Preparation of Rodent Temporal Bones for Scanning Electron Microscopy

1. Fix tissue in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.3 with 3mM CaCl2 (2 - 3 hours minimum, but better if done for 24-48 hrs. can be done at room temp or 4C, ideally in a sealed container in a fume hood). If possible, perfusion through the round window and/or an open fenestra at the helicotrema)

2. Rinse in 0.1M cacodylate buffer (3 x 5 min, room temp)

3. Fix in 1% OsO4 in 0.1M cacodylate. (30 – 60 min, room temp, in fume hood)

4. Rinse in cacodylate buffer (3 x 2 min), then transfer to ddH2O (2 x 5 min, room temp)

5. Remove most of the H2O, add saturated aqueous solution of thiocarbohydrazide (0.5% in water, stirred, filtered just before use) dropwise, if solution turns opaque brown, re-rinse in ddH2O, if solution is translucent or clear, keep adding until tissue is immersed (20-30 min., room temp)

6. Rinse in ddH2O (3 x 5 min)

7. Fix in 1% OsO4 in 0.1M cacodylate. (30 min, room temp)

8. Rinse in ddH2O (3 x 5 min)

9. Repeat steps 5-7 (if you have the time, you should repeat these steps again for a total of 3 iterations of OTO).

10. Dehydrate in ethanol series to anhydrous 100% ethanol
    - 25% EtOH, 5 min
    - 50% EtOH, 5-10 min
    - 75% EtOH, 5-10 min
    - 95% EtOH, 5-10 min
    - 100% EtOH, 3 X 10 min

11. At this point, dissect away the bone to reveal the sensory tissue. Then Critical point dry using liquid CO2. Proceed immediately to mounting and sputter coating.

11.5 (OPTIONAL) If critical point drying is unavailable, samples can be solvent dried in hexamethyldisilazane (HMDS). Add enough HMDS to completely cover your sample and let sit in a closed well plate overnight in the fume hood. Evaporate residual HMDS from the sample in a 37C oven for 1-3 hours. Proceed immediately to mounting and sputter coating.


13. Sputter coat (between 5 – 15 nm, depending on type of coating being used and structures to be visualized).
**Supplies:**
Glutaraldehyde solution (25%): 18426 from Ted Pella
Sodium cacodylate
CaCl₂
OsO₄; 4% aqueous solution – 18459 (10x2ml) from Ted Pella
Thiocarbohydrazide (from Sigma; No. T-2134)
Anhydrous ethanol
Specimen support stubs for SEM (12.5 mm: 16232, pack of 50, from Ted Pella
PELCO Carbon conductive tabs, double sided (16084-1 from Ted Pella)