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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 prepare isolated hair cells from which we record membrane currents in response to voltage steps. The isolated cells appear to retain their distinctive morphologies, and therefore are identifiable as either type I or type II. Preliminary results reveal that in some cells, the currents elicited by voltage steps are qualitatively similar to those previously described in frog and chick vestibular hair cells.

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Annual Report on "Stimulus processing in vestibular hair cells"
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Date of Report: 1 January 1990

Author of Report and Principle Investigator: Ruth Anne Eatock
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Introduction

The vestibular organs of mammals, birds, and some lizards possess an unusual kind of sensory cell, the type I hair cell, in addition to the type II hair cell which is ubiquitous in acousticolateralis organs (Baird and Lowman, 1978; Jorgensen, 1974; Jorgensen, 1989; Wersall, 1956). The two types of cell are morphologically defined. The type II cell is generally cylindrical and receives bouton synaptic contacts from the primary afferent neurons. The type I cell is described as "amphora-like" in shape, and receives a large afferent synaptic contact called a calyx, which may entirely surround the basolateral surface of the cell. The two cell types are differently distributed within the sensory epithelia. Most afferents make contact with both types of hair cell - i.e., they form bouton endings on some cells and calyceal endings on others (Baird et al. 1988; Fernandez et al. 1988).

What is the function of the unusual type I hair cell and afferent contact? The mixing of type I and type II input at the level of the afferent nerve has made it difficult to answer this question. The large calyceal contact is reminiscent of the end-bulbs and calyces of Held found at higher order synapses in the auditory system. These large synaptic contacts have been associated with pathways involved in temporal encoding of sound location in space: they are thought to ensure rapid and faithful transmission of timing information (Yin and Chan, 1988). Other clues to the special role of the type I cells can be found in studies of primary vestibular afferents and vestibular neurons within the central nervous system, which show a range of evoked and spontaneous properties. At one extreme are found cells that (among other properties) adapt strongly to vestibular stimuli and whose spontaneous discharge is highly irregular; at the other extreme are cells that are tonically active during vestibular stimulation and which fire very regularly in the absence of stimulation (Fernandez and Goldberg, 1971; Goldberg and Fernandez, 1971a,b; Fernandez and Goldberg, 1976a,b,c). It appears that there is segregation of these response properties within the central nervous system; for example, vestibular inputs to vestibulocollic reflexes appear to be largely those with irregular spontaneous discharge (Bilotto et al. 1982), and the adapting and tonic inputs to the vestibuloocular reflex may take different pathways (Lisberger and Pavelko, 1986).

A plausible possibility is that some of the functional variation within the vestibular central nervous system arises at the sensory periphery, as occurs in the visual and somatosensory systems. We wish to investigate this possibility directly, by recording the electrical signals of individual hair cells. In particular, we can ask whether type I hair cells adapt more rapidly to mechanical stimulation than type II cells, and whether the ionic currents underlying their voltage signals have different properties?

Methods

In the past year we have used two experimental approaches: (1) intracellular voltage recording from hair cells and primary afferents in excised vestibular organs; (2) whole-cell current recording from isolated vestibular hair cells.

(1) Intracellular voltage recording in excised vestibular epithelia

Recordings were made from type II hair cells and primary afferents in saccules excised from bullfrogs, and from hair cells (of unknown type) in utricles excised from guinea pigs. Techniques were largely similar to those described in Eatock et al. (1987) (Eatock et al. 1987), with the following differences: The guinea pig utricular maculae were maintained in either L-15 or Hanks' Balanced Salt Solution (HBSS), at pH 7.4, rather than in the artificial perilymph used for the frog saccules. In some experiments individual hair cells were stimulated by moving their hair bundles with a glass probe mounted on a piezoelectric bimorph element (Corey and Hudspeth, 1980), and the hair cells' receptor potentials were recorded with intracellular microelectrodes. In most of the experiments on bullfrog saccules, recordings were made from single saccular nerve fibers while the entire otolithic membrane was displaced by a bimorph-mounted stimulus probe. Both spikes and postsynaptic potentials were recorded, amplified, filtered and stored on computer. In some of these experiments we eliminated spikes by adding tetrodotoxin (TTx) to the bath (10 μg/ml), in order to record postsynaptic potentials alone.

