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## Electrical coupling differs in the in vitro and in vivo organ of Corti

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Electrical communication between the supporting cells of the guinea pig organ of Corti was studied. For in vitro experiments, the inner ear was rapidly removed and placed in a heated perfusion chamber. Medium 199 was used. The bony cochlea and the lateral wall (spiral ligament and stria vascularis) were removed to expose the top two coils of the organ of Corti. In vivo experiments were performed upon anesthetized animals whose cochleas were exposed surgically. A tiny fenestra was made in the bony cochlea which permitted the passage of electrodes through the lateral wall and into the organ of Corti of the third turn. Coupling was assessed by impaling neighboring cells with 3 M KCl electrodes, and noting the spread of intracellularly injected current. Coupling ratios in the in vitro preparation were consistently greater than those obtained in vivo ( $0.58 \pm 0.17$  vs.  $0.104 \pm 0.064$ ). Differences exist between the in vitro and in vivo preparations which might account for these results. In vivo the supporting cells are bathed in two different media, endolymph apically, and perilymph basally. Consequently, on their apical side the supporting cells are exposed to fluid high in  $K^+$ , low in  $Ca^{2+}$  and at a potential of 80 mV, the endolymphatic potential. In vitro the cells are bathed on all sides in fluid similar to perilymph. Intermixing the fluids in an in vivo preparation, by tearing away the stria vascularis and Reissner's membrane, increases the magnitude of the coupling ratio ( $0.455 \pm 0.209$ ). Thus the unique microenvironment of the inner ear maintains lower coupling ratios, and smaller space constants for the supporting cells.

organ of Corti, supporting cell, electrical coupling, in vivo, in vitro, gap junction

### Introduction

The organ of Corti, the sensory epithelium of the mammalian inner ear, is composed of hair cells and supporting cells. The extracellular fluid environments facing the epithelium's apical and basal surfaces are markedly different in composition. For example, the apical environment, the endolymphatic space, has concentrations of  $K^+$ ,  $Na^+$ , and  $Ca^{2+}$  of about 140 mM, 1 mM and 25  $\mu$ M, respectively (Bosher and Warren, 1968, 1978); all are maintained by metabolic processes. In addition, the electrical potential of this apical compartment is about 80 mV relative to the basal compartment, a perilymphatic space, whose ionic composition is more typical of extracellular fluid. This unique compartmentalization is required for normal auditory function.

Adjacent supporting cells in vitro (wholly incubated in perilymph-like culture media) can have coupling ratios greater than 0.8 (Santos-Sacchi,

1984, 1985). This implies that supporting cells directly interact over a wide area of the organ, potentially permitting ionically mediated changes in supporting cell tonus to spread along the organ. If coupling in vivo is comparable, then such interactions may influence cochlear micromechanics. It is important, therefore, to determine if electrical coupling among supporting cells is as good under in vivo conditions as is indicated by the in vitro studies.

### Methods

#### *In vitro*

Guinea pigs were anesthetized with pentobarbital and killed by decapitation. The whole temporal bone was placed in a perfusion chamber on a Zeiss ACM microscope, and the bony capsule around the two most apical turns was chipped away. The stria vascularis and spiral ligament were removed. Medium 199 (with Hanks salts

[1.26 mM  $\text{CaCl}_2$ , 1.7  $\mu\text{M}$   $\text{Fe}(\text{NO}_3)_3$ , 5.36 mM  $\text{KCl}$ , 0.44 mM  $\text{KH}_2\text{PO}_4$ , 0.81 mM  $\text{MgSO}_4$ , 137 mM  $\text{NaCl}$ , 4.16 mM  $\text{NaHCO}_3$ , 0.33 mM  $\text{Na}_2\text{HPO}_4$ ], pH 7.2–7.4, Gibco, NY) was perfused at a rate of 0.8 to 1.5 ml/min under an air atmosphere. The temperature of the preparation was controlled by Peltier devices utilizing a Bailey Instruments (NJ) controller unit.

