AN ELECTROKINETIC MODEL OF TRANSDUCTION IN THE SEMICIRCULAR CANAL

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ABSTRACT Transduction in the semicircular canal was studied by focusing an infrared beam on either side of exposed ampullae from the posterior canals of *Rana pipiens*. The direction of fluid movement resulting from a stimulus was inferred by observing the polarity of the change in afferent impulse mean rate relative to the spontaneous value. On the basis of the accepted functional polarization of this receptor, the results indicate that fluid moved toward the warmer side of the ampulla. Convection and thermal reception were shown to be unlikely explanations for these results. Morover, cupular displacements toward the warmer side would not be expected. Because thermo-osmosis can cause fluid to move toward the warmer side in a gelatin membrane, the results can be interpreted as evidence that thermo-osmosis occurred in the gelatinous cupula and influenced the transduction mechanism. Thermo-osmosis of liquids appears to be due to an electric field that is set up in a charged membrane; hence, the hair cells might have detected an electric field that occurred in the cupula during thermo-osmosis. Electroreception might be an important link in the transduction of physiological stimuli also. Rotational stimuli could result in weak electric fields in the cupula by the mechanoelectric effect. Cupular displacements could be important for large stimuli, but extrapolations to threshold stimuli suggest displacements of angstrom amplitudes. Therefore, electroreception by the hair cells could be an explanation of the great sensitivity that has been observed in the semicircular canal and other labyrinthine receptors.

INTRODUCTION

The generally accepted "displacement" model of the transduction mechanism in the semicircular canal is based on classical mechanics. Hydraulic coupling of the cupula with the endolymph is thought to transduce fluid movements into displacements of the cupula which mechanically shear the cilia on the hair cells. The shearing of cilia is thought to influence the polarization states of the hair cell membranes resulting in a modulation of the afferent nerve activity that encodes the magnitude and direction of rotational stimuli (Lowenstein, 1967).

However, this model does not account for the great sensitivity attributed to this

receptor. De Vries and Schierbeek (1953) and de Vries (1956) attempted to determine the minimum perceptible angular velocity in human subjects and concluded that no true threshold existed: the sensitivity appeared to extend to the minimum theoretical threshold (about 1 kT, where k is Boltzmann's constant and T is the Kelvin temperature) determined by the thermal energy of a single molecule. By using ruff (*Acerina cernua*) trained to respond to vibrations of the lateral line organ, Kuiper (1956) determined the experimental threshold amplitude of the vibrating cupula by extrapolating from measured skin displacements. The resulting value of 25 A agreed well with the theoretically calculated threshold of 0.3 A considering that rough estimates were necessarily used in the extrapolation. De Vries (1949) calculated that, for a threshold cupular displacement, the minimum perceptible energy per hair cell was 0.6 kT if the available energy was divided equally among the hair cells; and he noted that, since this value was less than the thermal agitation energy, the latter would trigger spurious excitations in the sensory cells. He attempted to explain this apparent discrepancy by assuming that the signal energy was concentrated on fewer than the total number of hair cells. He assumed also that simultaneous signaling from several hair cells responding in phase would require about 400 msec for sufficient cancellation of random noise to result in a detectable signal in the afferent fibers. These assumptions could be incorrect, however, as suggested by Naftalin (1965) for de Vries' similar analysis of hearing. For displacements near the theoretical threshold, the cupula itself would have insufficient signal energy to compete with random molecular interactions; and, although the conscious limit may be 400 msec, the latency of afferent responses to a rotational or thermal stimulus is so short that it appears immeasurable (Ledoux, 1958) rather than being of the order of milliseconds.

Niels Bohr (1958) discussed the general problem of modeling with classical mechanics interactions of atomic dimensions: ". . . In physical systems on the ordinary scale representation of events as a chain of states described by measurable quantities rests on the circumstance that all actions are here large enough to permit neglect of the interaction between the objects and the bodies used as measuring tools. Under conditions where the quantum of action plays a decisive part and where such an interaction is therefore an integral part of the phenomena, there cannot to the same extent be ascribed a mechanically well-defined course." In the present context, transduction in the displacement model is represented as a chain of states in which angstrom movements of whole bodies are the measurable quantities, but in which thermal agitation would be the quanta of action that would obscure displacements of atomic dimensions. Hence, this model is not sufficiently well defined mechanically for a receptor whose sensitivity appears to be limited only by thermal agitation in the sensory cells.

