

Hemi-Cochlear Preparations to Permit *ex vivo* Electrophysiology of Post-Hearing Basal Hair Cells.

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Introduction

After the onset of hearing, basal hair cells and spiral ganglion neurons become increasingly difficult to dissect out of the mouse auditory system due to the progressive ossification of the cochlear spiral. Utilising cochlear hemi-preparations allows for access to adult basal and apical hair cells for electrophysiology and imaging, to either gain an understanding as to how basal hair cells function or understand how specific mutations may have a greater effect at the low/high frequency end of the cochlea.

Procedures

Mice were culled via cervical dislocation rapidly followed by decapitation, and the two cochleae were dissected out and cleaned in artificial perilymph solution (135mM NaCl, 5.8mM KCl, 1.3mM CaCl₂, 0.9mM MgCl₂, 10mM HEPES, 5.6mM Glucose, 0.7mM NaH₂PO₄, 2mM Na-Pyruvate, 1x Amino acids and vitamins from concentrate [Gibco]). Each cochlea was then glued in place and cut a single time such that the cochlear modiolus was halved. Cuts were performed at a vibration amplitude of 2.5mm, a rate of 60Hz and a progression speed of 0.01mm/sec. The section and patch of glue were then cut from the pedestal and mounted in a custom chamber, held in place by a mesh of dental floss attached to a steel ring. These

preparations can then be easily identified and navigated under a 10x objective and patched with a 63x objective (Fig.1. and Fig.2.).

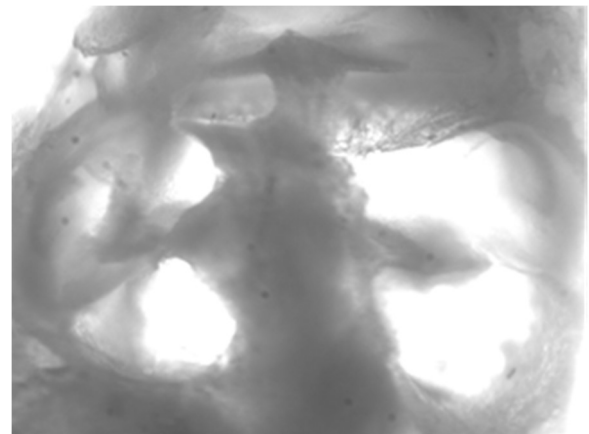


Fig.1. 10x image of cochlea hemi-preparation, showing modiolus at the centre approximately halved

Specific examples

While this technique has been only recently tested within the hearing group, it will no doubt be of use when trying to access basal hair cells or spiral ganglion neurons for electrophysiology. These experiments will likely be primarily aimed at understanding the potassium currents, capacitance or biophysical properties of these cells, as stimulation of

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Application Parameters

Amplitude	2.5mm
Frequency	60Hz
Advance Speed	0.01mm/s

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hair cell mechano-electrical transduction cannot be reliably achieved in this context due to the orientation of the cells.

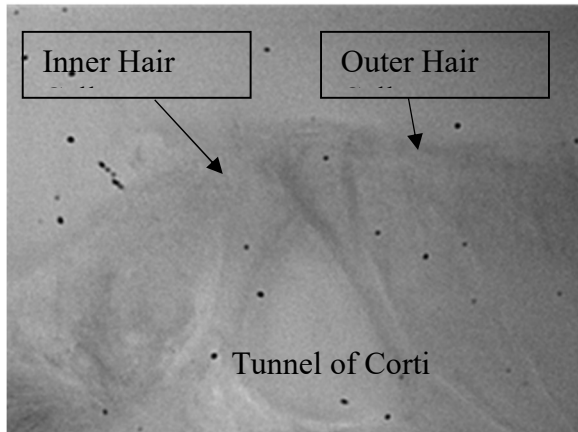


Fig.2. 63x image of the organ of Corti in a hemi-preparation. Contrast is difficult yet the location of the hair cells is clearly visible.

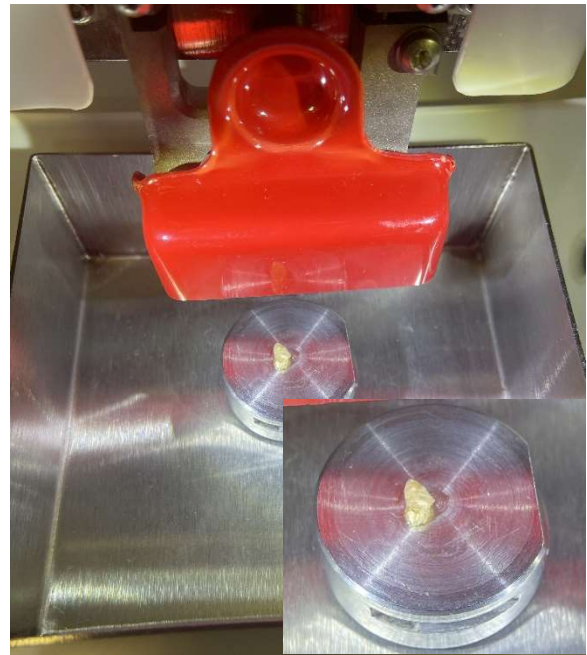


Fig.3. Tissue mounted using cyanoacrylate adhesive ready for slicing (red blade protector is in place, and bath would be filled with physiological buffer).