### *In vivo*

A ventrolateral approach was used to expose the right cochlea of anesthetized guinea pigs. The animals were artificially respirated during surgery and experimentation. A small fenestra ( $0.3 \times 0.5$  mm) was made in the bony cochlea overlying the lateral wall (stria vascularis and spiral ligament) of the third turn. Through this fenestra electrode penetrations were made into the organ of Corti with the aid of fiber optic backlight transillumination. In order to intermix cochlear fluids the lateral wall was torn away.

Coupling measurements were made with high input impedance devices (WPI KS-700, Dagan 8100-1) capable of constant current injection. Coupling was assessed by injecting negative current pulses of varying magnitudes into one cell and noting the voltage drop in the same and an adjacent cell. Electrodes (10–15  $\text{M}\Omega$ ) were pulled on a Narashige puller; double-barreled theta glass electrodes were used to separately inject current ( $I_1$ ) and record voltage drops ( $V_1$ ) in one cell, while a neighboring cell was impaled with a single-barreled voltage recording electrode ( $V_2$ ). For *in vitro* experiments, electrodes were separately impaled into neighboring cells under visual observation. For *in vivo* experiments, a single barreled electrode was bent according to the method of Hudspeth and Corey (1979) so that it and a theta electrode could be epoxied together with the shanks parallel and tips separated by 25–40  $\mu\text{m}$ . Because the Hensen's cells are 15–25  $\mu\text{m}$  in diameter, this design permits the penetration of adjacent Hensen's cells despite the inability to view the organ of Corti beyond the lateral wall.

Coupling ratio is defined as the voltage drop in cell 2 divided by the voltage drop in cell 1 in response to current injection in cell 1 ( $V_2/V_1$ ) (Bennett, 1966). Current pulses were generated by

a D/A convertor controlled by an IBM PC/XT. Coupling ratios were determined online using a Data 6000 waveform analyzer (Data Precision, MA) in conjunction with the IBM. Membrane potentials, coupling responses, and coupling ratios were recorded on a Gould four channel recorder. Individual coupling responses were digitally stored within the Data 6000 and saved to disk.

### Results

Adjacent Hensen's cells *in vitro* are very well coupled electrically. Fig. 1c illustrates electrical coupling in a pair of cells. Current injection of a  $-5$  nA 0.8 s pulse into one cell produces a voltage drop in that same cell and in an adjacent one. The coupling ratio in this case is 0.6. Coupling ratios averaged  $0.58 \pm 0.17$  ( $n = 17$  cell pairs, 9 preparations). The input resistance of these cells *in vitro* is  $0.47 \pm 0.15$   $\text{M}\Omega$  ( $n = 47$ , 5 preparations).

Unlike the *in vitro* preparation, the *in vivo* preparation consistently demonstrates poor coupling. Fig. 2c illustrates this point. Current pulses of  $-25$  nA were injected into one Hensen's cell and the voltage drops in that cell and an adjacent cell indicate a coupling ratio of 0.12. After pulling out of the cells the endolymphatic potential is encountered; a potential of zero is obtained upon further withdrawal of the electrodes outside the scala media. Coupling ratios obtained *in vivo* averaged  $0.104 \pm 0.064$  ( $n = 40$  cell pairs, 9 preparations). The input resistance under these circumstances is  $0.43 \pm 0.23$   $\text{M}\Omega$  ( $n = 47$ , 7 preparations).

One obvious difference between these two experimental conditions is the lack of physiologic compartmentalization *in vitro*. That is, the cells of the organ of Corti are bathed on both sides by perilymph-like media, unlike *in vivo* where the fluids bathing the apical and basal compartments differ drastically in ionic composition and electrical potential. In order to evaluate whether this may be the cause for the difference in coupling ratios, the normal compartmentalization of the *in vivo* preparation was disrupted by tearing away the lateral wall of the cochlear duct as illustrated in Fig. 3. By doing so the fluids of the three scalae are intermixed and the endolymphatic potential is abolished. Since there is a greater volume of per-