The most direct evidence that cupular displacements are relevant to transduction are light micrographs of stained cupula and endolymph obtained *in situ* that show

cupulae displaced in response to rotational, thermal, or pressure stimuli (Steinhausen, 1931; Dohlman, 1935; Ledoux, 1958; Trincker, 1962). This evidence, however, does not exclude the possibility that such cupular displacements could be absorbing excess energy, resulting from large stimuli, that might even be overloading a sensitive molecular mechanism responsible for the transduction of more physiological stimuli. Moreover, because cupular displacements resulting from threshold stimuli appear to be of atomic dimensions, light micrographs are not conclusive evidence that cupular displacements are necessary for the transduction of small stimuli. In conclusion it appears possible that the transduction mechanism is sensitive to more than one form of energy. In that case, cupular displacements might be effective stimuli for large accelerations whereas forces resulting from local electric fields in the cupula might be effective stimuli near the threshold.

The hair cells appear to be both functionally and morphologically polarized. Studies in which the canals were mechanically stimulated by either direct pressure or rotating the animal showed that the polarity of change in afferent firing rates was dependent on the direction of the net fluid displacement in the ampulla, and that this "functional polarization" was the same in all units innervating a given canal (Lowenstein, 1967; Lowenstein and Sand, 1940; Ledoux, 1958; Trincker, 1962). This polarization might be functionally related to the morphological polarization observed by Lowenstein and Wersall (1959). All hair cells in the vertical canal were oriented with their kinocilia located on the side of the hair bundle that pointed away from the utricle, but, in the horizontal canal, the kinocilia pointed toward the utricle. This suggested that the individual hair cell could be the unit of functional polarization. The displacement model should indicate how the moving of hairs in opposite directions could result in afferent responses of opposite polarities. Wersall (1967) attempted to explain this by speculating that the positions of the hairs could mechanically regulate "energy rich bonds" in oriented chains of protein molecules located in the cuticular region of the hair cells. Wersall assumed that movements of the hairs in opposite directions would then result in opposite polarization states of the cell membrane; and the membrane potential would then regulate the rate of synaptic transmission to the afferent nerve endings.

An alternative approach is to reject the premise that the bending or shearing of hairs is necessary for transduction. For example, some hypotheses have proposed mechanisms based on the elastic deformation of macromolecules. Dohlman (1960) suggested that the "bending or straining" of macromolecules by fluid movements could produce static electric charges on the hairs that would induce charges of opposite polarity inside the hair cells. This mechanism is analogous to energizing a capacitor. Other hypotheses have been suggested by the observation of Vilstrup and Jensen (1961) that acid mucopolysaccharide solutions exhibited stable potentials of a few mv to above 100 mv when the solutions were elastically strained in glass capillaries. Christiansen (1964) suggested that these displacement potentials were

caused by the bending of threadlike hyaluronate molecules. He proposed that if these molecules were attached at one end of the surface of the hairs, a displacement of the cupula with its subcupular substance would cause the macromolecules to bend, resulting in changes of current distribution over the external surfaces of the hairs. Christiansen assumed, with Dolhman, that the hairs could act as capacitors. Christiansen suggested that the external current distribution would then induce a distribution of opposite polarity inside the hair cell that would influence the polarization state of the cell.

In a related hair cell system in the cochlea, Naftalin (1965) proposed that the transfer of energy through protein-metal-water complexes in the tectorial membrane could be relevant to the transduction of mechanical energy resulting from sound stimuli. He noted that the hydrated gelatinous structure of the tectorial membrane suggests that it could exhibit both the icelike properties of structured solutions and the phonon-electron interactions of crystalline lattices. Naftalin concluded that the tectorial membrane could be considered a crystalline solid without centers of symmetry in the unit cells and should therefore exhibit piezoelectric properties.

