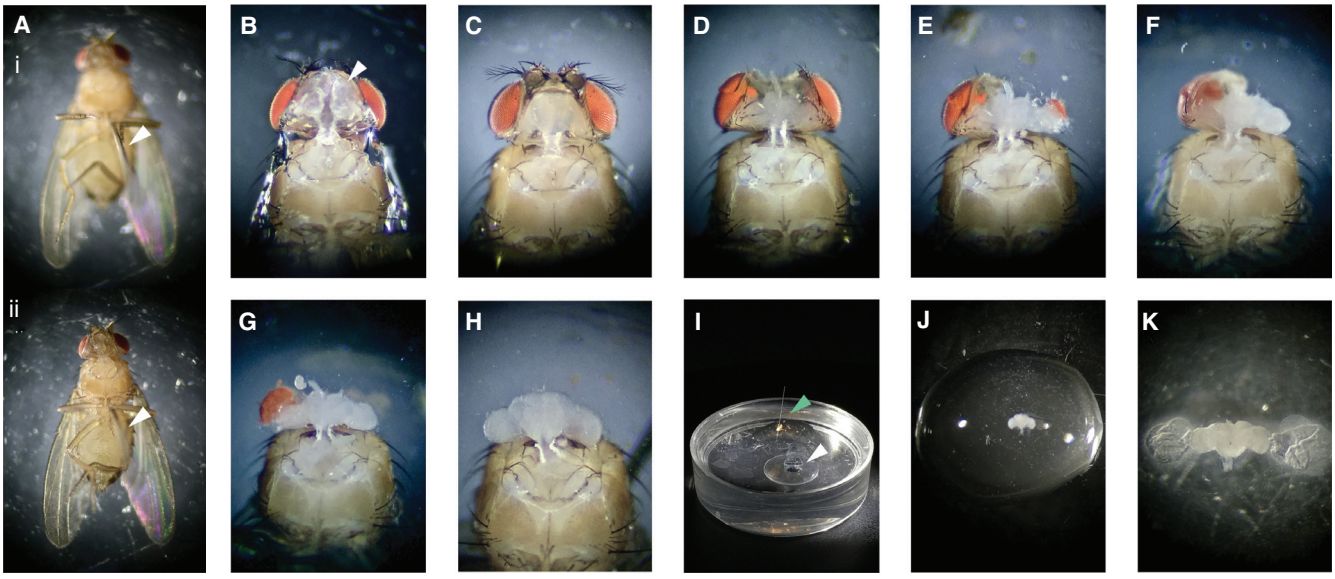

FIGURE 1. Step-by-step preparation of an adult brain for whole-cell patch-clamp electrophysiology. (*A*) Immobilized fly with an insect pin (white arrowhead) inserted through the thorax placed in a Sylgard-coated plastic dish. (i) Focused on the pin. (ii) Focused on the fly. (*B*) Fly head after removal of the proboscis; air sacs are exposed (white arrowhead). (*C*) Fly head with air sacs removed. (*D*) Initial stages of peeling the head's cuticle; the top part of the cuticle is removed. (*E*) Process of left eye removal (note that part of the retina is still attached to the brain). (*F*) The left eye is completely removed. (*G*) Process of right eye removal. (*H*) The brain is completely exposed and still attached to the body. (*I*) Sylgard-covered coverslip with a drop of adult external saline (white arrowhead) placed on the Sylgard-coated plastic dish where the dissection was performed; the preparation shown in *H* is nearby on the dish (green arrowhead). (*J*) The brain is removed from the body and transferred into the drop of adult external saline shown in *I* (white arrowhead). (*K*) The brain is glued to the Sylgard-coated coverslip using two drops of cyanoacrylate glue (one on each optic lobe).

Sylgard-coated plastic dish. Insert the pin at an angle such that it does not interfere with the head area; do not insert it perpendicular to the dish.

