1-1-2016

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Recommended Citation
Montgomery, Scott C and Cox, Brandon C. "Whole Mount Dissection and Immunofluorescence of the Adult Mouse Cochlea.." *Journal of Visualized Experiments* , No. 107 (Jan 2016). doi:10.3791/53561.

Video Article

Whole Mount Dissection and Immunofluorescence of the Adult Mouse Cochlea

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URL: http://www.jove.com/video/53561
DOI: doi:10.3791/53561

Keywords: Neuroscience, Issue 107, organ of Corti, hair cells, supporting cells, cochlea, inner ear, temporal bone, surface preparation, immunohistochemistry, immunostaining, immunolabeling, Neuroscience

Date Published: 1/1/2016

Citation: Montgomery, S.C., Cox, B.C. Whole Mount Dissection and Immunofluorescence of the Adult Mouse Cochlea. *J. Vis. Exp.* (107), e53561, doi:10.3791/53561 (2016).

Abstract

The organ of Corti, housed in the cochlea of the inner ear, contains mechanosensory hair cells and surrounding supporting cells which are organized in a spiral shape and have a tonotopic gradient for sound detection. The mouse cochlea is approximately 6 mm long and often divided into three turns (apex, middle, and base) for analysis. To investigate cell loss, cell division, or mosaic gene expression, the whole mount or surface preparation of the cochlea is useful. This dissection method allows visualization of all cells within the organ of Corti when combined with immunostaining and confocal microscopy to image cells at different planes in the z-axis. Multiple optical cross-sections can also be obtained from these z-stack images. In addition, the whole mount dissection method can be used for scanning electron microscopy, although a different fixation method is needed. Here, we present a method to isolate the organ of Corti as three intact cochlear turns (apex, middle, and base). This method can be used for mice ranging from one week of age through adulthood and differs from the technique used for neonatal samples where calcification of the cochlea is incomplete. A slightly modified version can be used for dissection of the rat cochlea. We also demonstrate a procedure for immunostaining with fluorescently tagged antibodies.

Video Link

The video component of this article can be found at http://www.jove.com/video/53561/

Introduction

The spiral-shaped cochlea of the inner ear, contained within the temporal bone, houses the organ of Corti, the auditory sensory end organ in mammals. The cochlea is tonotopically organized and commonly divided into apical, middle, and basal turns corresponding to different frequency regions with high frequency sound detection in the base and low frequency detection in the apex[1]. Hair cells, the mechanosensory cells of the organ of Corti, run the length of the cochlea, which is approximately 6 mm long in mice[2,3]. These cells convert the mechanical energy of sound waves, which are transmitted through the fluid-filled membranous labyrinth, into neural signals that are processed by central auditory structures. The technique described here provides a method for preparing whole mounts of the organ of Corti after calcification of the cochlea is complete (for samples ranging from one week of age to adulthood). We also present a method for immunostaining the whole mounted cochlear tissue. Cochlear whole mounts are crucial for visualization of all hair cells and surrounding supporting cells in their natural spatial arrangements and allow for analysis in three dimensions with the use of confocal microscopy.

Drs. Hans Engstrom and Harlow Ades originally described a whole mount cochlear dissection method in 1966. They detailed a technique to rapidly fix and dissect calcified cochleae submerged in liquid from a variety of mammals, preserving short intact segments of the organ of Corti for microscopic analysis[4]. The dissection of an unfixed, calcified rat cochlea has also been illustrated in an instructional video[5]. Drs. Barbara Bohne and Gary Harding at Washington University made several important modifications to this method. In their version of the cochlear whole mount method, the temporal bone was decalcified, embedded in plastic, and five half-turns or ten quarter-turns were dissected[6,7]. Dr. Charles Liberman and colleagues at Eaton Peabody Laboratories, Massachusetts Eye and Ear Infirmary, modified this technique so that plastic embedding was not required[8]. Further modification of the technique occurred in Dr. Jian Zuo's lab at St. Jude Children's Research Hospital[9-12] which informed the dissection method presented here. We use a different strategy to gain access to the organ of Corti than Bohne and Liberman, which allows isolation of complete apical, middle, and basal turns. Thus the dissected tissue is larger and less likely to be lost or damaged during the dissection or immunostaining processes. In addition, the current method facilitates measurement of the distance from the apical tip or basal hook to identify a frequency region.

Although many labs perform immunostaining of cochlear tissue, it is unclear where this method originated. As a result there are various recipes for blocking buffers and antibody incubation buffers that may affect the performance of individual primary antibodies. Here, we present one method for immunostaining with fluorescently tagged antibodies that is applicable to most commonly used antibodies in the auditory field.

