

Biology of the Inner Ear

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Laboratory Number 2 Dissection of the mammalian inner ear

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Laboratory Objectives

1. Observe and practice the dissection of the mammalian inner ear using neonatal mice as a model system
2. Observe and practice the dissection of the avian inner ear using neonatal and embryonic chickens as a model system
3. Observe a demonstration of dissection of the adult avian basilar papilla
4. Observe a demonstration of perfusion, fixation and dissection of the adult mammalian inner ear
5. Observe a demonstration of the paint-filling method for defining the three-dimensional morphology of the inner ear.
6. Review samples of paint-filled inner ears from embryonic chickens and mice

Dissection of the neonatal mouse inner ear (Matt Kelley)

The easiest way to gain access to the inner ear is from the dorsal or upper surface of the base of the skull. Following euthanasia, the animal should be decapitated and the skin and dorsal cranial bones should be removed to expose the brain.

Next, the brain should be removed to expose the dorsal surface of the base of the skull. Care should be taken to not damage the base of the skull. Once the brain has been removed, the ossifying basioccipital and posterior basisphenoid bones can be visualized. The bilateral petrous portion of the temporal bones, which contain the bony labyrinths, will be visible adjacent to the rostral portion of the basioccipital bone. The cochlear portion of the labyrinth is located directly adjacent to the basioccipital bone while the vestibular portion extends dorsally into the lateral wall of the skull. The sigmoid sinus outlines the rostral and caudal sides of the vestibular portion of the inner ear.

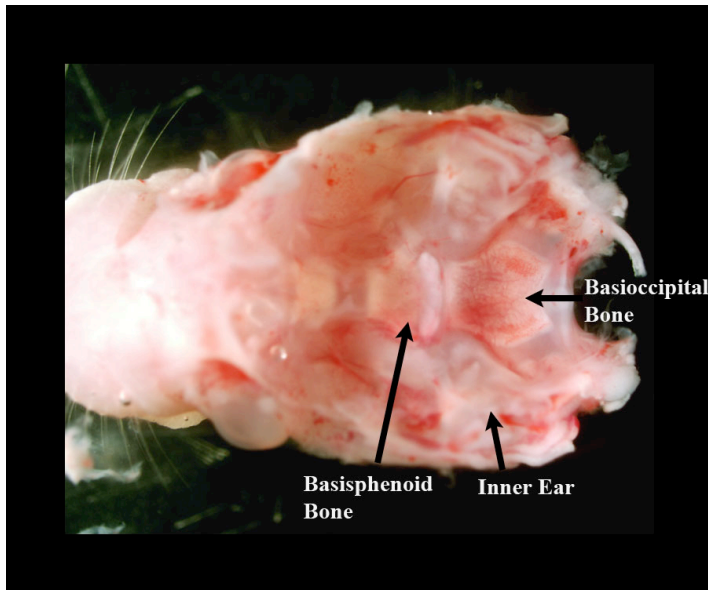


Figure 1.

To remove the bony labyrinth, start by bisecting the skull along the mid-line. Then use forceps to cut around the structure using the boundaries of the basioccipital bone and sigmoid sinus as guides.

Following isolation, the isolated labyrinth should be flipped over so that the lower (ventral) surface is facing upward (Figure 2). This orientation places the sensory structures of the inner ear distal to the area of dissection, decreasing the chances that they will be damaged during subsequent steps. All overlying tissue, including the surface skin and mesenchymal tissue should be removed to expose the tympanic ring. Once the tympanic ring is removed, the cochlear portion of the labyrinth will be visible. The middle ear bones are typically removed along with the tympanic ring. As a result, both the oval and round windows are usually exposed. At this point, depending on which tissues need to be preserved, the labyrinth can now be pinned down using minuten pins. The placement of the pins is dependent on the tissues to be dissected. For dissection the cochlea, saccule and utricle, the pins can be placed through the developing semicircular canals. It is a good idea to introduce the pins at an angle so that they will create a pincer effect that helps to keep the tissue in place. Once the labyrinth is pinned down, the oval window can be used as a guide to begin to remove the cartilaginous bony labyrinth in order to expose the membranous labyrinth. Carefully insert one tine of a pair of forceps into the oval window and slowly cut away small pieces of the bony labyrinth. Make sure that the membranous labyrinth is not adhered to the bony labyrinth before removal. Using this method, the cochlea, saccule, utricle and anterior and lateral cristae can be exposed. Finally, to expose the sensory epithelium, the luminal (endolymphatic) space must be opened. To do this, again use one tine of your forceps to open a small hole in the epithelium overlying the sensory structures. With the labyrinth pinned as described, this