4. Pour adult external saline using a Pasteur pipette around the pinned fly to continue the dissection in an aqueous environment.
5. Pull out the two forelegs of the fly with the forceps.
6. Remove the proboscis by pulling from its rim with one pair of forceps and holding its base with another forceps.
7. Remove air sacs by pulling them with the forceps through the hole left by the proboscis.

   Air sacs have a shiny appearance (Fig. 1B); when removed, the matte white tone brain will be apparent on the back (Fig. 1C).
8. Peel the head's cuticle to expose the brain using the two forceps.
   i. Remove the top (anterior in the fly) part of the cuticle (Fig. 1D).
   ii. Remove one eye by tearing gently all around it (Fig. 1E).
   iii. Continue peeling the head cuticle.

   The peeling of the head's cuticle is very personal, and, with time and practice, you may find a slightly different sequence of movements that you find more appropriate to achieve this. Explore the way that is easier for you; as long as the differences between Figure 1B and Figure 1H are brought about, it will be fine. One way is to peel the head cuticle, advancing from one side to the other (Fig. 1F,G). Another way is to first remove one eye and then the other, the top part of the cuticle (anterior in the fly), the back (dorsal in the fly), and finally the bottom part of the cuticle. Be careful when removing the bottom part of the head's cuticle, as the neck connective should not be damaged as it is needed intact for the cleaning step (Step 9) (tear this part by pulling toward the sides).

   At this point, you should have a very scary fly with a brain instead of a head (Fig. 1H).

   See Troubleshooting.
9. Pull additional air sacs and large trachea attached to the surface of the brain with forceps until the brain appears clean.

   The brain has a pastel/matte white tone, and the air sacs and trachea have a shiny white appearance.
10. Add a drop of adult external saline to a Sylgard-covered coverslip, and transfer the brain (handling from the neck connective) onto it (Fig. 1I). Place the brain on its posterior side in the middle of the saline drop.

    The tissue will naturally attach to the fresh Sylgard (Fig. 1J).

    Cyanoacrylate glue will harden as soon as it makes contact with the solution. To avoid clogging the pipette tip, apply some positive pressure (gently) as you insert the pipette into the saline.

    The fresh (unused in this context) Sylgard that coats the coverslips is mildly sticky; therefore, the brain will attach to it. However, this attachment to the Sylgard is quite loose; therefore, the brain can be repositioned if necessary. To do this, pull it off gently and replace it in the correct orientation on another part of the Sylgard-covered coverslip, as the area where it originally adhered will lose its stickiness.
11. Attach a blue tip to 30–40 cm of Tygon tubing to make a "gluer."
12. Prepare a glue container by cutting a lid from a 0.5-mL microcentrifuge tube (see Fig. 1D in Protocol: **Dissection of *Drosophila* Wandering Larval Brains for Patch-Clamping Neurons** [Fernandez-Chiappe and Muraro 2022b]) and adding cyanoacrylate glue to the lid.
13. Use cyanoacrylate glue to immobilize the brain.
    i. Place any electrode pipette at the end of the "gluer."
    ii. Break the tip by gently rubbing it on the bottom of a glue container, and apply negative pressure (from the blue tip of the gluer) to aspirate some cyanoacrylate glue into the tip (see Fig. 1E in Protocol: **Dissection of *Drosophila* Wandering Larval Brains for Patch-Clamping Neurons** [Fernandez-Chiappe and Muraro 2022b]).

iii. Use positive pressure to blow a small drop of glue onto each side of the brain, at the edge of the optic lobes (Fig. 1K).

See Troubleshooting.

Before the glue is completely set (be careful that it is no longer in its liquid form), it is possible to stretch the brain slightly to allow easier access to the accessory medulla, where the somas of LNvs are located. Do not overstretch the brain, as this will tear it open and disrupt neuronal circuits.

See Discussion.