The complex shape of the cochlea, delicate structure of the organ of Corti, and bony encasement provide a challenge for histological and biochemical analysis. A variety of techniques are currently used in the hearing field to surmount these difficult features and visualize the cells within the organ of Corti, each technique with its own advantages and disadvantages. The protocol presented here allows for whole mount dissection of the adult mouse cochlea and, with slight modification, can potentially be used to examine the critical structures within the cochleae from a variety of other model organisms used in the field.

Protocol

Ethics Statement: Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee at Southern Illinois University School of Medicine.

1. Extraction of Temporal Bones

1. Identify temporal bones in the base of the mouse skull[13] and scrape away the cranial nerves using standard pattern forceps.
2. Place standard pattern forceps at the tip of the otic capsule and with the thumb of the opposite hand press down on the posterior semicircular canal to dislodge the encapsulated cochlea.
3. Free the bottom half of the temporal bone from the skull manually with the thumb and index finger or by using 10.5 cm fine scissors.

2. Post-fix Temporal Bones

1. Place temporal bones into 2 ml microcentrifuge tubes containing 250 - 500 µl 4% paraformaldehyde (PFA) diluted in 10 mM phosphate buffered saline (PBS) pH 7.4 and incubate at RT for 2 - 20 hr.
   NOTE: No opening of the apical cap or injection of PFA into the round or oval window is needed. Recommend using methanol free, ultra-pure, electron microscopy (EM) grade PFA that can be purchased at a 16% concentration in glass vials. Once a vial is opened and diluted 1:4 in PBS, making a 4% solution, it can be used for up to 2 weeks when stored at 4 ^{o}C (Some antibodies require short fixation and others can tolerate O/N (O/N) fixation).

3. Decalcify Temporal Bones

1. After fixation, remove PFA using a pipette and replace with 120 mM ethylenediaminetetraacetic acid (EDTA), slightly more than 2 ml. Make sure to fill the entire 2 ml microcentrifuge tube to prevent air bubbles when the tube is closed.
   1. If not decalcifying immediately, replace PFA with 10 mM PBS pH 7.4 and store samples at 4 ^{o}C.
      NOTE: After fixation, temporal bones can be stored for variable amounts of time before decalcification depending on the antigens being examined.

2. Place tubes on end-over-end rotator and rotate at 4 rpm at RT. Change EDTA solution daily using a pipette. Length of decalcification depends on the age of the sample and the preference of the scientist doing the dissection.
   1. Incubate temporal bones in EDTA using the following guidelines for incubation times:
      For samples postnatal day (P) 8 to P15: 2 - 4 hr; For samples P15 to P21: O/N; For samples P21 to P30: 2 O/N; For samples older than P30: 3 or more O/N.
      NOTE: Decalcification times are subject to users' preference and can be extended as needed. Decalcification time can be reduced by changing the EDTA solution twice a day, about 8 hr apart. Some labs add 1% PFA to the EDTA solution when decalcifying for longer than 3 days to prevent contamination.

3. To determine if the sample is adequately decalcified, place temporal bones on a silicon elastomer-coated dissection dish and gently press forceps onto the snail-shaped cochlea. If the tissue is spongey, then decalcification is complete.
4. Once decalcification is complete, remove EDTA using a pipette and add 500 -1,000 µl of 10 mM PBS pH 7.4. Store samples at 4 ^{o}C until ready to dissect.

4. Create Silicone Elastomer-coated Dissection Dish

1. Combine the base and curing agent of a silicone elastomer encapsulant kit according to the manufacturer's instructions and mix thoroughly.
2. Add approximately 2 - 3 tablespoons of powdered charcoal until the solution is black and mix well.
   NOTE: Liquid ink or liquid charcoal will not mix with the solution and cannot be used. Silicone elastomer encapsulant kits can be purchased in black, allowing step 4.2 to be omitted.
3. Pour the solution into 60 mm glass or plastic petri dishes, filling enough to coat the bottom. If bubbles are present, puff with air on the surface. Let stand at least 24 hr at RT to set.
   NOTE: Silicone elastomer-coated dishes can be used repeatedly for years. However do not use ethanol for cleaning, as this will cause the silicone elastomer to crack.

5. Whole Mount Dissection of the Cochlea (for P7 and Older Samples)

1. Separate the basal turn from middle/apical turns
   1. Place one decalcified temporal bone in a silicone elastomer-coated dissection dish filled two-thirds full with 10 mM PBS pH 7.4 and use a stereo dissection microscope for the following steps.

2. Hold the temporal bone in the vestibular region with #4 or #5 straight jeweler's forceps and using 5 mm Vannas-Tubingen spring scissors, cut away excess otic capsule tissue along the sides and above the apex.
3. Using 2.5 mm Vannas spring scissors, insert one blade into the oval window and make several small cuts along the spiral ligament/ lateral wall of the basal turn.
4. Using 5 mm Vannas-Tubingen spring scissors, insert one blade into the region just cut and place the other blade on the outside the temporal bone, medial to the oval window. This cut separates the basal turn from middle and apical turns.