Models of transduction that included electrical, as opposed to purely mechanical, forces could be particularly relevant in view of the evidence that certain hair cell receptors are sensitive to weak electric fields. Dijkgraff (1967) has reported evidence based on the slowing of heartbeats indicating that dogfish can detect electric field gradients of the order of 0.01 μv/cm; the sites for electroreception are the ampullae of Lorenzini that are found in the skin of elasmobranchii (Dijkgraff, 1967). When local electric fields were applied with stimulating electrodes near the skin, Murray (1967) reported a threshold sensitivity of 1 μv/cm gradient for the impulse responses of single units that innervated ampullae of Lorenzini in the ray. These electroreceptors have hair cells in the base of a jelly-filled ampulla, and each hair cell has a single cilium that extends into the jelly (Murray, 1967). Although electroreceptor hair cells differ morphologically from hair cells that are found in the labyrinth, the latter might also be sensitive to weak electric fields. Lowenstein (1955) found evidence that afferents from the ampullae of the ray exhibited responses to galvanic stimulation of the ampullae. The responses reversed in polarity when the stimulus polarity was reversed.

This report will describe an experiment designed to test whether displacements of the cupula are a necessary part of the transduction mechanism. Experimental results which support an electrokinetic hypothesis of transduction will be presented and discussed.

THEORY

When an animal is rotationally accelerated, endolymph is displaced around the canal relative to a coordinate system fixed in the head. According to the displacement model, the hydraulic coupling between the cupula and endolymph would result in

cupular displacements that precisely followed the endolymph displacements. However, for incomplete momentum transfer from the endolymph to the cupula, the effect of an endolymph displacement would be to establish a pressure difference between the two boundaries of the cupula. An alternative to the displacement model would be one in which the pressure gradient across the cupula was the initial link in the transduction mechanism that, because of the cupular microstructure, established an electric field inside the cupula.

Anatomical evidence, reviewed by Iurato and de Petris (1967), suggests that the cupula has a microstructure resembling that of a dispersed reticular gel. A polyelectrolyte gel would have charged sites located internally; and this charged structure would be expected to exhibit electrokinetic effects similar to those that have been observed in charged membranes. Three of these effects, the mechanoelectric effect, electro-osmosis, and thermo-osmosis, are relevant to an electrokinetic model of transduction.

Electrokinetic effects can occur in charged membranes as a result of interactions among mechanical and electrical flows and forces. For a membrane maintained at temperature T, these interactions can be described by the phenomenological relations (de Groot and Mazur, 1962)

$$J_m = -\Lambda_{vv}\frac{\Delta p}{T} - \Lambda_{ve}\frac{\Delta \varphi}{T} \tag{1}$$

$$i = -\Lambda_{ev}\frac{\Delta p}{T} - \Lambda_{ee}\frac{\Delta \varphi}{T}, \tag{2}$$

where J_m is the mass flux, i is the current, the Λ's are phenomenological coefficients and Δp and $\Delta \varphi$ are the pressure and potential differences, respectively, across the membrane. The validity of these relations is independent of assumptions about the microstructure of the system.

In the steady state $i = 0$, and $\Delta p \neq 0$, the relations of equations 1 and 2 describe the "mechano-electric effect" (de Groot and Mazur, 1962)

$$\left(\frac{\Delta \varphi}{\Delta p}\right)_{i=0} = -\frac{\Lambda_{ev}}{\Lambda_{ee}}. \tag{3}$$

Equation 3 shows that a potential gradient that is proportional to a pressure difference across the membrane can occur in a charged membrane. On the basis of his detailed analyses of electrokinetic effects in charged membranes, Teorell (1966) suggested that mechanoelectric coupling similar to that shown in equation 3 could be influential in the transduction mechanisms of mechanoreceptors.

When the conditions $\Delta p = 0$ and $i \neq 0$ (resulting from an external field) are main-

tained, equations 1 and 2 then describe electro-osmosis as a mass flux that is proportional to the steady-state current (de Groot and Mazur, 1962)

$$\left(\frac{J_m}{i}\right)_{\Delta p=0} = \frac{\Lambda_{ve}}{\Lambda_{ee}}. \tag{4}$$

