

Mesencephalon Dissection
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See Neural Transplantation Methods (Dunnett and Bjorkland, Eds: IRL Press, 2000, pp. 11-14).

Gather supplies from tissue culture room, if needed:

Ice and ice bucket
1 and 10cc syringes
absorbent pads
petri dishes
HBSS

In procedure room:

- Add halothane to gauze in bottom of bell jar; cover with plexiglass.
- Turn on hood lights (never turn on UV light, it will destroy microscope optics).
- Turn hood outlet on.

- Put rat in bell jar (takes several minutes).
- Put out 2 absorbent pads in front of bell jar and one in front of microscope.
- On pad in front of bell jar, place large scissors, large toothed forceps and 3 petri dishes with HBSS (newly opened).
- On pad in front of scope will need black plastic (stored on shelf next to conical tubes), watch glass (rinse with HBSS), small dissecting scissors and small forceps (2nd smallest), plus 3 petri dishes with HBSS.
- Scope should be 1x, light from sides of watch glass.
- Put on sterile gloves.

- Remove rat from bell jar. Break neck by placing closed scissors at base of skull and pushing head forward while pulling in the opposite direction on the base of the tail.

- Squirt 70% ethanol on skin of abdomen, starting at tail and going towards chest, saturating fur in a back and forth motion.

- Grasp skin of abdomen with forceps, use large scissors sideways to cut a flap through skin and muscle wall. Make about a 3 inch cut, angling up towards ribcage on sides.

- Grab uterus with forceps, cut ligament at uterine horn (midline). Cut connective tissue away from uterus as necessary and at each end cut between end embryo and ovary. Put uterus in petri dish of HBSS.

Perform renal artery exsanguinations by cutting renal artery (near midline, along backbone); you will see blood pool immediately.

- Discard body by wrapping it in absorbent pad and putting it in a plastic bag. Add embryos after following steps. Carcasses are disposed of in walk-in freezer near cage cleaning.
- Pick up one end of uterus with forceps, use scissors to cut between end embryo and the next one in uterus. When separated, insert tip of scissors in end of uterus opposite of forceps, cut up through uterine wall; embryo will drop out through opening, still attached to placenta. Use scissor sideways to gently pull down on embryo until it separates and let fall into second dish of HBSS. Let fluid fall into 1st petri dish before embryo separates.
- Repeat with remaining embryos.
- At scope, take petri dish of embryos. Use small forceps to pinch off heads into third petri dish of HBSS.
- Add HBSS to watch glass using 10cc syringe. Use small forceps to put a head in each well. Remove skull, by preferred method. I use "face-off" method. With nose of embryo facing left, I grab chin with forceps. Use scissors to cup up the side of the jaw, stopping ventral to the brain. Make another cut horizontal towards nose, above eyes. Grab a pinch of skull and skin with forceps near where the two cuts met, insert tip of scissors underneath skull and skin and cut towards dorsal midline, pulling outward with scissor tip, and dorsal and over the top of the brain towards the other side. Peel away skin and skull rostrally and caudally; discard. Dissect mesencephalon (located in floor of mesencephalon, along the mesencephalon flexure). Make a cut through hindbrain just caudal to the tectum and cut just rostral to the thalamus (behind cerebral hemispheres). Make a cut along the dorsal midline. Trim away sides, and rostral caudal edges, leaving the mid-most ventral area (can see circular area) in a butterfly shape. Transfer mesencephalon with 1 cc syringe to chilled HBSS in 15 ml conical tube.