(2) Whole-cell current recording from isolated vestibular hair cells

Hair cells were isolated from frog saccules and from rat utricles and semicircular canal organs, using the following dissociation protocols.

The bullfrog saccules were superfused for 20 minutes with a low-Ca^{2+} artificial perilymph (in mM: 120 Na^+, 2 K^+, 0.1 Ca^{2+}, 122 Cl^-, 3 D-glucose, 5 HEPES; pH 7.25) containing 50 μg/ml of Sigma protease type XXVII. The otolithic membranes were then removed, and the saccules were superfused for 15 minutes with low-Ca^{2+} artificial perilymph containing 500 ng/ml of papain (Sigma crude) and 2.5 mM l-cysteine. This was following by superfusion for 5 minutes with low-Ca^{2+} artificial perilymph containing 500 ng/ml of bovine serum albumen (BSA; Sigma). Cells were dislodged from the sensory epithelium using an eyelash and plated onto a clean glass coverslip. During recording the cells were superfused with a high-Ca^{2+} artificial perilymph, which was identical to the low-Ca^{2+} solution except that it contained 4 mM Ca^{2+} and 128 mM Cl^-.

The protocol for the rat vestibular organs differed in the following ways: (1) The low-Ca^{2+} medium was HBSS, buffered with HEPES and to which 1.16 mM EGTA was added; pH 7.3. (2) The initial protease treatment lasted 10 minutes. (3) The papain treatment was for 30 minutes.

Whole-cell currents were recorded using the giga-ohm seal technique (Hamill et al. 1981). The pipettes had impedances of 5-10 MΩ. In recordings from frog saccular cells, the pipettes were filled with a solution comprising (in mM) 120 K^+, 2 Mg^{2+}, 47 Cl^-, 43 Asp^-, 10 EGTA and 5 HEPES; pH 7.3. In recordings from rat vestibular cells, the pipette solution contained (in mM): 140 K^+, 2 Mg^{2+}, 131.5 Cl^-, 5 EGTA and 5 HEPES. Currents were amplified, low-pass filtered at 10 kHz, digitized and stored on computer.

Results

(1) Intracellular voltage recording from hair cells and primary afferents

Using the bullfrog saccule, we have recorded receptor potentials from hair cells and postsynaptic potentials and spike discharges from the primary afferents. When stimulated with sinusoidal displacements of varying frequency, some nerve fibers were found to be tuned with best frequencies between 20 and 80 Hz; others appeared to be low-pass with a cut-off frequency below 20 Hz. Our goal is to use this kind of preparation to study signal processing by the afferent synapse, e.g. by comparing the receptor potentials and postsynaptic potentials evoked by similar stimuli.

The experiments on the bullfrog saccule were preparatory to similar experiments on excised mammalian vestibular organs. In a preliminary set of experiments, we recorded from hair cells of the excised guinea pig utricle. Although normal resting potentials were frequently encountered, responses to imposed hair bundle motions were rare. An example is shown in Figure 1.

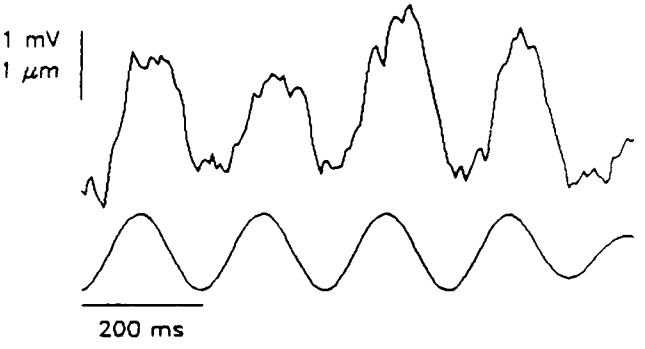

Figure 1. Intracellular potential (upper trace) in a hair cell of the guinea pig utricle, during sinusoidal deflection of its hair bundle (lower trace).

As an alternative approach, we have recently focussed on developing a preparation of isolated mammalian vestibular hair cells, which can be studied with the giga-ohm seal technique. Results of this approach are described next.

