Microdissection and Electrophysiology of Mouse End Organs

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Microdissection of the mouse utricle:

Utricles are excised from postnatal (P0-P6) mice. Temporal bones are removed and the bony labyrinth opened medially to expose the utricle. The tissue is then bathed in MEM solution (Invitrogen, Carlsbad, CA) supplemented with 10 mM HEPES (pH 7.4) containing 0.1 mg/ml protease XXIV (Sigma, St. Louis, MO) for 15 to 20 minutes. After the otolithic membrane is removed, the utricle is excised and the nerve fibers are trimmed close to the epithelium. The tissue is then mounted onto round glass coverslips and held in a flat position by two glass fibers previously glued to the coverslip with a small drop of sylgard.

Microdissection of the Organ of Corti:

Organs of Corti are excised from postnatal (P0-P6) mice. The temporal bones are rapidly excised and bathed in MEM (Invitrogen, Carlsbad, CA) supplemented with 10 mM HEPES (pH 7.4). The organ of Corti is gently dissected away from its accessory structures. Consecutively the tectorial membrane is impelled without the use of enzyme. The excised organ can consecutively be mounted on round glass coverslips. A pair of thin glass fibers previously glued to the coverslip can be placed on the edge of the tissue to stabilize it in a flat position. Alternatively the tissue can be placed on Cell tack (BD Biosciences, San Jose, CA)-coated coverslips.

Solutions:

Dissections are performed in standard MEM solution (Invitrogen, Carlsbad, CA) supplemented with 10mM HEPES (pH 7.4). Electrophysiological recordings are performed in an artificial perilymph solution that contained (in mM): 144 NaCl, 5.8 KCl, 10 HEPES, 0.7 NaH_2PO_4, 1.3 $CaCl_2$, 0.9 $MgCl_2$, 5.6 D-glucose, vitamins and amino acids (added from concentrates: Invitrogen, Carlsbad, CA), pH 7.4 and 320 mOsmol/kg. Recording pipettes are filled with an intracellular solution that contained (in mM): 135 KCl, 5 EGTA-KOH, 5 HEPES, 2.5 K_2ATP, 3.5 $MgCl_2$, 0.1 $CaCl_2$, pH 7.4 and 290 mOsmol/Kg.

Organotypic cultures and viral infection:

To produce organotypic cultures of mouse organ of Corti, tissues are harvested as outlined above. Tissues are maintained at 37°C in MEM (Invitrogen) supplemented with 10 mM HEPES (pH 7.4), 50mg/l Ampicillin, +/- 5% BSA. Media are replaced every 48 hours. For viral experiments, viral vectors are applied generally after 24h in culture, directly to the culture media for 4 hours at titers that range from 1×10^5 to 1×10^8 viral particles/ml. Tissues are placed back in culture for a minimum of 24h to allow stable and robust expression of the transfected construct.

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).

Electrophysiology. The sensory epithelia are placed onto a microscope chamber and viewed with a 63x water-immersion lens with differential interference contrast optics (Axioskope FS, Zeiss, Germany). Recording pipettes are pulled from borosilicate capillary glass (Garner Glass) with resistances that ranged from 2-5 MΩ. The apical surface of the epithelium is viewed from above as the recording pipette is advanced. Positive pressure is maintained as the recording pipette is lowered into the epithelium. When the pipette touch the membrane, positive pressure is released and a tight-seal formed on the basolateral membrane of the cell. Recordings are obtained at room temperature (22-24°C). The cells are held at –64 mV and data are acquired using the whole-cell tight-seal technique in both voltage-clamp and current-clamp modes using an Axopatch multiclamp 200B amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz with a low-pass Bessel filter, digitized at 5 kHz with a 12-bit acquisition board, and collected using pClamp 8.0 software (Axon Instruments).

Material and solutions required:
- Dissecting tools
- Cell culture dish (35 and 60mm)
- Hot plate
- Sylgard kit Corning
- Glass coverslips fisher 18mm (#2)
- Glass pipettes for chamber: VWR Calibrated pipettes 100ul #53532-921
- Glass pipettes for electrophysiology: R6 glass (size according to pipette holder)
- Syringe filter 0.22um pore; 33mm and 13mm
- Vacuum grease Corning
- MEM (with earle's salts and glutamax)- Invitrogen #41090-101 (10x500ml)
- Ampicillin (powder-In vitrogen)
- Various Salts + HEPES
- Methanol (to clean glass pipettes and for dissolving FM1-43)
- Protease XXIV Sigma P8038
- Poly-L-Lysin or Cell tack (may be used instead of fibers)