

Larval brain dissection
Adapted from K. Ito's "Dissection of Drosophila CNSs", Per M. Sniffen, 2005

Tools:

Two fine-tipped forceps

Two thicker-tipped forceps

Dissection Plate

Glass wells, one filled with 95% EtOH

PBT, PBS, or Ringer's solution

Dissecting microscope (with gooseneck fiber-optic lights positioned at a very low angle for best contrast)

A note about forceps: It is critical to have a good set of forceps for dissecting. Be careful with your forceps. They are not cheap.

About handling CNS tissues: The CNS is flexible but it takes some time to know how much force it can withstand without tearing apart. You can clean off extraneous bits of tissue once the CNS is floating free.

Dissection of wholemount 3rd instar larval brains

1. Collect 3rd instar larvae that are crawling out of the food.
2. In a glass well, soak larvae in cold 95% ethanol over ice until the larvae stop moving.
3. Place a small puddle (about 100 μ l) of cold PBT onto the center of the dissection plate.
4. Place a larva into the droplet of PBT. Under the microscope, use thicker-tipped forceps to grab the base of the mouthparts [Fig. 1] and grab about one quarter down the body from the head with your other forceps. [Fig. 2]
5. Gently pull your forceps longitudinally in opposite directions. Ideally, the cuticle will break at the base of the mouthparts or neck, and the internal organs will spill out (i.e. the oesophagus, gut, brain, and salivary gland.) [Fig. 3]
6. Locate the CNS. Using fine-tipped forceps, remove other tissues. [Fig. 4]
 (Optionally, you can remove the other organs, but leave the mouthparts attached, so that those can be used as a "handle" to move the CNS around through fixation and washes. The mouthparts can be removed while mounting.

Post-dissection and mounting: Same as with adult CNS dissection.