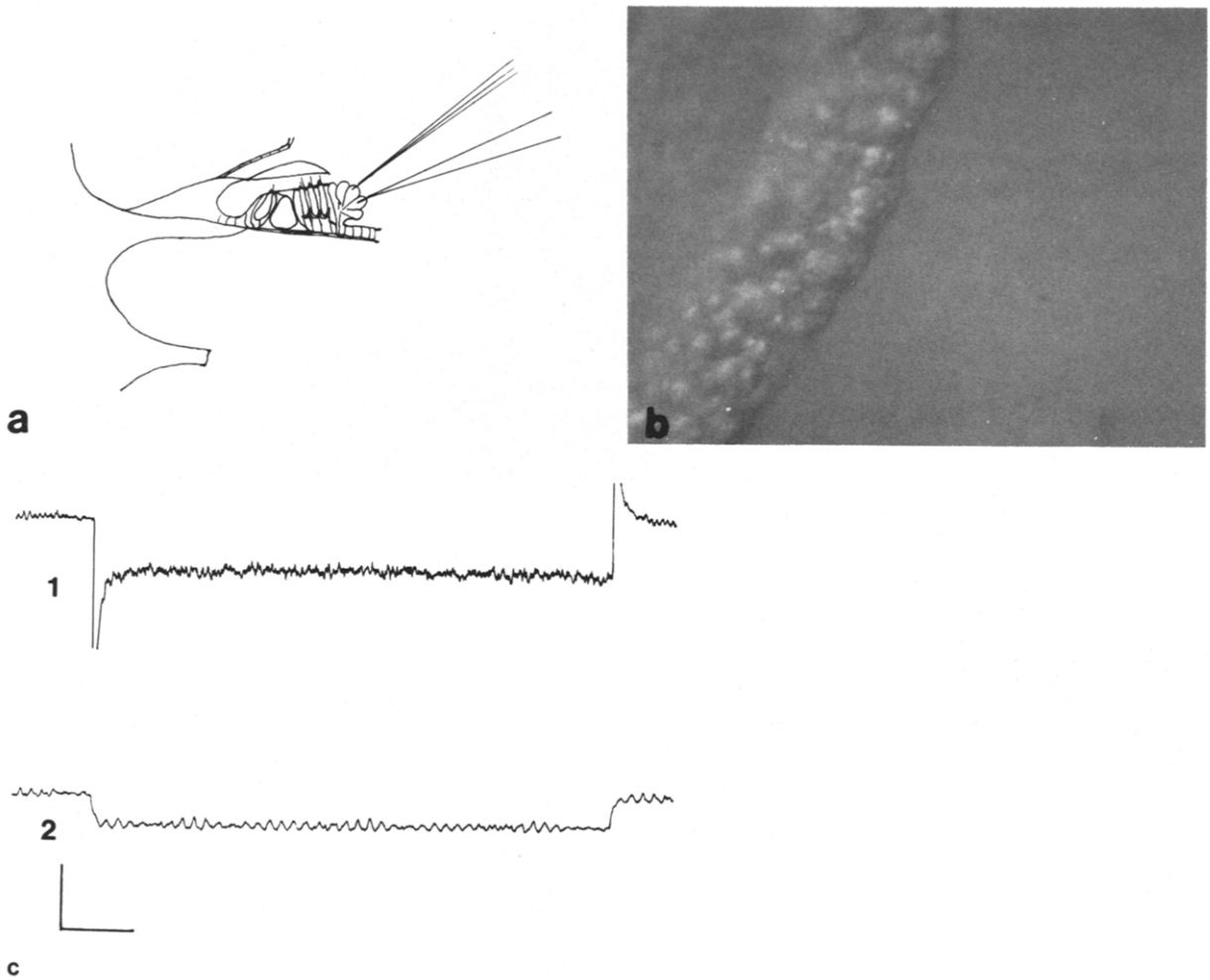


Fig. 1. (a) A schematic drawing showing the in vitro experimental setup. (b) A photomicrograph of the Hensen's cells within the perfusion chamber. (c) A trace of the voltage drop in cell 1 and the coupling response in cell 2, in response to a current pulse (0.8 s) of  $-5$  nA in cell 1. The coupling ratio in this example is 0.6. Horizontal scale, 0.1 s; vertical scale, 6 mV.

ilymph, this fluid, albeit diluted somewhat with endolymph, will surround the organ of Corti. Under such conditions it is demonstrated that coupling ratios between Hensen's cell are increased in magnitude. Fig. 3 illustrates a case where  $-20$  nA current pulses were delivered intracellularly into one Hensen's cell. The voltage drops in that cell and an adjacent one indicate a coupling ratio of 0.69. Upon pulling out of the cells, the potential drops to zero because the stria had been torn away. Coupling ratios under these conditions averaged  $0.455 \pm 0.209$  ( $n = 42$  cell pairs, 7 pre-

parations). The input resistance was  $0.50 \pm 0.21$  M $\Omega$  ( $n = 29$ , 5 preparations).

