Microdissection and organotypic culture of neonatal mouse utricle

Material and solutions required:

- Dissecting tools
- Cell culture dish (35 and 60mm)
- MEM (with earle's salts and glutamax)- Invitrogen #41090-101 (supplemented with 10mM HEPES (pH 7.4), 5mg/100ml Ampicillin and 1mg/100ml Ciprofloxacin, warmed to room temperature.
- FBS (Invitrogen)
- Protease XXIV Sigma P8038 at ~1mg/10ml

Optional:

Glass fibers chambers (Poly-L-Lysin or Cell tack may be used instead of fibers)

- Glass coverslips Fisher 18mm
- Glass pipettes for chamber: VWR Calibrated pipettes 100ul #53532-921
- Puller
- Hot plate
- Sylgard kit Corning

Fine eyelash or equivalent (fine fur from your favorite pet works but remember to acknowledge them when publishing!)

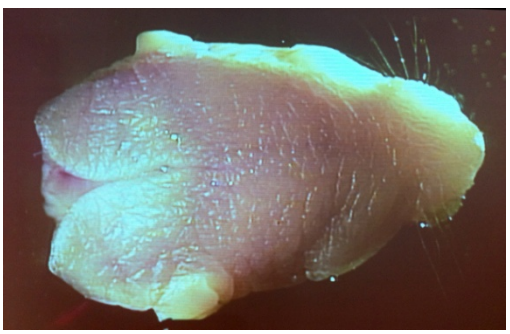
Microdissection of the neonatal P0-P6 mouse utricle:

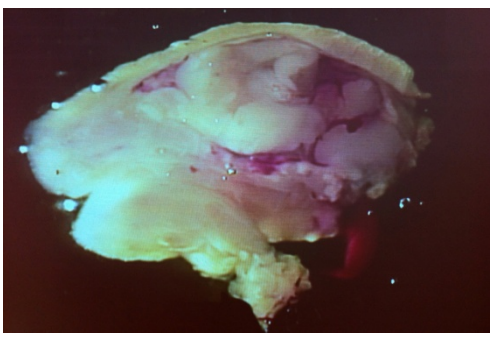
1-Temporal bones preparation:

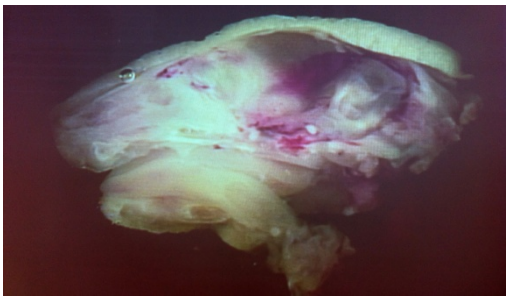
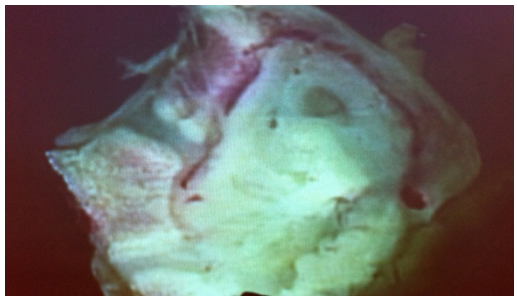
Neonatal mice P0-P6 are euthanized rapidly by decapitation below the ear bones. The head is then bisected along two cuts, one from the foramen magnum towards the top of the skull up to the nose and the other one from the foramen magnum to the middle of the mouth roof and up to the nose. Once the head is bisected, remove the nose from both sides and quickly place the preparations in fresh supplemented MEM.

Note: A key to preserving healthy tissues is to keep fresh media during the entire dissection. For this reason I do not hesitate to transfer to a new petri dish every time the media starts to appear cloudy or more pinkish.

The next part of the preparation is a cleanup phase with removal of the brain and extra bone and cartilage that may get in the way. Part of the skull is preserved to serve as an anchor during the dissection. At this point the temporal bones should appear clearly under the dissecting scope.

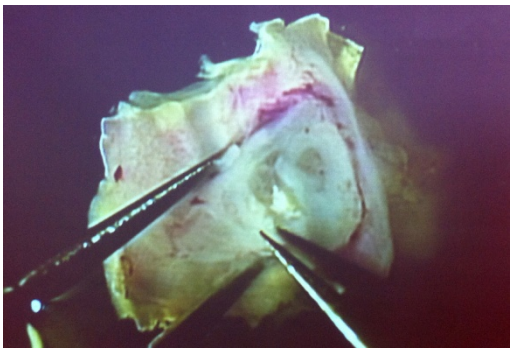
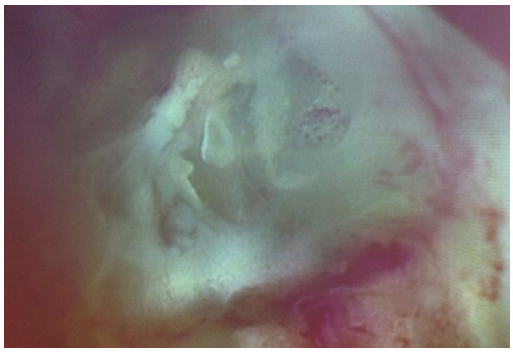




2- Preparation of the tissue for enzyme treatment:

The utricle is just beneath the bony medial surface, therefore caution should be used when opening the bony labyrinth to expose the utricle. In older animals the bone is more calcified and harder. In young mice I use forceps to pop up a portion of the bone. When working on older mice (P10 or more), I might use a small scalpel and gently scrape away the bone layers. When working on pigmented mice the location of the utricle is very obvious. Not so much in Albino mice. Nevertheless, once the bones and cartilages are removed the presence of a bright white otoconial mass should be obvious.