The phenomenon of thermo-osmosis was first described and named by Lippmann (1907). A gelatin membrane separated two masses of water that were maintained at different temperatures, and a flow of water occurred through the membrane from the cold to the hot side. The rate of flow through a round gelatin membrane 6 cm in diameter was about 50 mg/min for a temperature difference of about 80°C. Subsequent extensions of Lippmann's experiment, reviewed by Carr and Sollner (1962), indicated that the effect depended on the presence in the membrane of water-soluble substances and that the effect occurred only while these solutes remained within the membrane. Carr and Sollner (1962) studied the thermo-osmosis of electrolytes through charged membranes and found that the direction and rate depended on the charge of the membrane and the nature and concentration of the electrolyte. They concluded that their observations strongly supported Lippmann's hypothesis that the thermo-osmosis of electrolyte solutions through charged membranes is electrokinetic in nature and that its mechanism is probably closely related to that of electro-osmosis in isothermal systems. A theoretical study of thermo-osmosis by Kobatake and Fujita (1964) was in close argeement with this data and led to the conclusion that thermo-osmosis is an electro-osmotic flow caused by the electric field which is set up in the membrane so that no net electric charge is allowed to be transported across it.

An electrokinetic model of semicircular canal transduction would be the following: a rotational stimulus would result in a pressure gradient in the cupula, and, as predicted by the mechanoelectric effect (equation 3), an electric field would be generated. This field would then interact with the hair cells by a mechanism to be considered in the discussion.

An experimental test of this model was suggested by the observation that the mechanoelectric effect, electro-osmosis, and thermo-osmosis (considered as a form of electro-osmosis) should each occur in the cupula, as in a porous charged barrier, when the proper set of boundary conditions is maintained. Indeed, because of Onsager's relation, $\Lambda_{ev} = \Lambda_{ve}$, the effects described by equations 3 and 4 can be related by Saxen's relation (de Groot and Mazur, 1962)

$$\left(\frac{\Delta p}{\Delta \varphi}\right)_{J_m=0} = -\left(\frac{i}{J_m}\right)_{\Delta \varphi=0}, \tag{5}$$

which has been experimentally verified. If thermo-osmosis in the cupula would cause mass to flow toward the warmer side, analogous to Lippmann's results with gelatin

membranes, the following experiment would exclude one of the two models of transduction represented in Fig. 1.

The cupula is represented as a frictionless adiabatic piston in the displacement model (Fig. 1 *a*, *c*, *e*) and as a stationary gelatin membrane in the electrokinetic model (Fig. 1 *b*, *d*, *f*). The canal is in the vertical plane with the ampulla at the top. A rotational acceleration, approximated in Fig. 1 by its tangential component $\ddot{x}$, would cause a mass flux J_m in the same direction in both models. Heating the fluid in one side of the ampulla would, however, cause opposite effects. Indeed, the mass flux J_m should be directed toward the *cooler* side in the displacement model (Fig. 1 *e*) because of thermal expansion and toward the *warmer* side in the electrokinetic model (Fig. 1 *f*) because of thermo-osmosis. It might be argued that the piston in