It is interesting to note that the membrane potentials of the Hensen's cells in vitro are reduced initially after stripping away the lateral wall, but that these potentials recover over time close to those recorded under normal in vivo conditions (Santos-Sacchi, 1985). This is also the case for the membrane potentials recorded in the in vivo condition after removal of the lateral wall. For example, the membrane potentials of the preparation illustrated in Fig. 3 initially measured

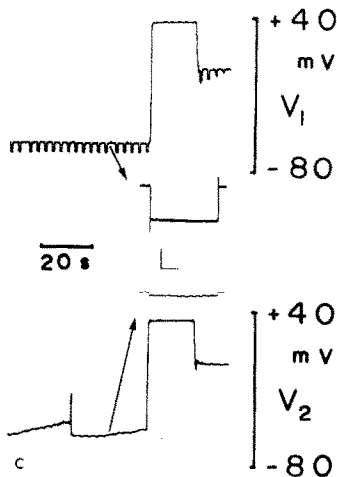
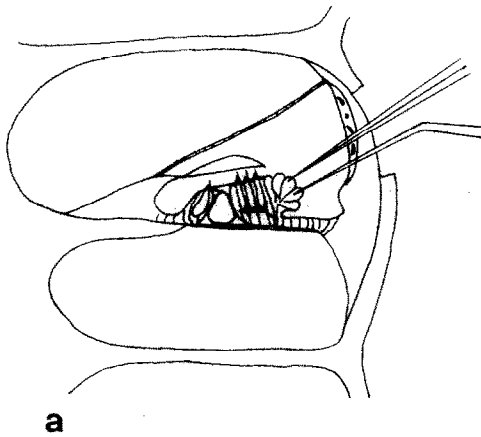


Fig. 2. (a) A schematic drawing showing the in vivo experimental setup. (b) A photo through the operating microscope showing the cochlea backlit with a fiber optic light source. The electrodes can be seen penetrating through a fenestra made in the bone overlying the third turn of the cochlear duct. (c) Traces depicting electrical coupling in vivo. Cell 1 received current pulses of  $-25$  nA. After recording coupling ratios, the electrodes were withdrawn into the scala media where the resting potential is positive. Upon withdrawal from the cochlea the potential drops to zero. The arrows indicate that region of the traces from which the expanded insets are taken. The coupling ratio in this case is 0.12. Inset horizontal scale, 0.2 s; vertical scale, 6 mV.

around  $-20$  mV following removal of the lateral wall; after a few hours they had returned to normal levels. This possibly signifies a process similar to the "healing over" which occurs in cardiac muscle and lens following injury to these tissues (De Mello, 1983; Bernardini et al., 1981). As with the in vitro inner ear preparation, coupling ratios in vivo are good throughout the recovery of membrane potentials.

## Discussion

The results demonstrate that coupling ratios in the in vitro and in vivo preparation of the organ of Corti differ in magnitude. In vivo electrical coupling is substantially poorer than in vitro. Fur-

thermore, this poor coupling appears to be due to the physiologic compartmentalization which characterizes the intact cochlea, since disruption of the organ's normal architecture (with concomitant intermixing of cochlear fluids) increases electrical coupling in vivo.

