

# Dissection of *Drosophila* CNSs

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English Version 1999 June

## A: Tools

### I: Forceps

A pair of INOX No.5

Three types of material are available : Stainless, Taxal and Titanium (from Fontax Inc. Switzerland)

Among them Taxal is the best. Titanium, which is the most expensive, is somewhat too soft.

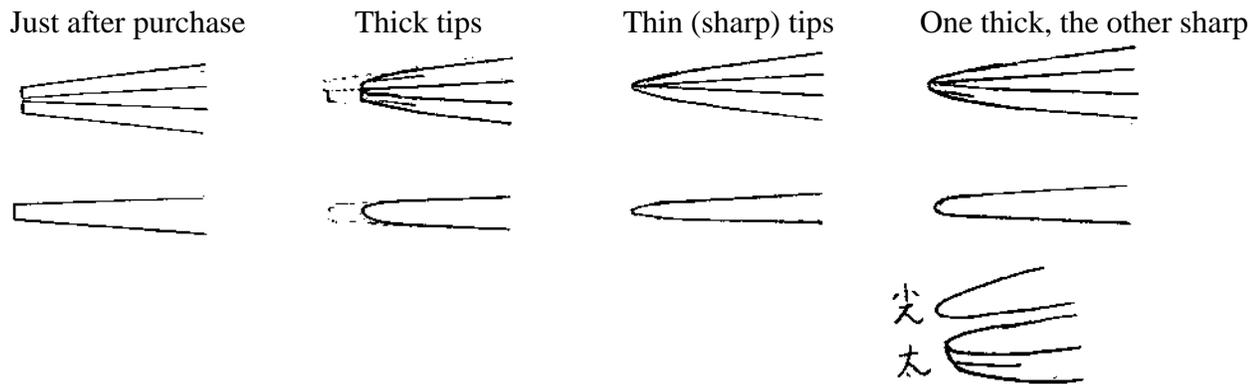
Sharpen the tip of the forceps:

Method A: sharpen with Arkansas stone (fine oil stone)

Can be obtained as the sharpener for drawing pen. A milk-white, translucent one is better than a black one.

Sharpen the forceps under a dissecting microscope.

Method B: sharpen with sandpaper. (use #1000 to shape the tip, #2000 for finishing.)



Thick-tip

to grasp larvae and pupae

Thin-tip type (2 pairs needed)

for CNS dissection

One thick, the other sharp

to peel the pupal cuticle without damaging the inside

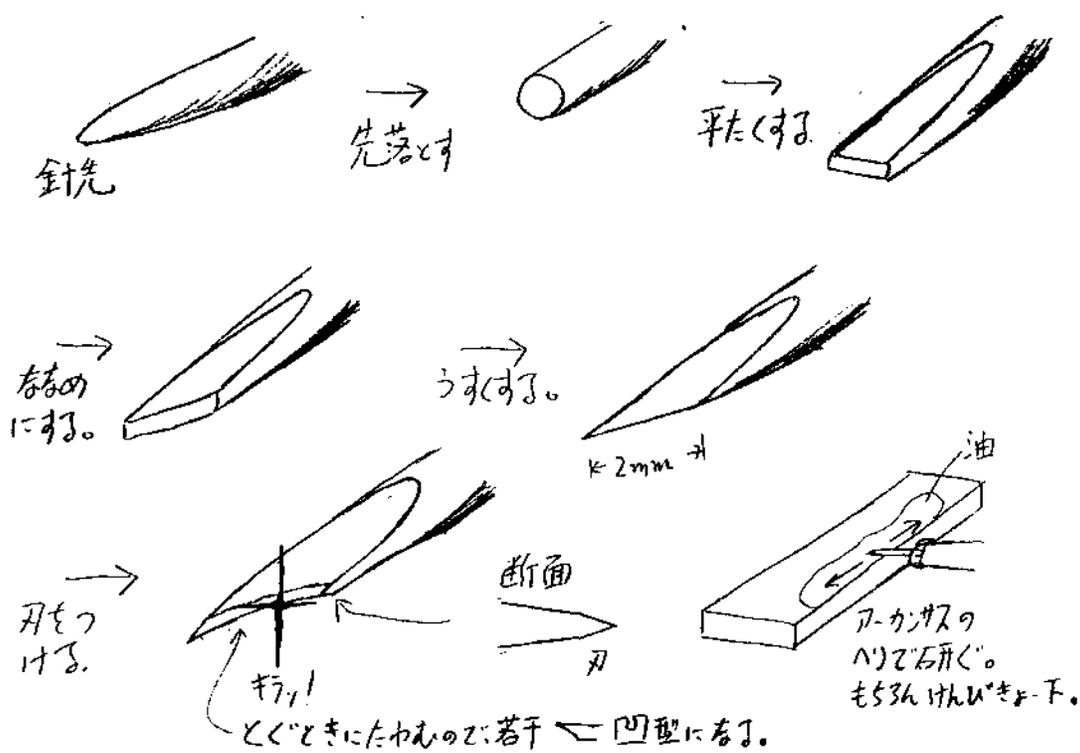
### II: microsscalpel (optional)