(2) Whole cell currents in vestibular hair cells

Because the whole-cell currents of bullfrog saccular hair cells have been studied in detail (Holton and Hudspeth, 1986; Hudspeth and Lewis, 1988), we used these cells in a series of experiments in which we developed a dissociation protocol and a whole-cell recording set-up. The whole-cell currents we recorded during depolarizing voltage steps displayed the same features described by Hudspeth and Lewis (1988) (Fig. 2). Depolarizing voltage steps evoked an early inward current and a later outward current. Both currents grew with increasing depolarization up to about +40 to +60 mV. The analysis of Hudspeth and Lewis showed that the early inward current is a voltage-dependent Ca^{2+} current, while the later outward current is a Ca^{2+}-activated K^{+} current. Both currents saturate with increasingly positive voltages as the driving force on Ca^{2+} declines. As shown in Figure 2, the kinetics of the currents varied in different cells. This variation underlies the variation in the frequency of the cells' electrical resonance. The electrical resonance in turn is important in determining the frequency at which the cells respond best to mechanical stimulation (Art and Fettiplace, 1987; Lewis and Hudspeth, 1983).

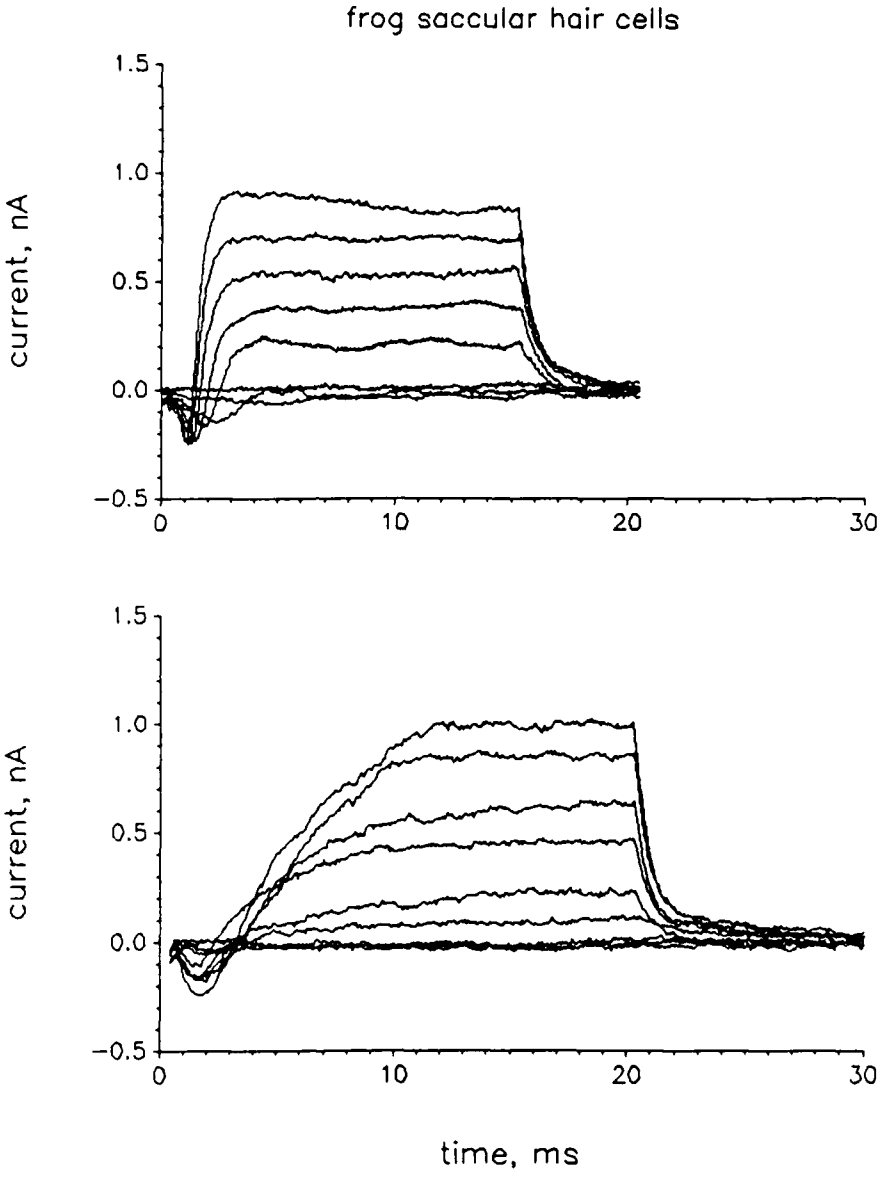

Figure 2. Whole-cell currents from two bullfrog saccular hair cells in response to depolarizing voltage steps of (in mV) 0, 20, 40, 60, 80, 100, 120, 140, and in the lower panel alone, 160; from holding potentials of -60 mV (upper) and -70 mV (lower).