epithelium will be facing up. In the cochlea, Reissner's membrane, the stria vascularis and basilar membrane form a triangular shaped tube that extends from base to apex. Starting at the base, carefully break Reissner's membrane at its medial boundary, and work upwards towards the apex. At the apex it will be necessary to dissect away the attachment at the future helicotrema. Once this is completed, return to the base and slowly break the connection between the stria vascularis and the lateral edge of the basilar membrane. Proceed towards the apex as before. It may be easier to separate Reissner's and then the stria for one turn at a time before moving upwards. Once this is completed, the organ of Corti will be exposed. To expose the saccule, continue the dissection from the base towards the saccule, which can easily be visualized by the presence of the otoconial membrane. See the addendum at the end of this document for some hopefully useful diagrams for this dissection.

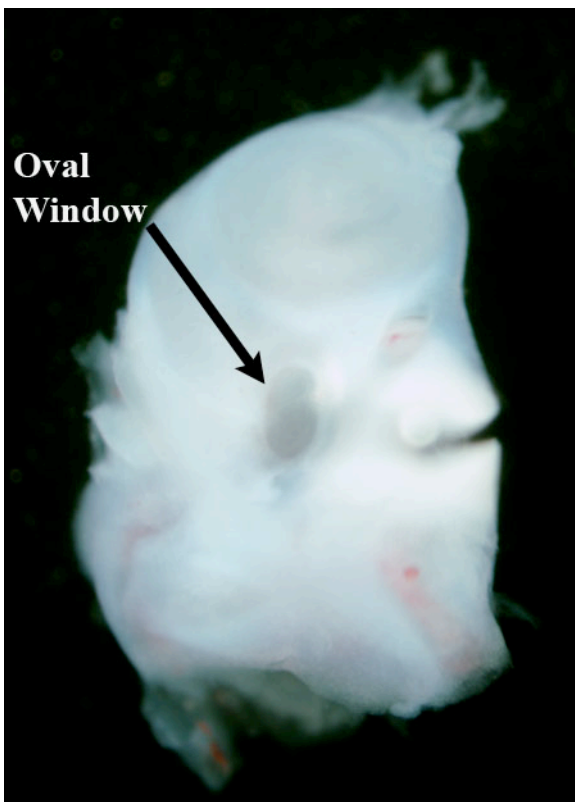


Figure 2.

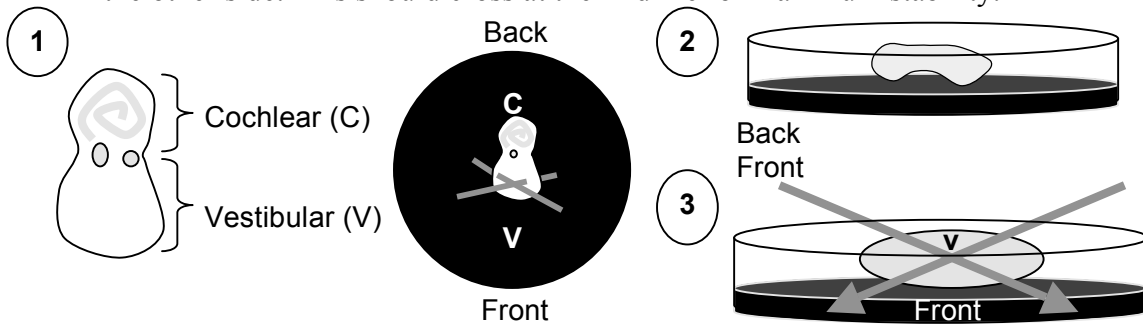
Addendum:

Cochlear dissection, diagrams (Bonnie Jacques, Kelley Lab)

This series of diagrams is intended to serve as a guide for the cochlear dissection.

Pinning:

1. Align temporal bone so that the cochlea is pointing towards the back of the dish and the vestibular portion is towards the front.
2. The convex side (ventral) of the temporal bone should point upwards with the concave (dorsal) side facing the bottom of the dissecting dish. A faint curving line should be visible beneath the surface of the cartilage; this is the developing cochlear duct.
3. Pins should be placed low and horizontal within the plane of the dish (rather than vertical) for maximum stability. Pin one side through to the sylgard before doing the other side. Pins should cross at the midline for maximum stability.