2. Complete dissection of the basal turn.
   1. Using 2.5 mm Vannas spring scissors, cut the spiral ganglion nerve fibers that connect to the modiolus to release the tension in the basal turn and cut below the basal turn to separate from vestibular organs.
   2. Using 2.5 mm Vannas spring scissors, make a series of small cuts to remove the spiral ligament/lateral wall from both above and below the organ of Corti. Use #4 or #5 straight jeweler's forceps to guide the tissue. Pin the spiral ganglion nerve fibers to the silicone elastomer-coated dissection dish, but do not hold onto this region as the tissue will tear.
   3. During the previous steps, some of Reissner's membrane will often be removed. If any still remains, grasp the Reissner's membrane with #4 or #5 straight jeweler's forceps and pull away from the organ of Corti.
      NOTE: The tectorial membrane is rarely visible and typically floats away without requiring a specific step for removal.
   4. Finally make several cuts to reduce the thickness of the spiral ganglion axons, to make the turn as flat as possible and use #4 or #5 straight jeweler's forceps to transfer the dissected basal turn, by grasping the remaining axons of the spiral ganglion, to a 48-well plate (or chamber slide) containing ~500 µl of 10 mM PBS pH 7.4.

3. Separate middle and apical turns
   1. Place the remaining two-thirds of the cochlea, apical side down.
   2. Using 2.5 mm Vannas spring scissors, insert one blade into the scala media where the middle turn used to be connected to the basal turn, and make several small cuts along the spiral ligament/lateral wall of the middle turn.
   3. Using 5 mm Vannas-Tubingen spring scissors, insert one blade into the region just cut with the middle turn placed on top of the blade, and place the other blade on the outside the bony labyrinth, at a 90^{o} angle from the apical tip. This separates the middle turn from the apical turn.

4. Complete dissection of the middle turn in a similar manner to the basal turn (see steps 5.2.2 to 5.2.4).
5. Complete dissection of the apical turn.
   1. Using 2.5 mm Vannas spring scissors, open the cap that covers the apical turn. Complete dissection in a similar manner to the basal turn (see steps 5.2.2 to 5.2.4).
      NOTE: Dissected cochlear turns can be stored in 10 mM PBS pH 7.4 in a 48-well plate (or chamber slide) at 4 ^{o}C for several weeks before immunostaining. However PBS will evaporate and needs to be periodically replenished and storage longer than 2 - 3 weeks can result in bacterial or fungal growth on the tissue which can decrease the quality of the image. We recommend long-term storage of samples as undissected temporal bones.

6. Immunostaining with Fluorescently Tagged Antibodies

1. After the dissection, store each cochlear turn in a separate well in a 48-well plate (or chamber slide), submerged in ~500 µl of 10 mM PBS pH 7.4. For each of the following steps the cochlear turn should be submerged in liquid, not floating on top or stuck to the side of the well.
   NOTE: When removing liquid from each well, it is easy to lose the cochlear turns or suck it up in the pipette tip. Changing solutions with a 200 µl pipette tip using a dissection scope will help prevent this.
2. Using a pipette, remove PBS from each well and replace with ~200 - 300 µl per well of blocking/permeabilization solution (1% Triton X-100, 1% bovine serum albumin (BSA), and 10% normal goat serum (NGS) diluted in 10 mM PBS pH 7.4). Incubate 1 hr at RT on a 3D rotator.
   NOTE: If any primary antibody used was made in a goat host, then a secondary anti-goat antibody will be needed and NGS should **NOT** be used for any of the steps. Normal horse serum can be used as a replacement for NGS.
3. Remove blocking/permeabilization solution using a pipette and replace with ~100 µl per well of primary antibody solution (0.1% Triton X-100, 1% BSA, and 5% NGS diluted in 10 mM PBS pH 7.4). The dilution factor for each primary antibody varies. Incubate O/N (minimum 14 hr) at 4 ^{o}C on a 3D rotator.
   NOTE: If more than one primary antibody is used, all can be combined into the same solution for incubation, just make sure that each primary antibody has a different host.
4. Remove primary antibody solution using a pipette and perform 3 washes of 10 mM PBS pH 7.4 at ~500 µl per well. Each wash incubates a minimum of 5 min at RT on a 3D rotator.
5. Remove last PBS wash using a pipette and replace with ~100 µl per well of secondary antibody solution (0.1% Triton X-100, 1% BSA, and 5% NGS diluted in 10 mM PBS pH 7.4). The dilution factor for each fluorescently tagged secondary antibody is usually 1:500 or 1:1,000. Place 48-well plate in a black box to protect fluorescently tagged secondary antibodies from light. Incubate 2 - 3 hr at RT on a 3D rotator.
   NOTE: If more than one secondary antibody is needed, they can be combined into the same solution for incubation. Make sure there is no possibility of cross-labeling (*e.g.*, using goat anti-rabbit and chicken anti-goat secondary antibodies)
6. Remove secondary antibody solution using a pipette and perform 3 washes of 10 mM PBS pH 7.4 at ~500 µl per well. Incubate each wash for a minimum of 5 min at RT on a 3D rotator. Keep 48-well plate in a black box to protect from light.
7. Remove last PBS wash using a pipette and replace with ~100 µl per well of Hoechst 33342 (diluted 1:2,000 in 10 mM PBS pH 7.4) to label nuclei. Incubate 15 - 20 min at RT on a 3D rotator. Keep 48-well plate in a black box to protect from light. Do NOT incubate longer than 20 min.
8. Remove Hoechst solution using a pipette and perform 3 washes of 10 mM PBS pH 7.4 at ~500 µl per well. Incubate each wash for a minimum of 5 min at RT on a 3D rotator. Keep 48-well plate in a black box to protect from light.
9. All steps can be extended, except Hoechst incubation, by several hours if needed. To pause the reaction at any stage, just submerge samples in ~500 ul of 10mM PBS pH7.4 and store the 48 -well plate (or chamber slide) at 4 ^{o}C until ready to resume the protocol hours or 1 - 2 days later.