To allow solutions to access the epithelium, the upper part of the membranous labyrinth is cut open on one side of the utricle. I use fine forceps to expose the entire surface while preserving the contacts on each end so the sensory epithelium stays in place in the temporal bone.

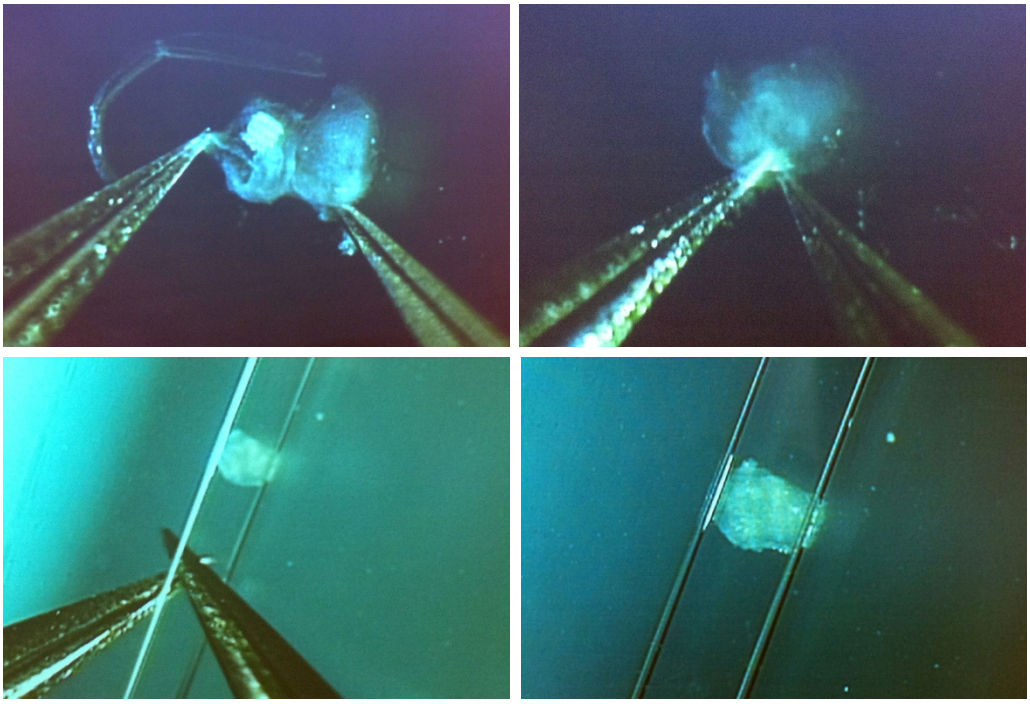
The temporal bone serves as a convenient scoop to bring the tissue to the enzymatic bath and after that back in the rinse solution. This will preserve the stereocilia as the epithelium will not be handled directly and will not pass through a meniscus.

The temporal bones are then bathed in MEM solution (Invitrogen, Carlsbad, CA) containing 0.1 mg/ml protease XXIV (Sigma, St. Louis, MO) for 15 to 20 minutes. The bones are placed now faced down so the otoconia (also called otholiths) may fall down naturally during the enzymatic treatment. The right concentration of enzyme will ensure success. Too much enzymatic treatment will result in a sticky epithelium, too little may pose problem while removing the otolithic membrane.

3- Final steps: Removal and mounting

After the otolithic membrane has been removed (either during the enzymatic treatment or using a fine fiber or eye lash), the utricle is excised using fine forceps. Since you want to

avoid touching the apical surface of the epithelium, you may try to grap it by the nerve or by one side. Sometime, it's better to pull it out via the anterior ampulla. The extra tissues and nerves are then trimmed away. The older the prep, the thicker the nerve. I grab it from underneath with two forceps and slowly trim it away to obtain a thin sensory layer. The tissue is then mounted onto a round glass coverslip and held in a flat position by two glass fibers glued to the coverslip with a small drop of sylgard.



Organotypic cultures:
To produce organotypic cultures tissues are maintained at 37°C in MEM (Invitrogen) supplemented with 10 mM HEPES (pH 7.4), 5mg/100ml Ampicillin, 1mg/100ml Cipro, +/- 3% FBS. Media are replaced every 2 to 3 days.

FM 1-43 fluorescence:
The styryl dye FM 1-43, or the fixable analog, FM1-43FX (Molecular Probes, Eugene, OR), are applied at 5μM for 10 sec. The dye that partitions into the outer leaflet of the membrane is washed out during three full bath replacements. Cells are imaged 5 minutes after exposure to FM 1-43 using the Axioskop FS microscope (Zeiss) equipped with differential interference contrast optics, and FM 1-43 filter set (Chroma Technologies).