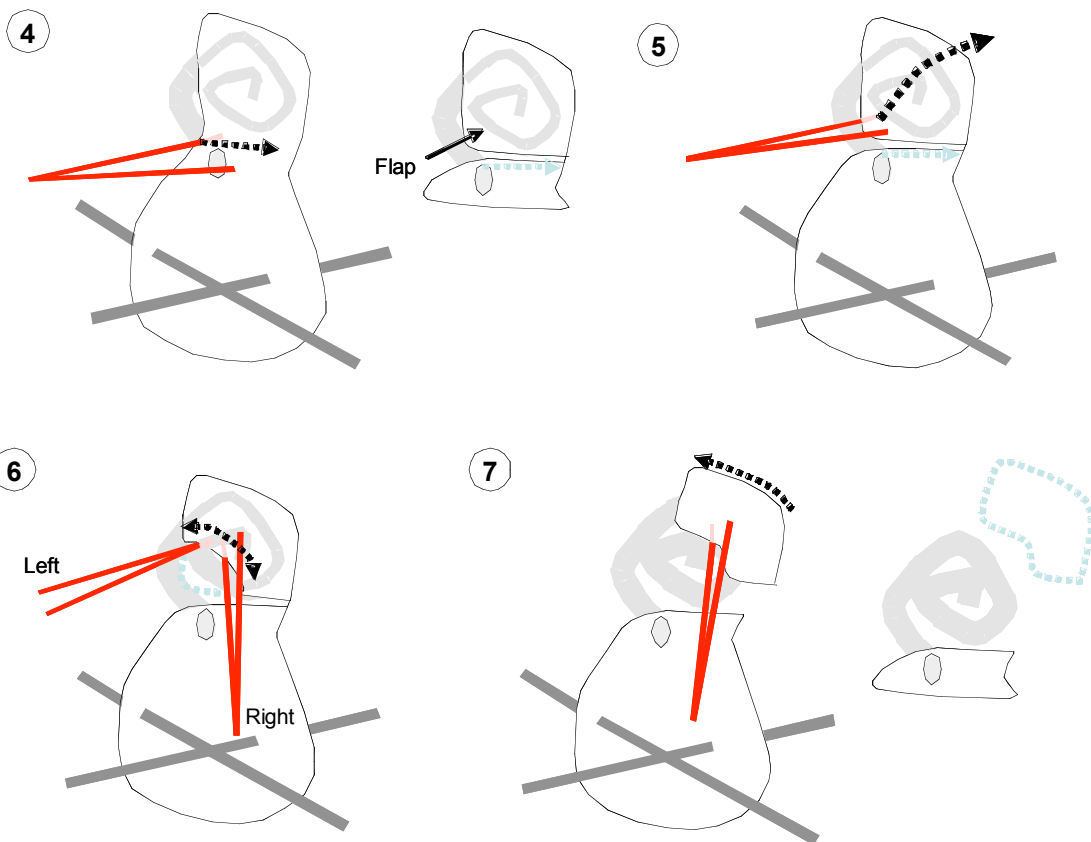


Exposing the Cochlear Duct (cont.):

4. Next, using the oval window as a guide, snip in a horizontal plane with one tine of the forceps just below the surface of the cartilage until you reach the opposite side of the cochlea.
5. Now grab the new flap with your right forceps and gently lift upwards.
6. Use your left forceps (keeping the tines pinched together) to carefully pry the apical regions of the cochlear duct from the cartilage using a gentle side-to-side scraping motion right beneath the lip of the cartilage.** Using this same motion, work your way across the cartilagenous “cap” until the apex comes loose.

** NOTE: During development, the cochlear duct begins to become more fused and incorporated into the turns of the bony/cartilagenous casing, so in older animals it may be necessary to use more scraping/prying to release the cochlear duct intact from the cartilage, whereas in younger tissue you may not need to do it at all.

7. Pull back further on the flap until it comes off; this may require some snipping at the top edge. At this point the entire cochlear duct should be exposed.



Exposing the Sensory Epithelium:

8. The next step is to remove Reisner's membrane which will open the cochlear duct and expose the sensory epithelium. Use your right forcep to pinch the very surface of the Reisner's membrane at the basal-most region then pull upwards following the turn of the cochlear duct. The Reisner's membrane should pull cleanly away.
9. If the entire membrane does not come off in one piece keep repeating the process pinching each time at the spot where it broke off during the previous attempt until you reach the apex.
10. Be careful when pulling at Reisner's membrane at the most apical piece as part of the sensory may become detached along with the membrane. It may be best to use some snipping motions in this area to separate the membrane from the cochlear duct.

