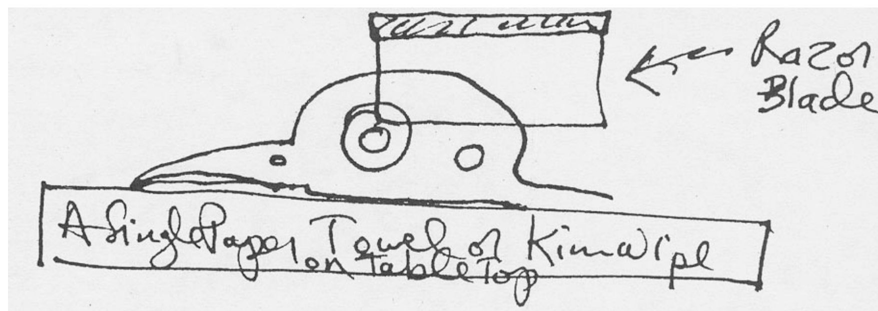
Chick Utricle Dissection Method

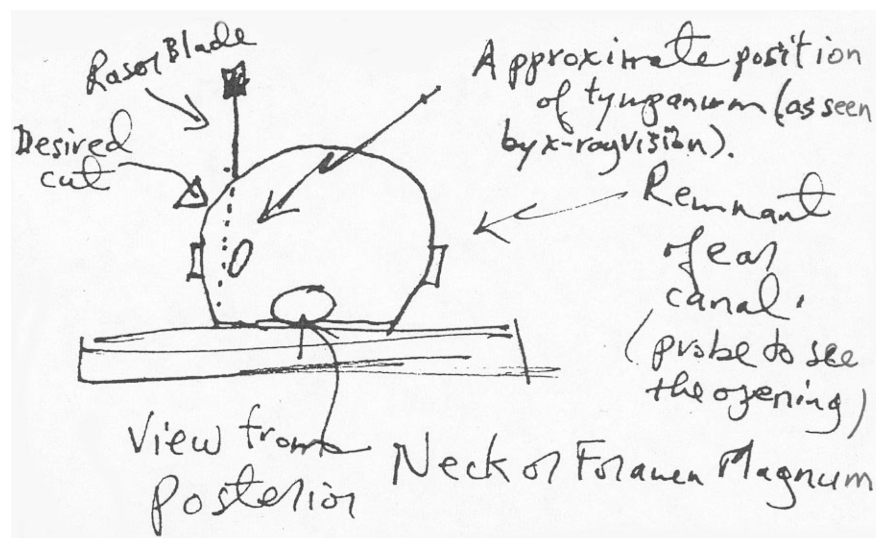
Step 1: Proceed after removing the head.

Step 2: Insert tips of scissors beneath skin along the side of the head so as to clip through the canal of the external ear on each side of the head. Pull skin off by everting from posterior to anterior. Remove lower jaw (this can best be done by pulling off with brute force (it's gross, but the best way)) or with scissors. (Okay, the worst is over).

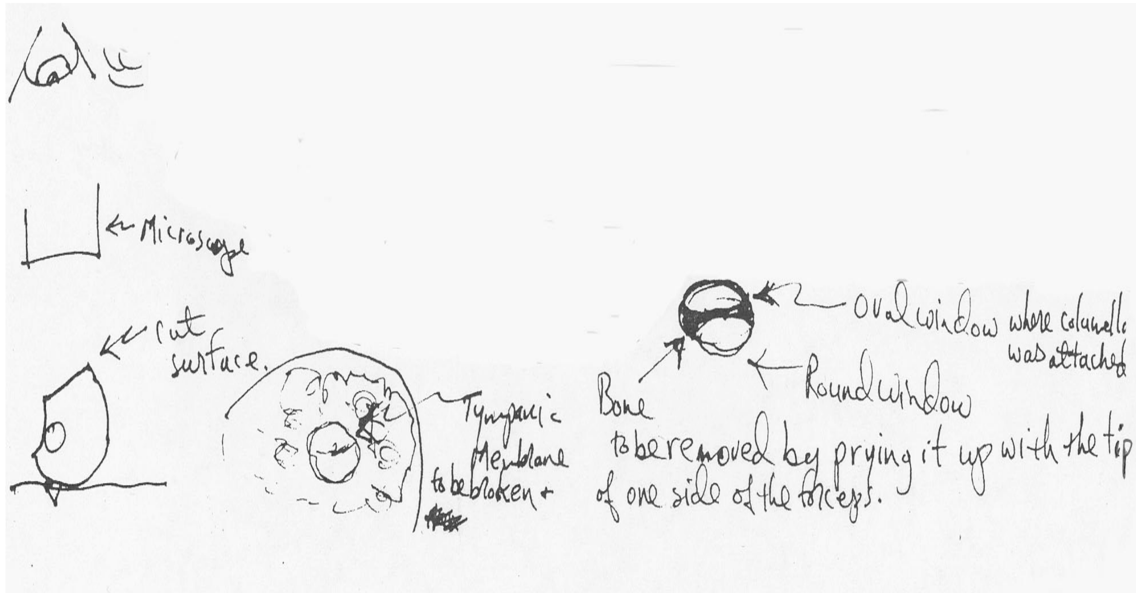
Step 3: Place heads in ice-cold 70% EtOH for at least 10 minutes but up to 20 minutes. Immersing the head in liquid that is chilled to 0 to 4° C avoids the chance of freezing and slows the metabolism of the cells in the vestibular epithelia to preserve their health. The immersion in ethanol also helps to kill bacteria, fungi, and other microbes that would be present on the surfaces of the head, the inside of the ear canal, and the pharynx.