14. Once finished with the dissection, orient the preparation under the dissecting microscope.

For preparation of adult flies, the dorsal part should be on top of the field of view with the optic lobes toward the sides (Fig. 1K).

15. Transfer the coverslip with the glued brain preparation to the electrophysiology setup, keeping in mind its orientation.

Proceed immediately to Protocol: ***Patch-Clamping*** **Drosophila** ***Brain Neurons*** *(Fernandez-Chiappe and Muraro 2022c).*

TROUBLESHOOTING

Problem (Step 8): The brain is damaged during dissection.

Solution: The dissection described in this protocol is not easy, and many brains will be damaged before the technique is learned. Even people who have experience dissecting adult brains for immunofluorescence techniques will probably need to practice for some time before they can do it correctly. This is because dissecting fixed tissue (normally performed for immunofluorescence techniques) is much easier than dissecting live tissue needed for electrophysiology. Fixed tissue is much harder, making it easier to manipulate than fresh tissue, which is softer and stickier. Moreover, small incisions made in fixed tissue may not render the brain unusable for immunofluorescence techniques if the damaged part is not close to where the neurons of interest are located. On the contrary, a brain dissected for electrophysiology should be completely undamaged. The only way to master this technique is to practice the dissection. With time and practice, damage to the brains will occur less frequently, but it will still happen, and, in this case, discard the brain and start a new dissection from the beginning.

Problem (Step 13.iii): The glue at the tip of the glue pipette hardens before it can be blown out.

Solution: If clogging still occurs, the pipette may be saved by breaking the tip a little more to remove the clogged portion. To do this, insert the pipette into the Sylgard down to the coverslip and rub the tip on the glass at the bottom to break the clogged tip. When pulling the pipette out of the Sylgard, the liquid glue will flow out of the pipette again, allowing you to glue the brain. Be careful with this technique, however, because if the tip is broken too much, all the glue will flow out at once, damaging the preparation by overgluing. If this happens, discard the brain and start a new dissection from the beginning.

DISCUSSION

Electrophysiological recordings require the tissue to be immobilized by some means. To achieve this, harps (tiny pieces of metal in the shape of a harp that hold down tissue such as a mouse brain slice) can be used, but these are not convenient for small fly tissues such as larval or adult brains. Pinning down the tissue is another means of immobilization, but this causes extra damage to the tissue. Also, pins may obstruct access when approaching the tissue with the electrode or the protease pipette.

Both of our dissection protocols (this one and Protocol: **Dissection of *Drosophila* Wandering Larval Brains for Patch-Clamping Neurons** [Fernandez-Chiappe and Muraro 2022b]) propose gluing down the tissue instead of holding it down by other means. This is a quick and easy way to immobilize the tissue, but an adequate substrate onto which the tissue can be effectively glued down is necessary. Sylgard is a silicone product that perfectly serves this purpose. Once prepared following the manufacturer's instructions (using the Sylgard 184 Silicone Elastomer Kit [Dow Corning]), Sylgard can be poured into small Petri dishes (plastic, 35-mm in diameter) to create a perfect arena for dissections; these can be washed and recycled over and over. Sylgard can also be poured onto small coverslips on which the desired tissue can be glued down.

Gluing brains to coverslips is a challenging part of this procedure. To master gluing down a brain, you must first master gluing. Take time to accustom yourself to how strongly you need to rub the tip of the pipette to cause a good amount of glue to flow in when you aspirate from your gluer (as shown in Fig. 1E in Protocol: **Dissection of *Drosophila* Wandering Larval Brains for Patch-Clamping Neurons** [Fernandez-Chiappe and Muraro 2022b]). Next, add a drop of adult external saline with no fly to a Sylgard-covered coverslip and place it under the dissection microscope and simply practice blowing glue by using positive pressure onto the Sylgard surface, aiming to produce small drops of glue in a row. If you feel adventurous, attempt to write your name in glue on the Sylgard surface. If you accomplish this, you are ready to glue some brains! Patch pipettes need to be freshly polished (see Steps 8–13 in Protocol: **Preparation of Pipettes and Pipette-Filling Devices for Patch-Clamping *Drosophila* Neurons** [Fernandez-Chiappe and Muraro 2022d]), so patch pipettes left unused from a previous patching session or the halves of pipettes left over when setting up the appropriate puller settings (see Steps 20–28 in Protocol: **Preparation of Pipettes and Pipette-Filling Devices for Patch-Clamping *Drosophila* Neurons** [Fernandez-Chiappe and Muraro 2022d]) can be used for gluing.