Sharpen the tip of a sewing needle

Recommended product : #A102.9, No. 20 needle made by Milward Needles Co. (United Kingdom)

A: Put the needle to a needle holder

B: Sharpen the tip under a dissecting microscope. First, keep the holder upright and cut the tip of the needle to make it blunt. Then, shape two sides of the needle to make it like a small knife.



## B: Dissection of the CNS

The way of dissecting the central nervous system (brain and the thoracic ganglia) depends on the developmental stages.

Dissection is performed in a small dish filled with PBS. Put sheets of tissue paper on both sides of the dish. Transfer the remnants of dissected bodies (legs and wings, thorax and abdomen, head capsule, etc.) to this paper and clean the forceps. Otherwise the PBS may become full of debris.

After Dissection, transfer the specimen to an Eppendorf tube filled with a small amount (ca. 200  $\mu$ l) of fixative with a 200- $\mu$ l pipette. Pool the specimen for up to 20 minutes. (i.e. 5 to 20 CNSs depending on how fast you can dissect.) in case of Formardehyde fixation, which should last for 50-70 min. (Pool for a shorter time for the fixation with shorter incubation period.) Each time you transfer the specimen, the fixative is diluted with the PBS you bring with the specimen. After pooling all the specimen, remove the diluted fixative and add enough amount of fresh fixative.

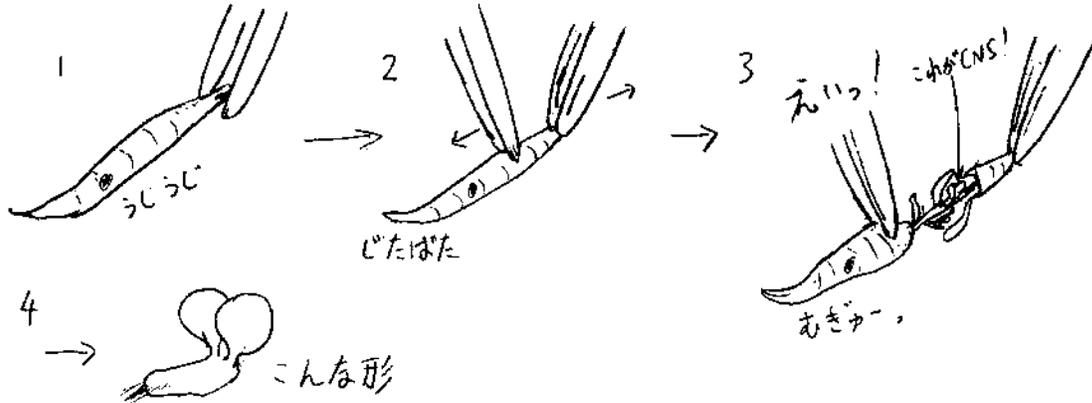
Speed is more important than perfection. After the head cuticle is removed, transfer the specimen to the fixative as soon as possible. Discs, tracheae and fat bodies can be removed *after* fixation. (Note: Air sacs of the adult brains are better removed, since the air inside the sacs may put the specimen afloat in the staining solution.)

## I: Dissecting larval CNSs

Essentially the same as the dissection of salivary gland.

Compare your forceps. One pair of them may have thicker tips than the others. Thicker tips are convenient for grasping the animal without damaging their body. Sharper ones are convenient for removing small tissues.