Step 4: Place the head on a flat surface and use a single-edge razor blade to cut away the bone and tissues down to the tympanum as shown.

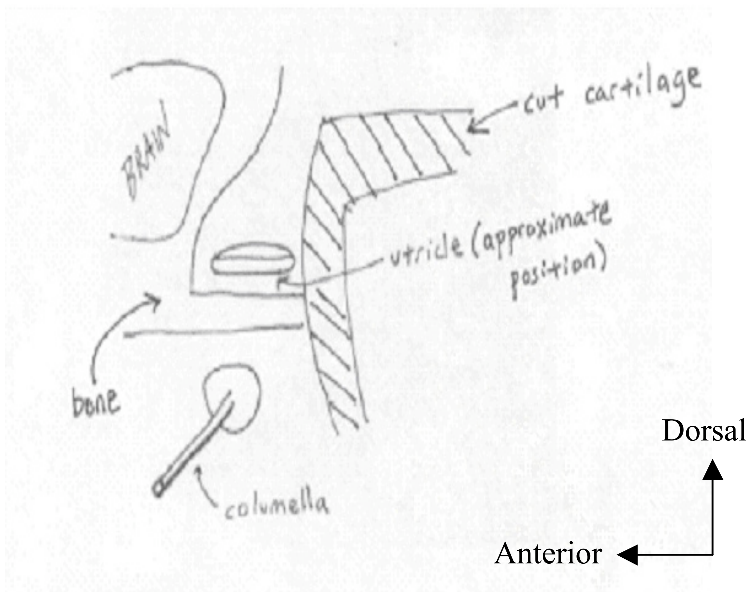




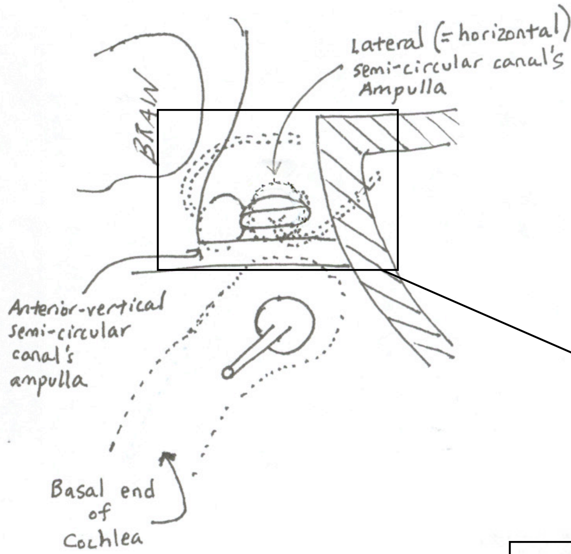
Step 5: Use a dissection microscope from here on. Using the beak as a "handle", tilt head to look at the cut surface.



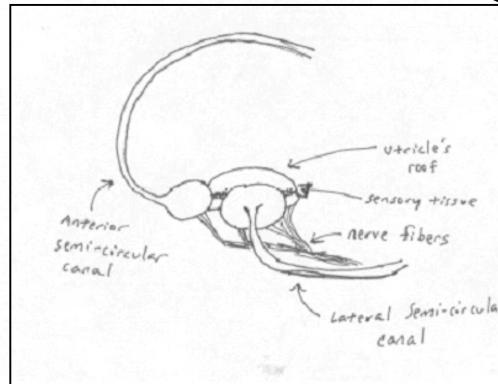
Step 6: After you have removed the tympanic membrane locate the columella (= single ossicle that is the stapes equivalent in birds). The utricle lies above this structure. You can approach it from various angles now that you have located it; you'll have to decide which is best for you. <u>Side View of Left Ear, Utricle Exposed</u>



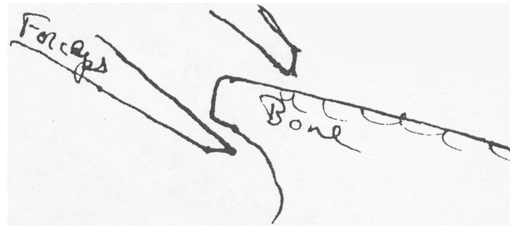
Side View of Left Ear, Utricle Covered



(The utricle is not visible at this point. It is under bone and medial to the lateral semi-circular canal's ampulla, but shown diagrammatically in its relative location.