7. Mount Cochlear Turns on Slides

1. Label slides with pertinent information about the sample and antibodies used.
2. Pipette ~50 µl of mounting media onto each slide and be careful to prevent bubbles. Centrifuge the tube containing the mounting media to remove any bubbles.
3. Using #4 or #5 straight jeweler's forceps, grasp the axons of the spiral ganglion to gently transfer one cochlear turn from the 48-well plate to the slide and place in the mounting media. Mount one cochlear turn per slide to prevent light exposure and photobleaching during the imaging process.
4. Use a stereo dissection microscope to ensure that cochlear turn is not folded, twisted, or near an air bubble. If any of these conditions occur, use #4 or #5 straight jeweler's forceps to reposition the cochlear turn.
5. Place one end of a coverslip on the slide and gently release to let the coverslip fall.
6. Use a stereo dissection microscope to ensure that the cochlear turn is not folded, twisted, or near an air bubble. If any of these conditions occur, gently move the coverslip back and forth to reposition the cochlear turn.
7. Place slides in a slide folder so that they lay flat. Let mounting media cure O/N at RT (keep in the DARK)
8. Seal coverslips with clear nail polish and store at RT or -20 ^{o}C until imaged. Slides can be stored in a slide folder or slide box.
   NOTE: Slides can be stored long term at -20 ^{o}C or -80 ^{o}C and fluorescence will be maintained for several months.
9. Image slides using a confocal microscope with the appropriate wavelength based on the secondary antibodies used during the immunostaining procedure. Brightness and contrast adjustments can be performed using the imaging software provided by the confocal vendor.

Representative Results

We present a method to isolate the organ of Corti as three intact cochlear turns (apex, middle, and base) from cochlear tissue that is calcified, with key dissection steps presented in **Figure 1**. During the first postnatal week of development, calcification of the mouse cochlea is incomplete and a more simple dissection method can be used[13]. Using the neonatal whole mount dissection method with cochlea from P7 and older mice results in tears and shredding of the organ of Corti. The spiral ligament/lateral wall is now more firmly attached and cannot be peeled away from the sensory epithelium without causing damage. Thus the "adult" whole mount dissection method is needed for samples older than P6. We present an example of the middle turn of a P15 mouse cochlea that has been dissected and immunostained with hair cell and supporting cell markers (**Figure 2**). Optical cross-sections can also be obtained with the whole mount technique (**Figure 3**).

Several problems can occur during the whole mount dissection or when mounting the cochlear turns on slides. During the removal of the spiral ligament/lateral wall, there is a narrow window between cutting too much or not enough. Cuts that occur next to the last row of outer hair cells may cause the hair cells in this last row to mount at varied angles (**Figure 4A**). Cuts that are too large can remove sections of the organ of Corti (**Figure 4B**). Handling the sample with forceps takes great care and often there are holes in the organ of Corti where forceps were misplaced (**Figure 4C**). Finally when mounting the cochlear turns, the organ of Corti can fold which obscures the image (**Figure 4D**).

