Mouse Utricle Dissection

If you are going to be setting up cultures, then before sacrificing the mouse you should have ice-cold DMEM/F12 without phenol red waiting in the hood.

1. Kill the mouse by placing it in the isoflurane-filled chamber after covering the chamber bottom with paper towels. This allows the mouse to die with the least amount of trauma because it will slowly become anesthetized, go unconscious, and then will die.

2. Once the mouse has stopped breathing, check for the absence of eye-blink reflexes, by gently touching near the eye, then on the eye. If there are any hints of remaining blink reflexes return the mouse to the isoflurane chamber and allow more time for over anesthesia to occur, before repeating the test for the absence of blink and toe pinch reflexes. Once the mouse exhibits no reflexes take the mouse out and hold it with the head uppermost as you spray the head and the neck down with 70% ethanol. Briefly (~5 to 10 seconds) holding the mouse in that orientation will allow some of the blood to move from the head down into the body, so that you'll have less interference from residual blood when you dissect the ear. Use stout scissors to cut off the head.

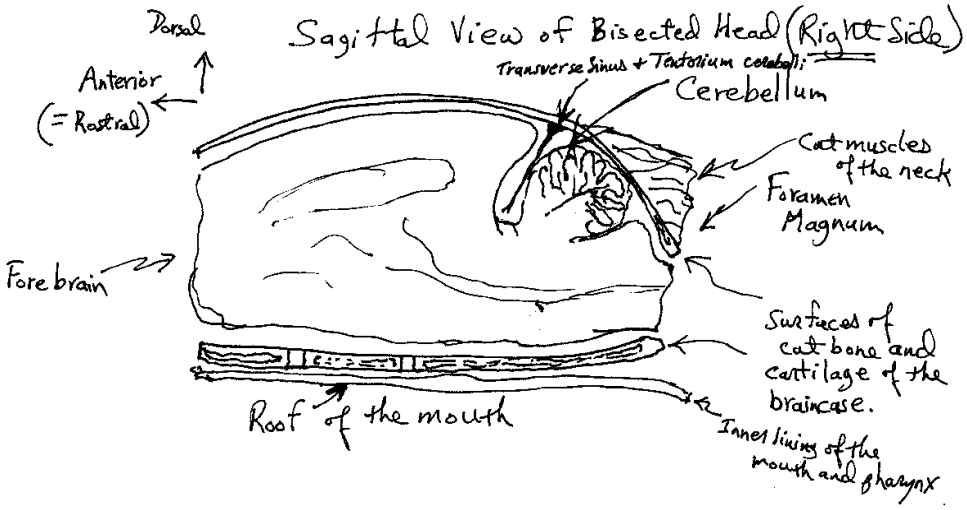
3. At this point the amount of dissection that is required depends on the age of the mouse. For P6 and younger pups it is not necessary to trim away the skin lower jaw or snout before placing the head in ethanol. For P7 and older mice it is advisable to cut away the lower jaw and cut through the ear canal on each side of the skull. Next, remove the skin from the head, by inverting it from posterior towards the snout. When you have the skin up to the level of the eyes, cut through the skull transversely just posterior to the eyes and discard the rostral tissues and skin. Trim away excess muscles and tissue from the neck.

4. Place the head in ice-cold 70% EtOH for 10 to 20 minutes in a container on crushed ice. As with nearly all solutions and washes we use in the lab, the volume of the solution should be at least 20 times the volume of the tissue you put into it. (No need to measure. Just try to add enough liquid to achieve at least that 20 to 1 ratio for the solution to tissue volumes.)

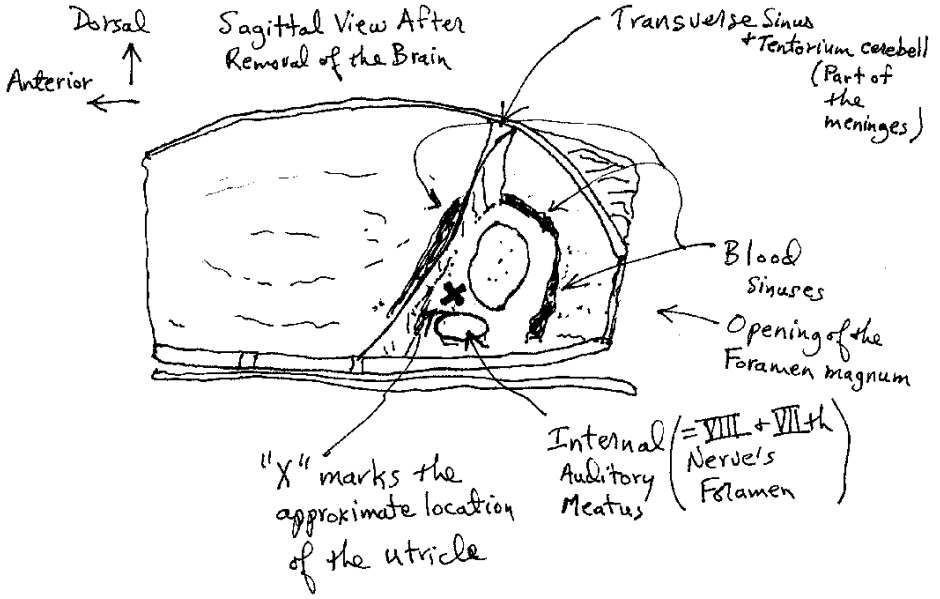
5. Repeat steps 1 through 4 for all the mice you would like to dissect at this sitting.