- 1: Grasp the mouth part of a larva with the thicker forceps.
- 2: Grasp the “neck” (about one fourth from the head) of the animal with the sharper forceps, and pull the body longitudinally.
- 3: Neck cuticle is torn apart. Internal organs, i.e. the oesophagus, gut, brain and salivary gland will spill out.
- 4: Locate the CNS. Remove other tissue.



## II: Dissecting early pupae (before pupation: 0-12 hours APF)

In diptans such as *Drosophila*, the cuticle of larvae remains after the final molt to become the puparium. The hardening of the larval cuticle is called “puparium formation”. After 10-15 hours, the pupal cuticle is formed beneath the larval cuticle (puparium). This is called “pupation” and corresponds to the pupation of other insect species. Then, the adult cuticle is formed beneath the pupal cuticle. Thus, a late pupa has three layers of cuticles. The eclosion occurs at around 95 - 105 hours after puparium formation (APF).

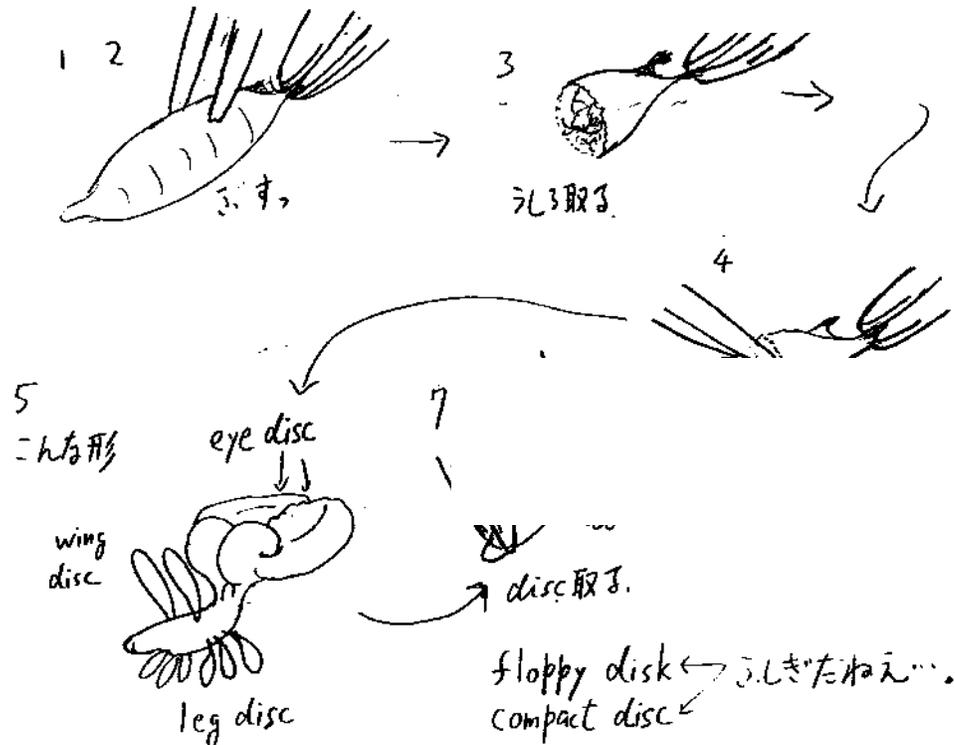
In early pupae, there is only one layer of cuticle. The pupal cuticle is not yet detached from the larval cuticle, which forms the puparium. Fat bodies become granulated and spill out when the body is dissected, making the handling a bit more cumbersome than for larvae. Also, the imaginal discs, which are attached to the CNS, become more prominent and more difficult to remove.

- 1: Grasp the head of a pupa with the thicker forceps. (If you use the sharper forceps here, the pupal cuticle of the head, rather than of the neck, will be torn apart at step 4.)
- 2: Stick a tip of the sharper forceps to the dorsal or lateral surface of the pupa at about one fourth from the head.
- 3: Move the sharper forceps to make a nick around the body wall. Cut apart the posterior body half (i.e. three fourths).