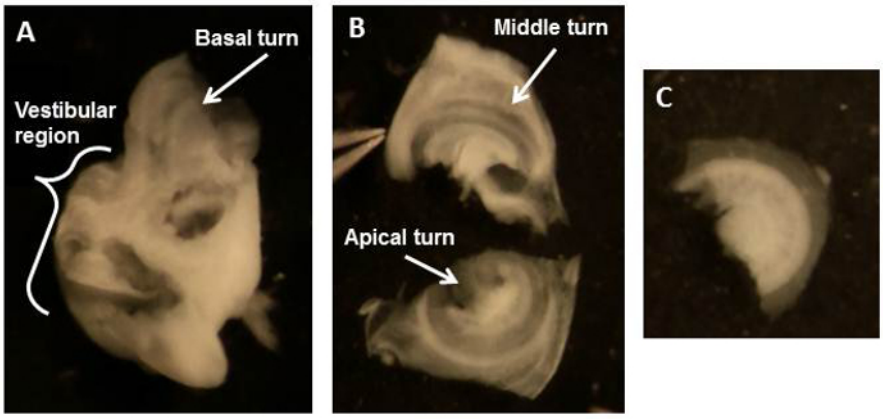

Figure 1. Important Steps in the Whole Mount Dissection of the "Adult" Mouse Cochlea. (A) After protocol step 5.1.4, the basal turn of the cochlea is separated from middle/apical turns, yet still attached to the vestibular region. **(B)** After protocol step 5.3.3, the middle turn is separated from the apical turn. **(C)** Example of the completed dissection of a middle turn where the spiral ligament/lateral wall is removed. Please click here to view a larger version of this figure.

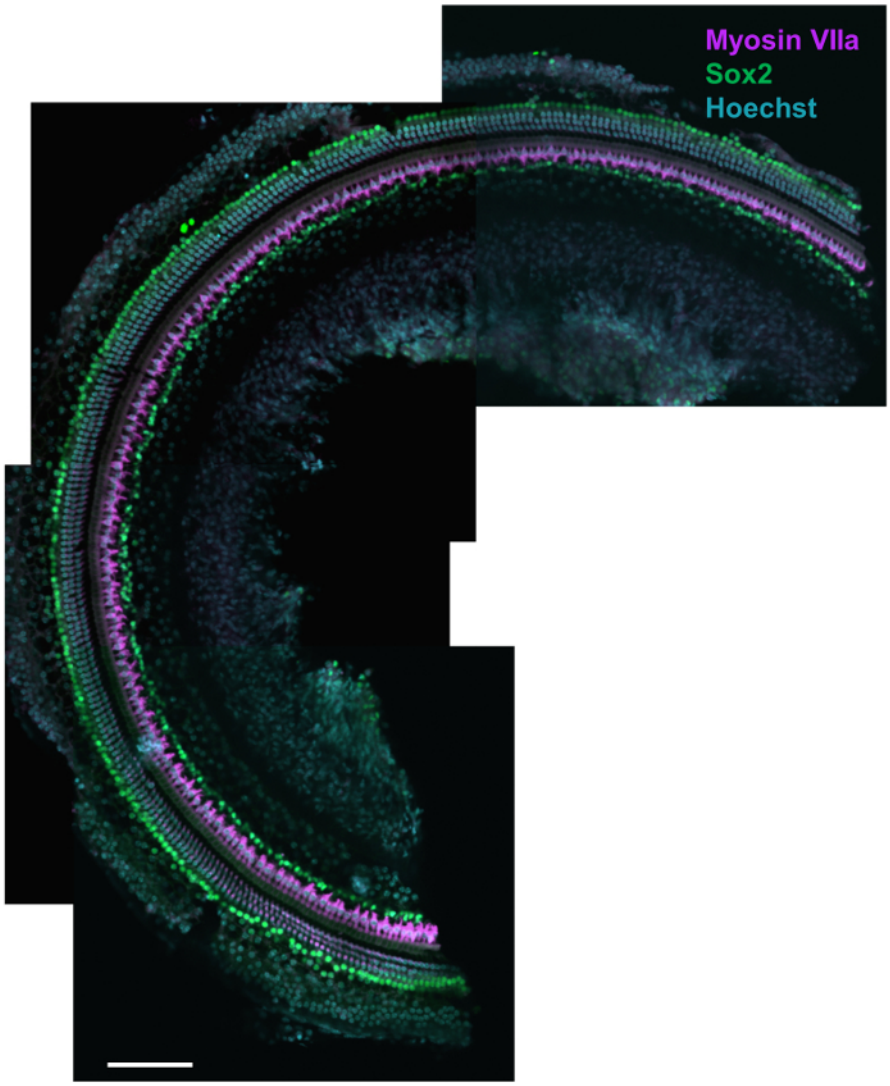

Figure 2. Confocal Slice Image of the Middle Turn Isolated from a P15 Mouse. Four 20x images are overlaid to reconstruct the whole middle turn. Hair cells are labeled with a rabbit anti-myosin VIIa primary antibody (1:200 dilution) combined with a donkey anti-rabbit Alexa 488-conjugated secondary antibody (1:1,000 dilution) (magenta). Supporting cells are labeled with a goat anti-Sox2 primary antibody (1:500 dilution) combined with a donkey anti-goat Alexa 568-conjugated secondary antibody (1:1,000 dilution) (green). Hoechst (blue) labels all nuclei. Image was taken using a Zeiss LSM 700 confocal microscope with 405, 488, and 555 wavelengths. Scale bar = 100 µm. Please click here to view a larger version of this figure.