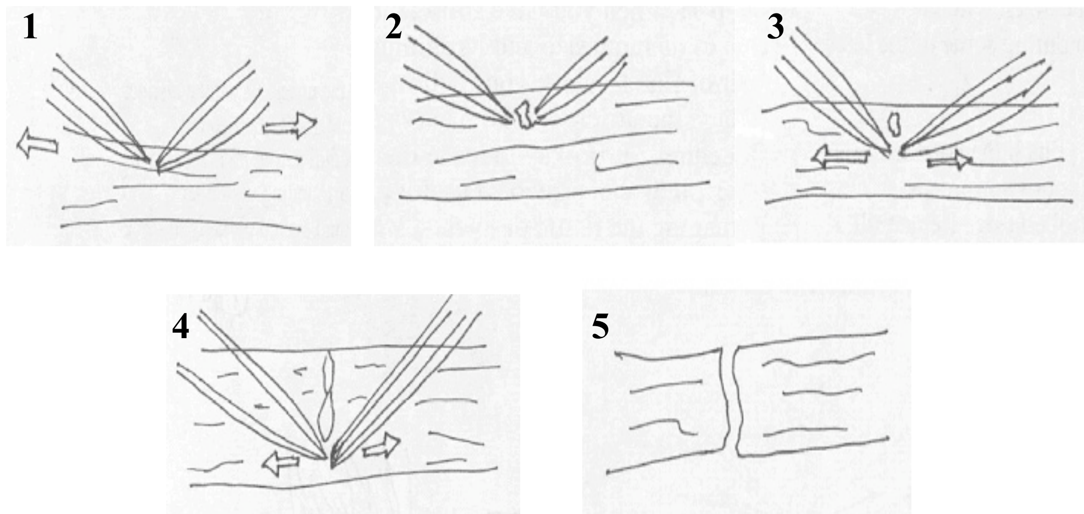
A common method is to gently remove the bone that lies on top of the utricle when viewed from this position. The best way seems to be inserting the tip of the "chisel forceps" under the edge of the bone *just slightly* then prying up that bone to get a small fragment to break free.



The trick is to try to break off only small bits of bone at a time and then progressively enlarge the opening.

Step 7: When you have sufficiently exposed the utricle (it will be distinct due to its large size and its shining white complement of otoconia), use a pair of fine forceps (your dullest #5 or even a dull pair of #55 forceps) to detach the utricle from the nerve tissue and surrounding vestibular structures. It is okay to leave them attached and even preferable in some cases (they can provide a useful place to grab the specimen without damaging the tissue of interest. This can be of particular use during some of the later and more delicate procedures).

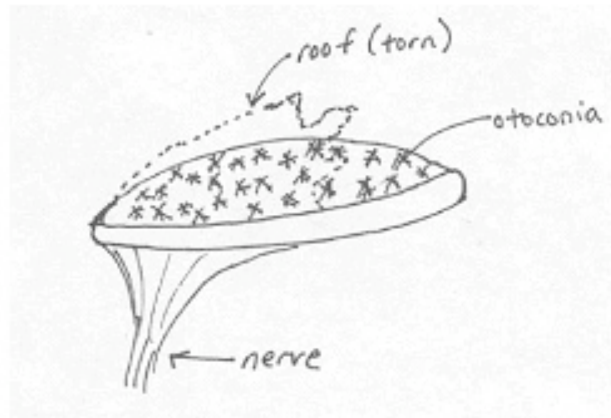
To safely remove these structures, use two forceps. Pinch the connecting tissue at two places (very close to each other) with your two forceps simultaneously. Gently pull apart to create a small controlled tear. Move to a point near the end of the tear and repeat the process of creating a controlled 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.



Step 8: Pull the utricle out of the cavity and immediately transfer it to a black Sylgard dish that you have already filled with ice-cold M199 containing Hank's salts.

Step 9: Clear otoconia and part of the utricular roof using #5 forceps.

Picture of Utricle



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.

Step 10: Using a Moria #35 stainless steel spoon, transfer the utricles to a black Sylgard dish containing room-temperature thermolysin solution. Incubate the pieces at 37°C and 5%CO_2 for 45 minutes.