- 4: Put the forceps into the head cuticle (anterior one fourth) and remove internal organs from within it. Usually the brain is located near the anterior end of the head capsule.
- 5: Locate the brain. Remove oesophagus and tracheae.
- 6: (option) If needed, exchange the thicker forceps with the microscalpel.
- 7: The eye imaginal discs protrude from the brain hemisphere. Leg and wing discs protrude from the thoracic ganglia. Grasp these discs, cut the nerve with either the sharper forceps or the microscalpel to remove the CNS.

(Do not try to grasp the CNS when removing the discs. This way you would surely damage the specimen.)

\* Discs may be kept until after fixation/staining.



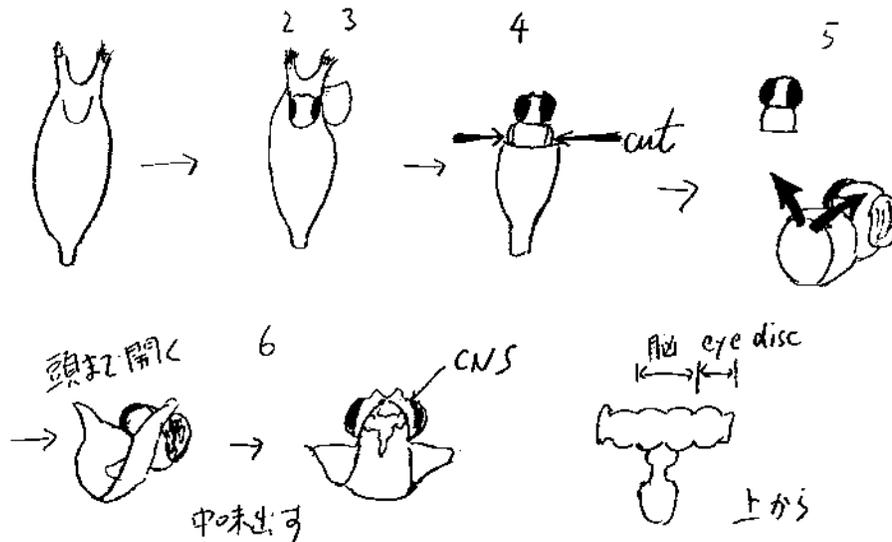
## II: Dissecting mid pupae (12-50 hours APF)

The pupal cuticle is already formed beneath the puparium. But the neck part of the body is not yet narrowed. The leg and wing discs are already everted to form the adult legs and wings. Only thin nerves connect them with the CNS. The retina of the eye is not yet tightly connected to the body wall, making it easy to separate the brain from the head cuticle.

- 1: Choose the sharper forceps.
- 2: Grasp the posterior part of the pupa. Open the opeculum (the lid of the puparium head) with the other pair of forceps.
- 3: Put the tip of the forceps into the space between the puparium (pupal case) and pupa. Remove the puparium up to the level of the thorax.
- 4: Grasp the dorsal area of the anterior thorax with both forceps. Put the body apart to cut at the level

of the thorax.

- 5: Open the dorsal thorax anteriorly towards the neck.
- 6: Push the anterior end of the head towards the bottom of the dish, and then squeeze the head capsule. The internal organs of the head, including the CNS, will spill out. Remove oesophagus and fat bodies carefully to expose the CNS. Pipetting will help to remove debris. Be careful not to damage the connecting fibres between the brain and the thoracic ganglia.
- 7: Grasp attached debris and discs, cut the region between them and the CNS. Then remove the CNS.
- 8: Cut the region between retinae and laminae.



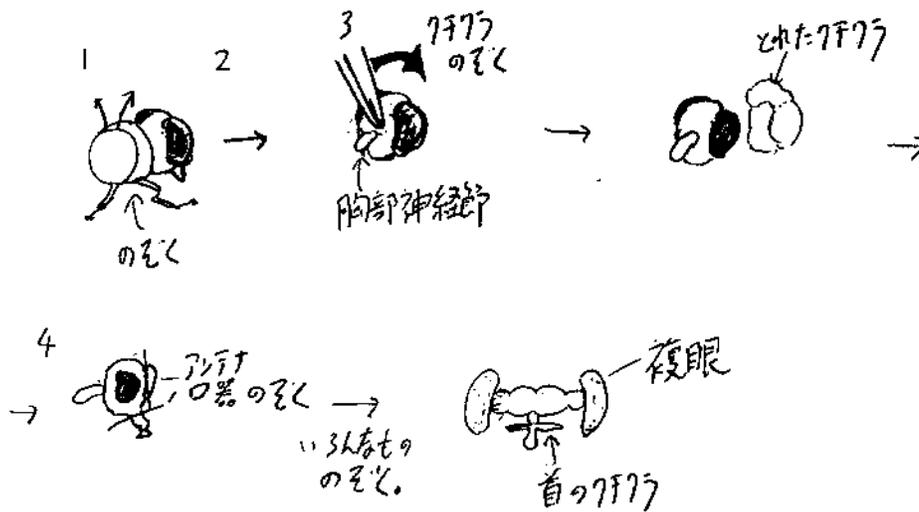
### III: Dissecting late pupae – adult (50 hours APF –)