We have recently begun recording from rat vestibular hair cells. An encouraging finding has been that the morphologies of the dissociated hair cells fall into two broad categories: (1) more or less cylindrical cells, reminiscent of the frog saccular cells in shape but about half the size, which we tentatively assign astype II cells; (2) cells with a pronounced amphora-like shape (Fig. 3), which we assume are type I cells. Data from a cylindrical cell are shown in Figure 4. The whole-cell currents elicited by depolarizing voltage steps appear qualitatively similar to those in chick vestibular hair cells (Ohmori, 1984), in some pigeon type II vestibular hair cells (Lang and Correia, 1989) and in frog saccular cells, albeit faster than any of our records from frog cells. Again, an inward current is followed by a sustained outward current; the latter can be seen to saturate at about +40 mV, consistent with its being dominated by a Ca^{2+}-activated K^+ current. The speed of activation of the inward and outward currents is interesting; by analogy with observations on frog saccular and turtle cochlear hair cells, the fast activation may indicate an electrical resonance that is high-frequency relative to the mechanical best frequencies of squirrel monkey primary afferents (Fernandez and Goldberg, 1971; Fernandez and Goldberg, 1976) and more in line with the mechanical best frequencies of pigeon vestibular afferents (Dickman and Correia, 1990). We have also recorded from some cells with much smaller, slower outward currents than those shown in Figure 4, and with no discernable inward current.

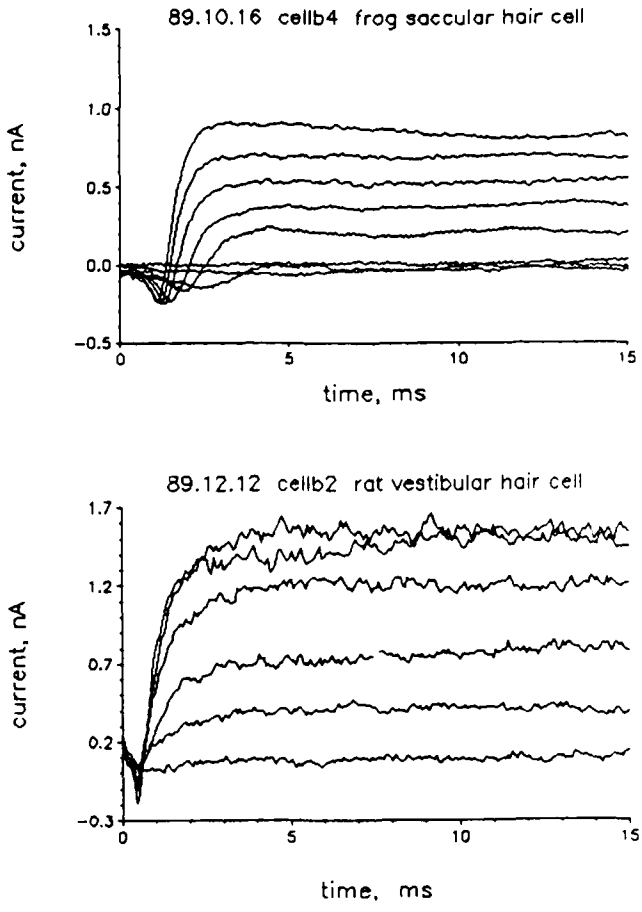

Figure 4. Whole-cell currents in vestibular hair cells from a frog (upper) and a rat (lower) during depolarizing voltage steps of (in mV) 20, 40, 60, 80, 100, 120, 140, and in the upper panel alone, 0; from holding potentials of -60 mV.

Concluding Remarks

Our preliminary results with rat vestibular hair cells suggest that we shall be able to identify isolated hair cells as being probably type I or type II, and to compare their whole-cell currents. We wish to examine whether either the mechanosensitive currents or the voltage- and ion-dependent currents differ in the two cell types, and if such differences could contribute to the documented variation in the stimulus-evoked responses of higher-order vestibular neurons.