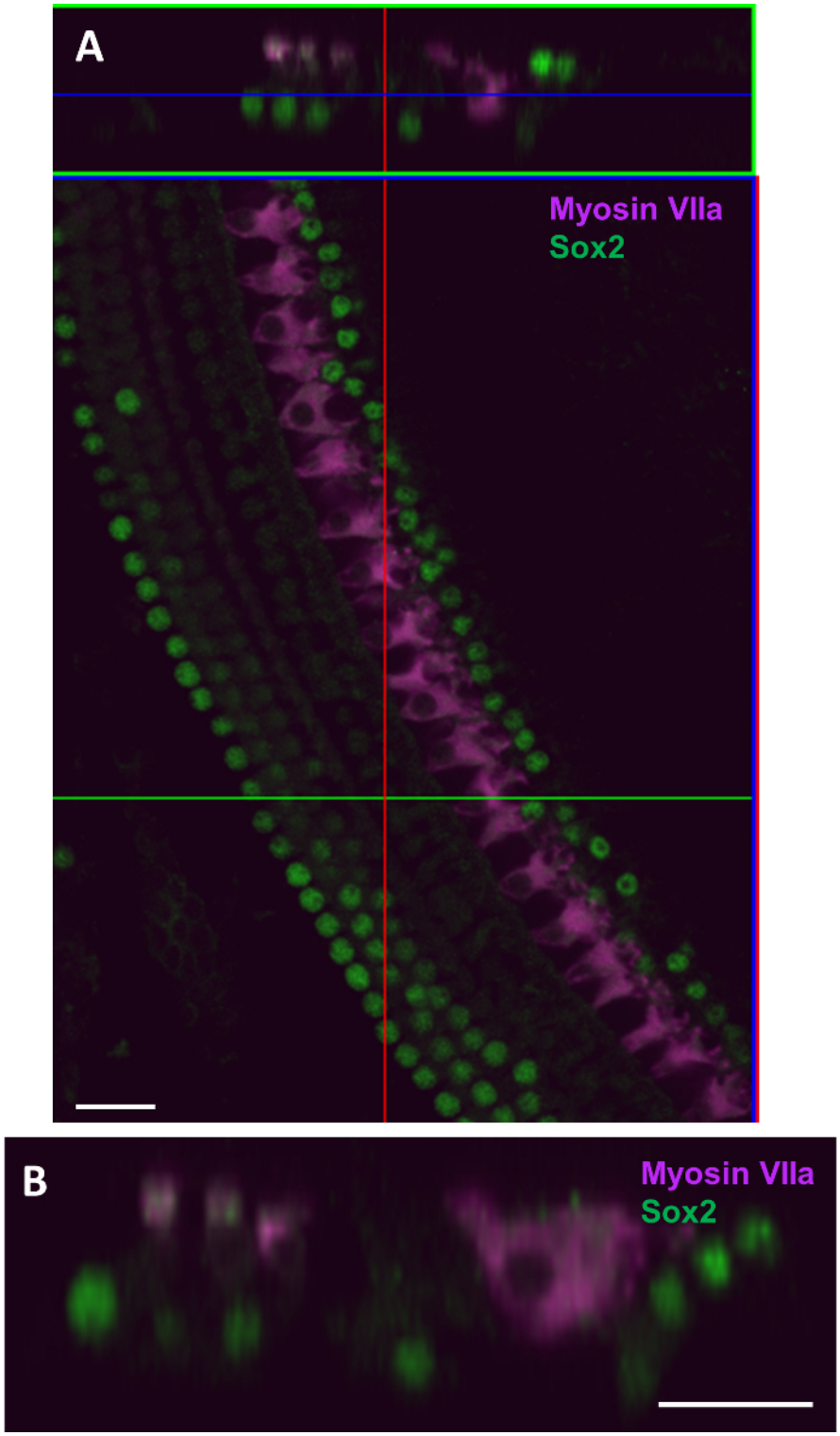

Figure 3. Optical Cross-section of the Middle Turn Isolated from a 6-week Old Mouse. (A) Confocal slice image of the whole mount preparation (bottom) and optical cross-section in the XZ plane (top). **(B)** Increased magnification of the top panel in **(A)**, with the crosshairs removed. Hair cells are labeled with a rabbit anti-myosin VIIa primary antibody (1:200 dilution) combined with a donkey anti-rabbit Alexa 647-conjugated secondary antibody (1:1,000 dilution) (magenta). Supporting cells are labeled with a goat anti-Sox2 primary antibody (1:500 dilution) combined with a donkey anti-goat Alexa 568-conjugated secondary antibody (1:1,000 dilution) (green). Image was taken using a Zeiss LSM 700 confocal microscope with 405, 555, and 647 wavelengths. Scale bars = 20 µm Please click here to view a larger version of this figure.