RECIPES

Adult External Saline

Reagent	Final concentration
NaCl	101 mM
KCl	3 mM
$CaCl_2 \cdot 2H_2O$	1 mM
$MgCl_2 \cdot 6H_2O$	4 mM
NaH_2PO_4	1.25 mM
$NaHCO_3$	20.7 mM
Glucose	5 mM

In advance of the experiment, prepare this solution as follows.

1. Combine the above reagents without calcium chloride or glucose.
   The salts are not soluble at 10× the concentrations shown; do not attempt to make a 10× solution.
2. Adjust pH to 7.2 with 10% HCl.
   This solution can be stored for up to many weeks at 4°C.
3. Before use, add calcium chloride and glucose.

Sharpened Forceps

MATERIALS

Equipment

Dissecting microscope
Forceps (fine; e.g., Dumont #55, Fine Science Tools 11255-20)
Sharpening stone (e.g., Dan's Black Arkansas)

METHOD

1. Set the sharpening stone under a dissecting microscope, and, with the pair of forceps pressed closed, start rubbing one side and then the other to sharpen the tip, with the aim of making the ends meet again. Alternate sharpening a little on one side followed by a little on the other side; also change the angle between the forceps and the sharpening stone.

   A drop of H_2O on the sharpening stone may help to achieve better sharpening.

2. After a few minutes (depending on the material of the forceps and the extent of damage, it may take less or more time), wet soft tissue paper and wipe the forceps' tip clean from the handle toward the tip to remove the shredded metal.
3. Observe the result of the sharpening under the dissecting microscope. Test the forceps by attempting to pick up a small item such a dissection pin.
4. Continue sharpening (Step 1) if the tip is not yet good enough.

Sylgard-Coated Coverslips and Petri Dishes

MATERIALS

Equipment

Coverslips (small, square, 18 × 18-mm or round, 10-mm in diameter)
Falcon tubes (50-mL)
Incubator at ~50°C
Petri dishes (plastic, 35-mm and 100-mm in diameter)
Sylgard 184 Silicone Elastomer Kit (Dow Corning)
Syringes (1-mL)

METHOD

Sylgard-Coated Coverslips

1. Spread coverslips in many large Petri dishes (diameter of 100 mm or more) so that they are not touching each other, but the whole dish is covered.
2. Prepare the Sylgard following the manufacturer's instructions (basically by mixing the two components at a 10:1 ratio in a 50-mL Falcon tube) and mix well.
3. Fill a 1-mL syringe with the Sylgard mixture. Release a single drop on top of each coverslip.

   One drop is enough; do not add more or it will go over the coverslips, joining them all together and creating an unusable art installation.

4. Place Petri dishes flat in an incubator for a few hours or overnight at ~50°C until the Sylgard is hard.

   Sylgard will remain liquid if stored at –20°C for some time (months); keep leftover material at –20°C for future preparation of Sylgard-covered coverslips.

Sylgard-Coated Petri Dishes

5. Prepare the Sylgard following the manufacturer's instructions (basically by mixing the two components at a 10:1 ratio in a 50-mL Falcon tube) and mix well.
6. Pour Sylgard into a 35-mm Petri dish to cover its surface completely with a thick layer (approximately half its height).
7. Place the Petri dish flat in an incubator overnight at ~50°C until the Sylgard is hard.

REFERENCES

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