Recently, it has become clear that micromechanical interactions between outer and inner hair cells may underlie the inner ear's exquisite frequency resolving power (see Dallos, 1985). These interactions are thought to be mediated by hair cell cytoskeletal characteristics. It is also known, however, that supporting cells possess cytoskeletal elements (Slepecky and Chamberlain, 1983), which conceivably could influence organ of Corti micromechanics. Since the frequency resolv-

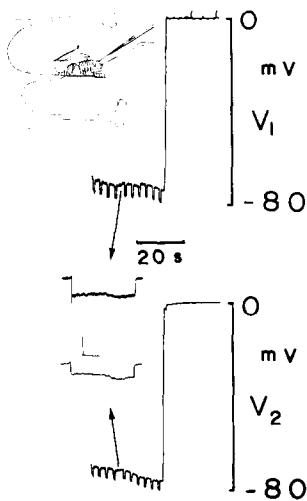


Fig. 3. After recording from the intact cochlea, the lateral wall of the cochlear duct was torn away. In the upper left portion of the figure is a schematic drawing showing the experimental setup. The traces depict electrical coupling *in vivo* after intermixing the cochlear fluids. Cell 1 received  $-20$  nA current pulses. After recording coupling ratios, the electrodes were withdrawn into the scala media. The positive resting potential of the scala media is abolished by the experimental treatment. The arrows indicate that region of the traces from which the expanded insets are taken. The coupling ratio in this case is 0.69. Inset horizontal scale, 0.2 s; vertical scale, 6 mV.

ing power of the inner ear is dependent upon the ability to physically perturb small, discrete portions of the basilar membrane, upon which the organ of Corti lies, it would appear non-advantageous that supporting cells ionically and electrically communicate over long distances. Thus it may be requisite for normal function that the inner ear's unique compartmentalization maintains low coupling ratios and space constants for the supporting cells.

Electrical coupling can be modified by alterations of cellular ionic environment. For example, acidification of cell cytoplasm can decrease coupling (Spray et al., 1981). This is also true for *in vitro* preparations of the organ of Corti (Santos-Sacchi, 1985). Although it is demonstrated here that the unique compartmentalization of the inner ear maintains low coupling ratios and space constants *in vivo*, it is not known what particular aspect of the physiological compartmentalization is responsible for this observation. We have preliminary evidence (Santos-Sacchi and Marbey, un-

publ.), however, that the endolymphatic potential is not a contributing factor. In addition, it is known that high  $K^+$  solutions do not decrease the coupling ratio *in vitro* (Santos-Sacchi, 1986b). Experimental modifications of the endolymph composition *in vivo* may provide a means to determine possible contributions.

Interestingly, Chuang et al. (1985) have found differences between electrical coupling in explanted vs. intact newt embryo epithelium. They suggested that the extraepithelial environment in the intact system promoted coupling as compared to the explant system. It is known that hormones and second messengers can influence the production and the conduction of gap junctions in sensitive tissues, such as the myometrium and sympathetic neurons (MacKenzie and Garfield, 1985; Wolinsky et al., 1985). It is conceivable that in the present experiments something similar is occurring. That is, perhaps the lateral wall produces some factor which acts via the endolymph to inhibit coupling, or the *in vitro* and *in vivo* stria removed condition provides some factor to enhance coupling. In the myometrium (MacKenzie and Garfield, 1985), estradiol treatment can actually increase the frequency of gap junctions between smooth muscle cells. It would be worthwhile evaluating, ultrastructurally, the frequency of occurrence and structure of gap junctions in the inner ear under high and low coupling conditions.

In contrast to our initial concepts (Santos-Sacchi and Dallos, 1983), it is now established that dye coupling is present in the *in vitro* preparation of the organ of Corti (Santos-Sacchi, 1986a). This suggests that metabolic cooperation may occur between the supporting cells of the organ. However, the present finding that electrical coupling in the normal *in vivo* preparation is poor could indicate that dye coupling *in vivo* is likewise poor. This may not be the case, however, depending on the reason for the poor electrical coupling *in vivo*. Coupling ratios are dependent not only upon the junctional resistance between cells but also upon the non-junctional membrane resistance. Thus coupling ratios may increase or decrease due to an increase or decrease in the non-junctional membrane resistance, without a concomitant alteration of junctional conductance. If the poor coupling *in vivo* is simply due to a lower membrane resistance

than is present *in vitro*, dye coupling may not be compromised. Yet, if the difference in coupling ratios is due to junctional conductance differences, then the degree of dye coupling may correspond with the degree of electrical coupling. Since the organ is an electrical syncytium, only input resistance can be measured directly and this measure is a function of both membrane and junctional resistances. Dye coupling studies performed *in vivo* will help determine whether junctional conductance is similar to that measured *in vitro*.

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