6. Bring the heads over to the hood and transfer them to a new 100 mm Petri dish filled with ice-cold DMEM/F12.

7. Next, taking special care so as to not put undue stress on the brain, bisect the head with two sets of cuts. The first set of cuts should begin at the foramen magnum and go straight down the middle of the top of the skull, ending at the nose. Use multiple cuts and movements of the scissors so as to avoid squishing the tissue. The second set of cuts should again start from the foramen magnum and go straight down the middle of the roof of the mouth and into the nose.



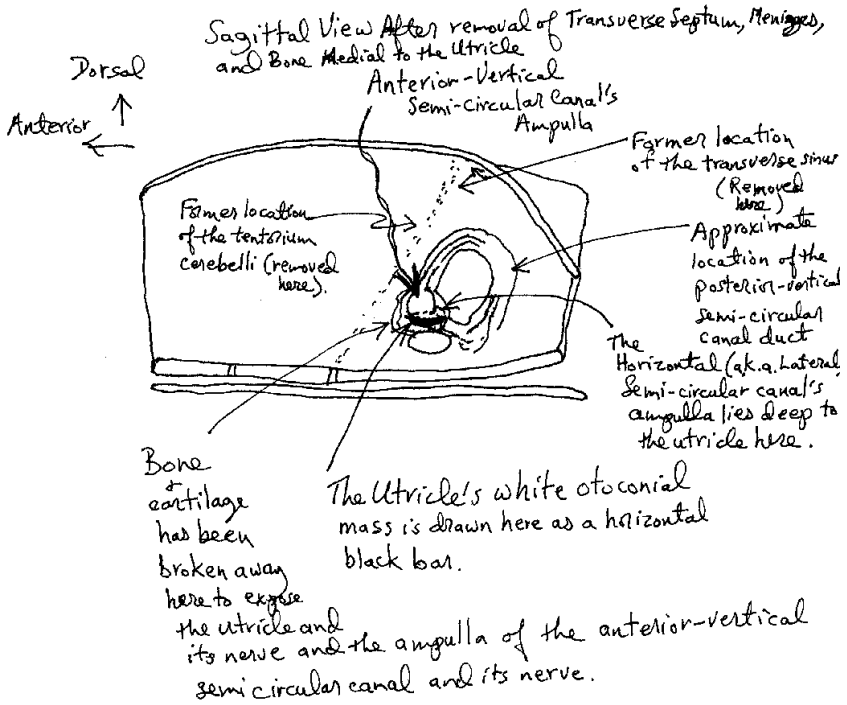
8. From each skull half, scoop out the brain and locate the “ear box.” Some people like to dissect out just the “ear box” at this point; others don’t. Whichever way feels comfortable to you is fine.