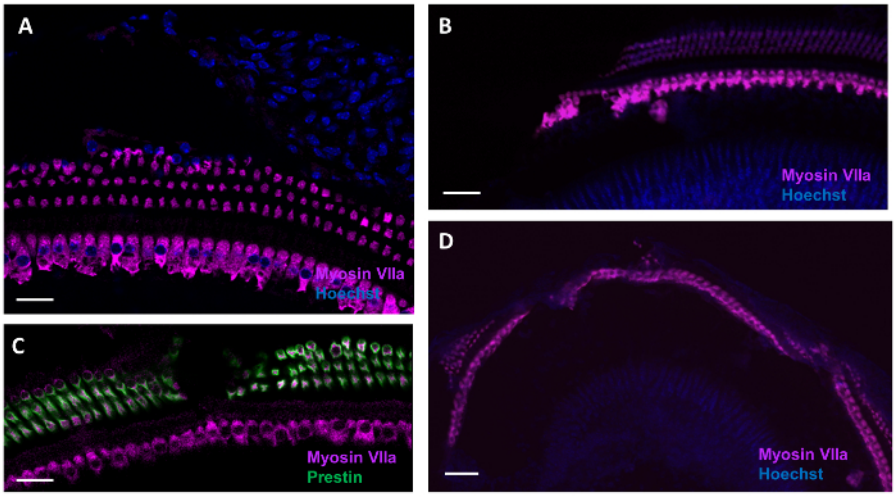

Figure 4. Examples of Problems that can Occur During the Whole Mount Dissection or When Mounting Cochlear Turns on Slides. (A) On the left side of the image, the cochlear tissue was cut next to the last row of outer hair cells causing many of these cells to be mounted at varied angles. **(B)** A section of the organ of Corti on the left side of the image has been cut off. **(C)** There is a hole punched in the outer hair cell region in the middle of the image. **(D),** The organ of Corti is folded in several places. Images were taken from 4 - 8 week old mouse cochleae. Hair cells are labeled with a rabbit anti-myosin VIIa primary antibody (1:200 dilution) combined with a goat anti-rabbit Alexa 488-conjugated secondary antibody (1:1,000 dilution) or a donkey anti-rabbit Alexa 488-conjugated secondary antibody (1:1,000 dilution) (magenta). In C outer hair cells are labeled with a goat anti-prestin primary antibody (1:200 dilution) combined with a donkey anti-rabbit Alexa 568-conjugated secondary antibody (1:1,000 dilution) (green). Images were taken using a Leica SP5 confocal microscope with 405, 488, and 555 nm wavelengths. Scale bars: in A-B = 20 µm; in C-D = 40 µm. Please click here to view a larger version of this figure.

Discussion

There are several critical steps for successful whole mount dissection and immunostaining. However before either of these methods are performed, proper fixation of the cochlear tissue is needed. We recommend using methanol free, ultra-pure, EM grade PFA. PFA made from powder can have traces of methanol and an unstable pH which decreases the quality of immunofluorescence. Other groups have also shown that similar dissections are possible using fixatives that do not contain formaldehyde[14-16]. The length of fixation is also important and is antibody specific. Some antibodies can tolerate an O/N fixation, while others do not work well with just 1 hr in PFA (however this is rare). Under-fixed tissue can be problematic for the dissection method as the tissue falls apart. In our experience a 3 - 4 hr fixation provides adequate fixation and does not interfere with the majority of primary antibodies commonly used in the hearing field.

It is also possible that EDTA can interfere with primary antibodies; thus some antibodies will work well in neonatal tissue, but not in P7 or older tissue that was decalcified. After fixation, temporal bones can be stored for variable amounts of time before decalcification depending on the antigens being examined. Some antigens require decalcification and dissection within days to weeks after fixation, while others may be stored for years (either before or after decalcification) without decreasing the quality of the immunostaining. We recommend storing samples as temporal bones due to the risk of evaporation of the storage media (PBS) from the 48-well plate and potential contamination with fungus or bacteria. We typically perform the whole mount dissection less than one week in advance to immunostaining.

Once fixed and decalcified, the whole mount dissection is performed with the temporal bone submerged in liquid. Removing excess bone and soft tissue surrounding the labyrinth early in the dissection will aid in removal of the spiral ligament/lateral wall at later stages by facilitating the manipulation of the tissue and providing a less obscured view of critical structures. When performing the first few steps, forceps can be used to hold the tissue in the vestibular region. However once the turns are isolated, it is important to avoid placing forceps on the organ of Corti or spiral ligament/lateral wall. Instead, keep the forceps closed and pin the spiral ganglion nerve fibers to the silicone elastomer-coated dissection dish. Do not hold onto this region as the tissue will tear. In general, once the tissue has been divided into the three turns, grasping and pulling maneuvers can cause unpredictable results, which are often damaging to the organ of Corti and should be avoided. Tissue from younger animals (P7-P21) tends to be more forgiving than tissue from animals older than P21. In addition, cochlear samples with hair cell damage are more difficult to dissect. If the mouse received noise exposure the tissue is especially fragile. Regardless of the state of the tissue, the dissection we present is technically demanding and requires many practice attempts for proficiency.

