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Mammalian vestibular organs have two types of sensory cells: Type I and Type II hair cells. To compare signalling properties of these cell types, we study them in vitro, where their voltage responses (receptor potentials) to controlled manipulations of their hair bundles can be examined. The guinea pig utricle is excised and maintained in medium; accessory structures are removed to expose the hair bundles of the sensory cells. A fine probe moves the hair bundle of an individual cell while a microelectrode inserted in the cell records its voltage. Although normal resting potentials have been recorded, we have not vet detected responses to the hair bundle motion. Keywords:

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Annual Report on "Stimulus processing in vestibular hair cells"
Vestibular Transduction Program: Project No. RR04108 Task No. 441m801
Date of Report: 1 January 1989

Author of Report and Principle Investigator: Ruth Anne Eatock
Contractor: University of Rochester
Start Date of Project: 1 January 1988

Our goal is to compare the signalling properties of the two types of sensory cell *(type I and type II hair cells)* in mammalian vestibular organs, in order to determine whether differences in these properties contribute to known functional differences among vestibular cells in the central nervous system.

The experimental approach is as follows: Mammalian vestibular sensory epithelia are isolated and maintained *in vitro* in medium. Individual hair cells are stimulated by moving their hair bundles with a glass probe. The hair cells respond with voltage signals *(receptor potentials)*, which are recorded with intracellular microelectrodes.

Currently we are focussing on the guinea pig utricle. Pigmented guinea pigs weighing about 200 g are anesthetized with sodium pentobarbitol. The temporal bone is removed and the inner ear perfused with chilled oxygenated medium (Hanks balanced salt solution (HBSS), pH 7.4). The utricle is excised and the endolymphatic chamber is opened to expose the sensory epithelium. Before removing the otolithic membrane overlying the hair cells, we superfuse the epithelium with medium (currently HBSS) containing protease (subtilopeptidase A, 15 ug/ml) for ten minutes in an effort to loosen bonds between the hair bundles and the otolithic membrane (see Hudspeth and Jacobs, Proc. Natl. Acad. Scis. USA **76**: 1506-1509, 1979). The membrane is then removed and the epithelium is viewed at 400-800x magnification, using a water-immersion lens with Nomarski optics on a Zeiss Standard 16 microscope.

Microelectrodes and stimulus probes are pulled from glass tubing and then bent (Hudspeth and Corey, Am. J. Physiol. **234**: C56-C57, 1978). The probe is fixed to a piezoelectric material which moves a calibrated amount when voltage is applied to it (Corey and Hudspeth, J. Neurosci. Meth. **3**: 183-202, 1980). The probe motion is calibrated using an eyepiece micrometer or by imaging the probe on a photodiode. The probe is brought into contact with a hair bundle under visual control. The microelectrode is inserted near the base of the hair bundle. Resting potentials between -10 and -60 mV have been recorded. Probe movements on the order of 1 micron and between 5 and 500 Hz have thus far failed to elicit receptor potentials.

We are taking several approaches to obtain receptor potentials. These include: (1) trying different culture media; (2) varying the protease treatment, as this may be damaging the hair bundles; (3) reducing noise sources to allow detection of small receptor potentials (microvolt range).

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