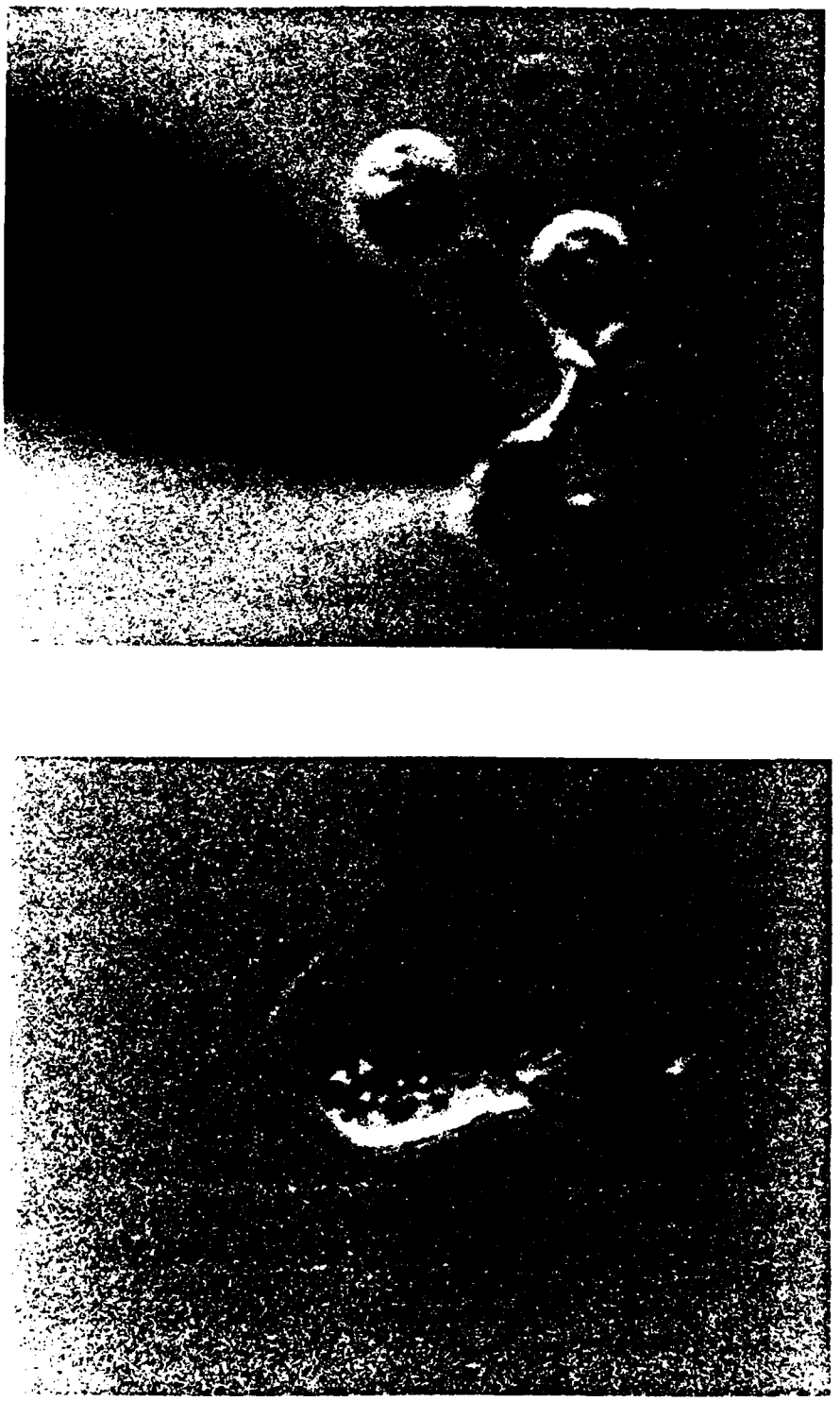

Figure 3. Rat vestibular hair cells viewed with Hoffman-modulation contrast optics at 600x. The cells are about 5 μm long. The amphora-like shape of the cell in the upper photograph suggests that it is a type I cell. A micropipette to the left of the cell is faintly visible. The cell in the lower photograph is probably a type II cell.

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