The adult cuticle is formed beneath the pupal cuticle. The head part is clearly separated from the thorax. The neck, as well as the cervical connective that connects the brain and the thoracic ganglia, becomes very thin. This makes it difficult to dissect the whole CNS. In many cases, it is more efficient to dissect only the brain, or the thoracic ganglia, separately. The fully-developed compound eyes connect the brain with the head cuticle. Removal of the brain becomes more difficult.

Adult flies secrete wax from their body, which makes them hydrophobic. Before transferring the adult flies to the dish with PBS, soak them into alcohol for 30 sec. to 1 min. to wash out this wax.

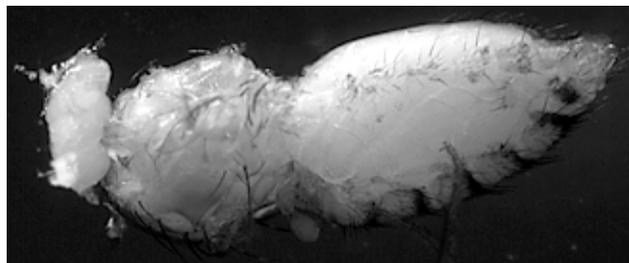
### IIIa: Dissecting the whole CNS

- 1: Remove the puparium case. Tear off the legs and wings with the forceps.
- 2: Turn the body upside down. Gently open the cuticle of the ventral thorax between the roots of the legs. (Like the way you take off your blouse.) Cut the side wall of the thorax and remove the muscles. The thoracic ganglia will be exposed.
- 3: Put the forceps into the region behind the dorsal posterior head near the neck (nape). Remove the transparent pupal cuticle like the way you take off your wig.
- 4: Remove the mouth part. Cut up the region between the antennae, forehead, and the top of the head. Gently separate the right and left halves of the head capsule. (Do not put too much stress to the brain, otherwise the optic lobes would be separated from the central brain.)
- 4': Alternatively, grasp the dorsalmost region of the posterior brain with both forceps and pull the cuticle towards left and right. Open up the nick from dorsal towards ventral (via forehead and antennae.)
- 5: Remove oesophagus, tracheae (air sacs) and fat bodies.
- 6: Remove the cuticle around the neck. Take extreme care not to damage the cervical connective.



### IIIb: Dissecting only the brain proper

If your purpose is to stain only the brain, it is sometimes a good idea to dissect only the brain, with the body attached intact. The body functions as a handle so that the small brain will not be lost when changing the staining and washing solution. The body alias handle is removed after staining. Legs and wings are better removed.



## C: Taking the pupa out of the puparium

- 1: Prepare black surface made of vinyl or plastic. (This is to make it easier to observe white larvae.)
- 2: Place a larva onto the black surface and paste with instant glue.
- 3: Open the lid of the head region of the puparium (operculum). The head will be exposed.
- 4: Put the thinner (sharper) tip of the forceps into the space between the puparium and pupa. Cut the side wall of the puparium towards the posterior. (The side wall is thinner than other puparium cuticle.) Cut the side wall a bit (one segment or two) and then open the area towards the other side of the puparium. The pupal body will gradually be exposed.
- 5: The head (dorsal part and side region) and the wing discs are pushed towards the puparium cuticle. Take extreme care not to damage the surface. For this purpose, place the thicker tip towards the pupal surface.
- 6: When all the body is exposed, put the forceps beneath the pupa. Since the pupa is wet, it will attach to the forceps. Never, ever, try to grasp the pupal body!