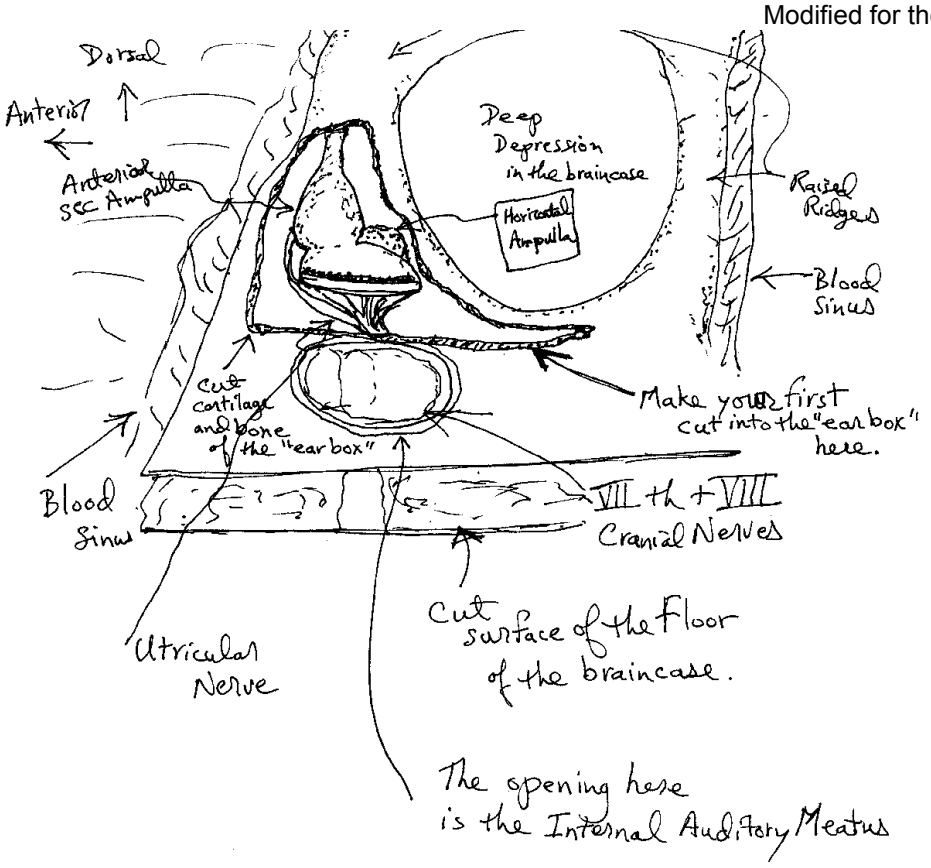
9. Place each “ear box” or cleaned braincase together with the rest in a new 60 mm dish sitting in crushed ice. The dish should be filled with ice-cold DMEM/F12 without phenol red.

10. Repeat Steps 7-9 until all the remaining “ear boxes” or braincases are dissected and placed in the storage dish sitting on crushed ice.

11. When all the “ear boxes” or braincases are out of all the pups, dissect the utricles from each. At this point it is important to remove the meninges that may still be adhering to the inner surface of the bone or cartilage. The meninges are thin membranes that line the braincase. Strong connective tissues in those membranes can interfere with getting clean breaks in the skeletal structure that surrounds the delicate inner ear tissue. It is easiest to begin to remove them by grasping and removing the tentorium cerebelli.

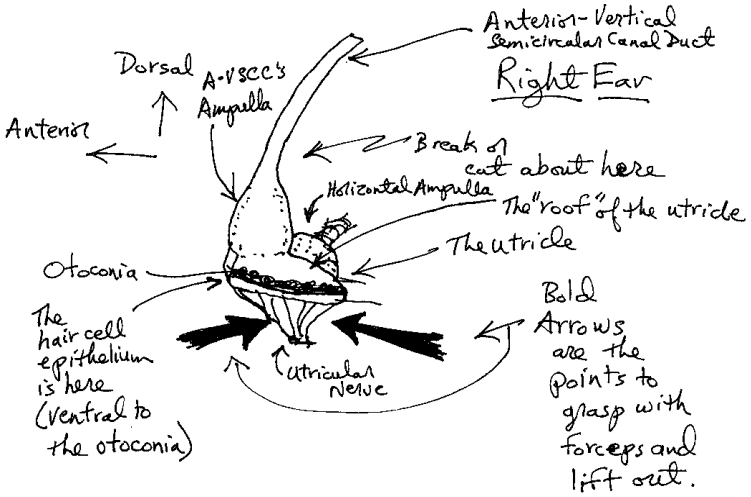


12. The easiest way to remove the utricle is to use specially sharpened #5 Dumont forceps or your “chisel forceps” to open up the cartilage at the medial side of the “ear box” above the point where the statoacoustic nerve passes through the internal auditory meatus in the wall of the braincase.



13. Take off the resulting flap of cartilage or bone thereby exposing the utricle, the saccule and the ampullae of the semicircular canals. The utricle is recognizable because it is white and lies at the convergence of the anterior vertical and the horizontal semicircular canals and is directly attached to the ampullae of those two canals. The utricle nerve is a small branch that comes off the same ramus of the statoacoustic nerve that connects to the anterior vertical and horizontal canal ampullae. The saccule is another white structure, but it is not near the point where the Anterior-Vertical semicircular canal and the Horizontal semicircular canals and their ampullae converge. The saccule is shaped a bit like an “S” and it is attached to the cochlea.