Step 11: Transfer tissue into ice-cold M199 with ***Earle's salts*** and 10% FBS (notice the change in media from now on). Keep on ice for 10 minutes before proceeding.

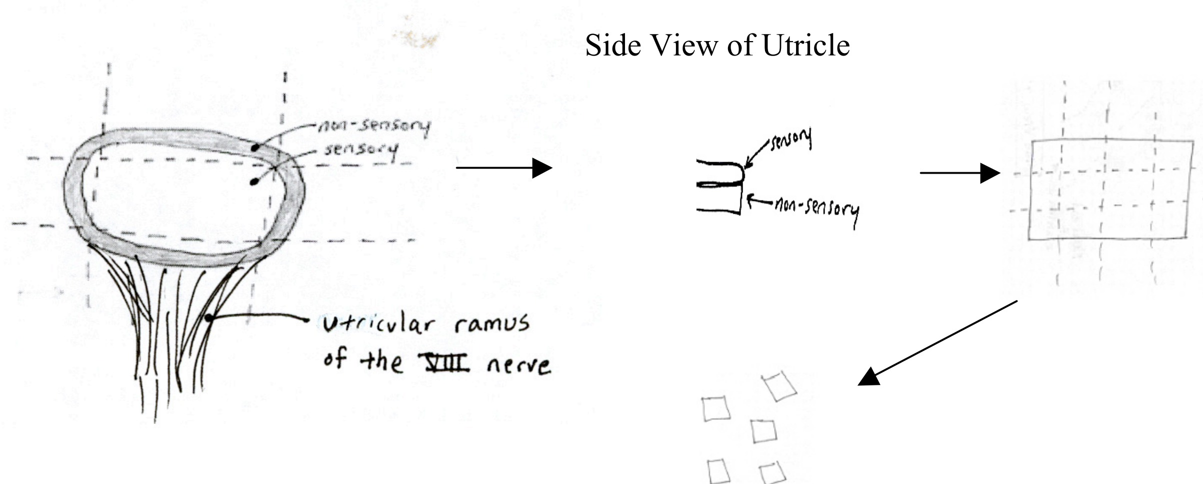
Step 12: To delaminate the utricle (i.e., to separate the sensory epithelium from the non-sensory epithelium), I do the following.

First, I use a sapphire knife to trim away the outside edges of the piece. This is important for two reasons: the first is that the periphery of the utricle is composed of non-sensory cells and we want to isolate only sensory epithelium, and secondly, cutting the edge makes it much easier to visualize the separation between the two layers of cells (i.e., the sensory epithelium vs. the underlying connective tissue stroma).

Next, peel or pry up the sensory epithelium using a sterile 30G ½-inch hypodermic needle attached to a 1ml syringe. Start from the nerve insertion side and take care to keep as much of the tissue intact as possible. This is the most difficult step in the procedure and requires the most patience and practice.

Once you have separated the sensory epithelia, remove all non-sensory debris from the working area. Using a fine pair of forceps to transport the debris from the working area to an unused area is usually sufficient. But if I am working with many utricles at one time or just want to clean up the area more thoroughly, I will aspirate the waste tissue using a Pipetman and discard it completely. Please don't hesitate to ask for help or guidance if you're having trouble with this.

Step 13: Once you have isolated pure sensory epithelium, cut it into 9-12 pieces using the sapphire knife (see diagram).



Again, clean the area and only keep the good (i.e. the right size and shape) pieces. Using a 100 µl Pipetman, transfer the pieces (4-5 per dish) into fibronectin coated poly-d-lysine MatTek dishes containing 120 µl M199 with Earle's salts and 10% FBS.

Step 14: Now that you have the pieces distributed amongst the dishes, you must orient the pieces bundle-side up. When illuminated from just the right angle, one side of the piece will appear to glow and shine due to light being refracted by the hair bundles. This is the side you want up. To be able to see this, you will have to play with the angle of the light source and the orientation of the tissue piece until you hit just the right combination.

Once you have determined the side you want up, flip the piece so that it is in the correct orientation and move on to the next piece.

Note: Do not touch the tissue too forcefully with your forceps during this step. The pieces are sticky and will adhere to your tools and are very difficult to recover. Instead, try to accomplish most of the work by generating little waves in the growth media.

Step 15: Place the MatTek dishes inside a 150 mm suspension culture dish. Include one small culture dish (but not a MatTek) without a cover and fill it with sterile distilled water (this is absolutely necessary to prevent the dishes from drying out during the 4-day incubation period). Place the dish in the incubator and allow it to rest undisturbed at 37°C and 5% CO_2 for 96 hours.