During immunostaining, it is important that each cochlear turn is submerged in liquid, not floating on top or stuck to the side of the well. This allows more complete penetration of triton and antibodies into the tissue. When removing liquid from each well, it is easy to lose the cochlear turn or draw it up into the pipette tip. Changing solutions with a 200 µl pipette tip using a dissection scope will help prevent this. Slowly extract the liquid and move the pipette tip if the cochlear turn gets too close. Also pipetteting waste solution into a clean tube can be a good strategy as this waste tube can be searched if a turn is accidentally drawn up into the pipette. If the turn is stuck in the pipette tip, the tip can be cut open with a razor blade, but often the organ of Corti will be damaged if this occurs.

When trouble-shooting antibodies for immunostaining, additional steps such as antigen retrieval or signal enhancement can be added. There are low pH and high pH antigen unmasking reagents that can be purchased. If an antibody does not work with the method described here, the first protocol change to try is one of these antigen retrieval methods. Alternatively, use a signal enhancer. There are commercially available solutions to use prior to immunostaining, or tyramide amplification kits that can be used to amplify the signal from the secondary antibody.

The significance of the technique we present is the ability to maintain the three-dimensional structure of the organ of Corti and to visualize all cells within the organ. The entire length of the cochlea is separated into only three turns while other similar techniques, namely the Bohne and Liberman methods, require division into 5 - 10 pieces[6-8], increasing the number of samples to maneuver in the immunostaining and imaging processes. The cochlear lateral wall dissection that was developed by Cosgrove and Gratton requires a similar level of skill and is possible in unfixed, fresh tissue, as well as in decalcified tissue, but the organ of Corti is stripped away and destroyed in the process of isolating the lateral wall[17]. Another group has performed similar dissections in unfixed, fresh cochlear tissue from three week old rats where the spiral ligament/lateral wall is grasped and stripped away from the organ of Corti, leaving the organ intact. However this was only achieved in the apical turn[5]. The method of peeling the spiral ligament/lateral wall away from the organ of Corti is routine for dissection of fixed tissue in a young mouse (< P7)[13]. However, in our experience with mice older than P6, after fixation and decalcification, this maneuver often tears the organ of Corti in an unreliable fashion. In addition the procedure described here allows isolation of middle and basal turns as well.

Cryosections and sections obtained after paraffin embedding are also commonly used in the auditory field. These methods allow visualization of other structures such as the stria vascularis, Reissner's membrane, and tectorial membrane, yet each section only allows visualization of a small region of the organ of Corti in each cochlear turn. Thus to investigate events that occur in a mosaic pattern, such as cell loss or cell division, with the sectioning method, 50 or more slides need to be stained and imaged to capture the entire length of the cochlea. In contrast, the whole mount dissection protocol has the advantage of preparing the entire organ of Corti in just three pieces. In addition to the benefit of preserving the lengthwise architecture of the organ of Corti, this technique allows for simplified data collection and storage. One limitation of the whole mount dissection is that it destroys surrounding structures such as the spiral ligament, stria vascularis, Reissner's membrane, and tectorial membrane. Another limitation is the technical difficulty and length of time for a single dissection. This is mostly due to the fragile nature of the organ of Corti and small margin for error when removing the spiral ligament/lateral wall. Once mastered, the whole mount dissection of one cochlea can be performed in about 20 - 30 min .

While the above protocol describes a whole mount dissection method for the adult mouse, we hope to apply this technique to other model organisms used in the auditory field. Our lab is currently modifying this technique for the dissection of the rat cochlea. The larger cochlea of the rat is encased in a thicker otic capsule that is more densely calcified than the mouse. Thus the temporal bone must be excised with large scissors. Before fixation and decalcification with EDTA, the otic capsule should be opened with scissors to allow better access to the cochlear tissue. Also the decalcification process is longer and can take up 3 weeks depending on the age of the sample. For the whole mount dissection, the size of the bony labyrinth affects the technique. The increased size of the rat cochlea provides a slightly larger distance between the spiral ligament/lateral wall and organ of Corti, providing a larger margin of error for individual cuts. However, while the larger tissue may provide more material to grasp for manipulation of the specimen, it also requires a greater number of cuts to remove the entire length of the spiral ligament/lateral wall. We believe that analogous modifications could be made for dissection of the cochlea from chinchilla, gerbil, and guinea pig.

Disclosures

The authors have nothing to disclose.

Acknowledgements

This work was supported in part by a grant from the Office of Naval Research (N000141310569). The Southern Illinois University School of Medicine Research Imaging Facility equipment was supported by the National Center for Research Resources-Health (S10RR027716).

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