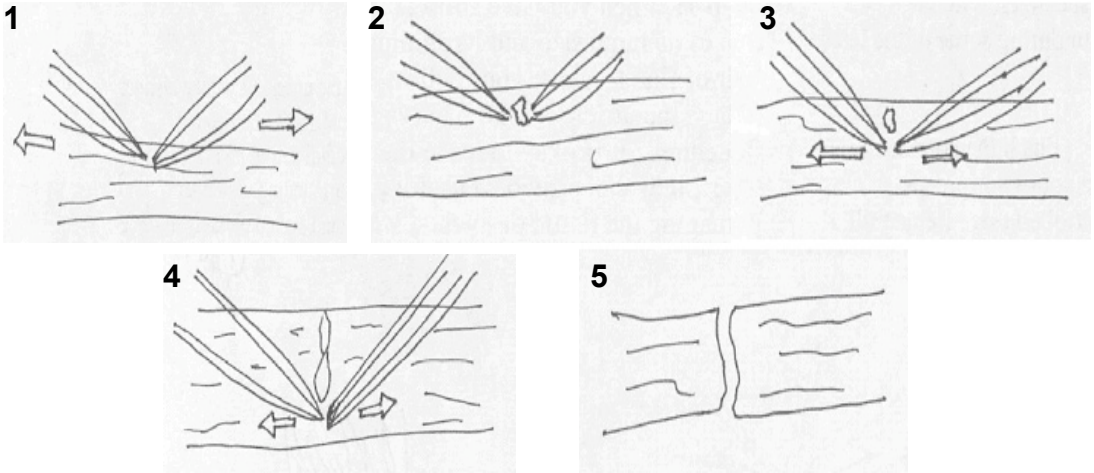
14. With sharp #5 Dumont forceps or with iridectomy scissors, gradually and carefully cut away the attachments of the utricle to the other parts of the inner ear, and lift the utricle out of the ear box. It is best to lift it from below, i.e., from ventral to its nerve. Many of us prefer to do that by grasping the utricular nerve in the forceps and lifting up. It is fine to have the Anterior-Vertical and Horizontal semicircular canal ampullae and parts of the canal ducts come out along with the utricle. From this point on, the utricle should never go through an air-water interface. It is quite delicate and should always be kept fully submerged in liquid. When transferring the utricle, use a 1.5 mm or 2 mm curette or a Moria stainless steel spoon to move it from one dish to another.



15. Transfer the utricles to a clean sterile black Sylgard dish containing ice-cold DMEM/F12 without phenol red.

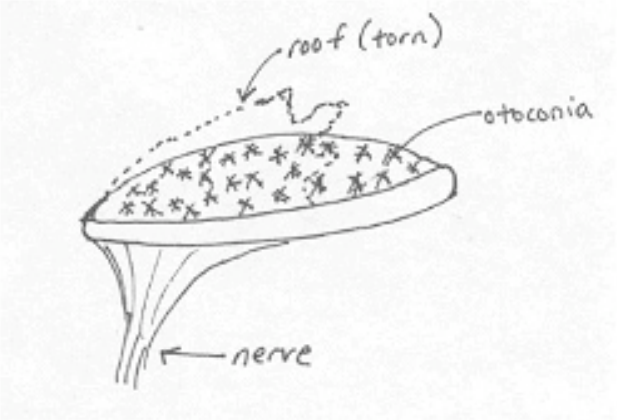
16. Carefully take off all of the roof membranes from each utricle so that only the floor of the utricle is left intact. Remove the otoconia and otolithic membrane using #5 forceps. If the utricle will be cultured in organ culture, then trim off the nerve at this point so that the utricle will lay flat when it is plated. If the utricle will be processed through thermolysin for harvesting of the sensory epithelium, then leave the nerve attached, since that will be the best holding place for grasping the utricle while you delaminate the epithelium.

17. To safely remove the ampullae and the roof from the utricle, use two sharp #5 forceps. Pinch the tissue at two places (very close to each other) with your two forceps simultaneously. Gently pull apart to create a small controlled tear. Move to a point near the end of the tear and repeat the process of creating a controlled tear. Continue repeating this process until the desired cut or opening has been made. Due to the delicate nature of the tissues, it is very important to take these precautions to minimize any stretching or strain on the utricle.



To clear otoconia from the utricle sensory epithelium, use your finest #5 forceps.

Picture of Utricle



18. To do this, use the same two forcep method described above to remove about half of the utricle's roof. Grab the utricle by either the remaining portion of the roof or by the remnants of the nerve, and using a dull pair of #5 forceps, begin to gently scrape away the otoconia from the apical surface of the utricle. Take great care in this step because it is very easy to puncture the underlying sensory epithelium! **Hint:** If you've cleaned most of the otoconia off, but a few spots remain, it is often helpful to grab a clump of the discarded otoconia and use it like a piece of tape to remove the remaining crystals. They tend to stick together.

19. You've now succeeded in dissecting out the utricle and should refer to other protocols for the specifics that follow from this point on.