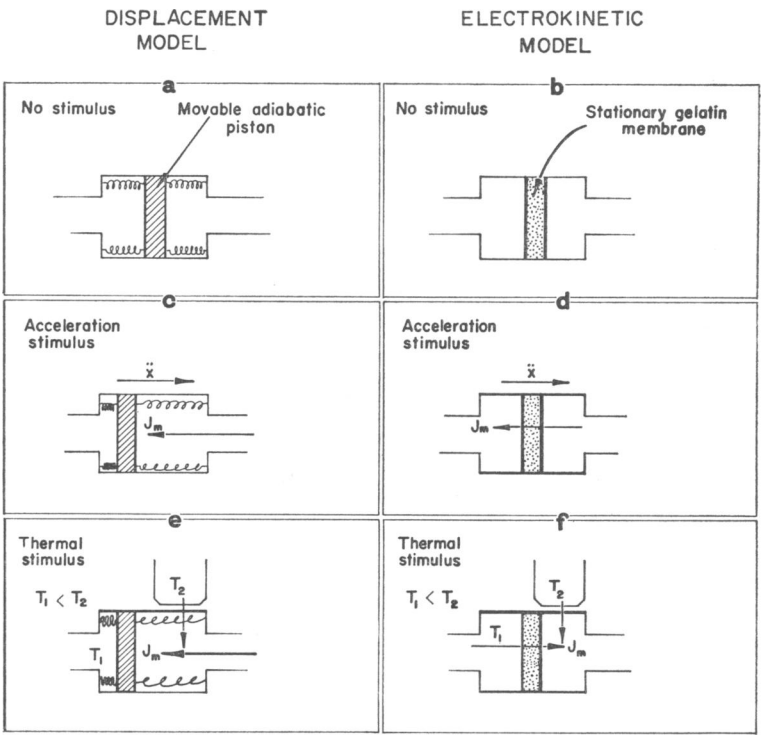

FIGURE 1 Flow responses of two alternative models of transduction in the ampulla. Left column: displacement model. Right column: electrokinetic model. (*a*) A movable adiabatic piston is centered in a cylinder in the absence of a stimulus. (*b*) No net fluxes occur through the gelatin membrane in the absence of a stimulus. (*c*) Accelerating the ampulla toward the right causes a flux of mass J_m and piston displacement toward the left; $\ddot{x}$ is the tangential component of a rotational acceleration of the ampulla. (*d*) Accelerating the ampulla toward the right causes a flux of mass J_m toward the left. (*e*) Heating the right side results in thermal expansion and this should produce piston displacement toward the left. (*f*) Heating the right side results in a flux of mass J_m toward the right because of thermo-osmosis.

Fig. 1 *e* should not be displaced because the shunt (not shown) formed by the canal and utricle would equalize the pressures on each side of the piston. This would be true if endolymph was an ideal fluid enclosed by rigid walls; but, even then, displacements toward the warmer side would not be expected because the piston should remain stationary. However, the great sensitivity of this receptor would allow it to detect the consequences of the nonideal hydrodynamic properties of this system (e.g. slight fluid compressibility), and thermal expansion in a compressible fluid would result in a piston displacement in the direction shown in Fig. 1 *e*.

The direction of mass flux J_m resulting from a heat stimulus could be inferred from the polarities of the afferent responses. Afferents from a vertical canal will respond with a decreased impulse frequency when J_m is directed from the center of the ampulla toward the side adjoining the utricle and with an increased impulse frequency when J_m is oppositely directed. This method would detect only those fluid movements that were transduced into afferent impulses, so it is a suitable criterion for excluding one of the two alternative models of transduction represented in Fig. 1.

METHODS

Adult *Rana pipiens* were pithed and their heads were severed at the joint of the first and second vertebra. The ampulla of the posterior vertical canal on the right side was exposed by enlarging the foramen of the endolymphatic duct. The bone covering the ampulla was first thinned with a dental burr and then carefully removed with a fine forceps. The endolymphatic sac and an overlying vascular plexus were removed in order to permit access to the posterior branch of the eighth nerve. The ampulla was placed uppermost in the vertical plane of the canal. In order to thermally insulate it, the ampulla was exposed to air by aspiration of the surrounding fluid before responses were recorded. Nerve activity from first-order afferents was recorded from the 1 mm portion of the dendritic branch that innervated only the posterior vertical canal. Multicell activity was recorded from small bundles of nerve fibers by suspending them in air on a silver wire electrode similar to the method of Ledoux (1958). Alternatively, glass pipette suction electrodes (Hartman and Boettiger, 1967) with fire-polished tips of 10–30 μ inner diameter and filled with saline, were used to record spike trains from single cells. Stable afferent activity was observed for 3–4 hr after severing the head in agreement with Ledoux (1958). Experiments were terminated after 3 hr. The heat source was light from a tungsten filament directed through a red filter. The infrared light was focused on the large end of a fiber optic image conduit (Edmund Scientific Co., Barrington, N. J.) that had been heated and hand drawn to form a tapered bundle of 0.5 mm diameter at the smaller end. The stimulus was applied by placing the smaller end of the image conduit above one side of the ampulla as shown in Fig. 2. Glass bead thermistors (Fenwal No. G170, Fenwal Electronics, Inc., Framingham, Mass.), 0.014-inches in diameter, were mounted on ceramic insulators and placed at each end of the ampulla in contact with the external surface of the membranous canal. The thermistor outputs were differentially amplified in order to show the approximate direction and magnitude of the temperature gradient along the major axis of the ampulla, considered as an ellipsoid, resulting from a heat stimulus. The thermistor record was recorded on one channel of analogue tape and the afferent spike activity was recorded on another channel. Afferent responses were observed with the thermistor circuit first on and then off in order to control potential artifacts resulting from the self-heating of the thermistors. The recorded spike records were later played back through a one-shot discriminator with its thresh-

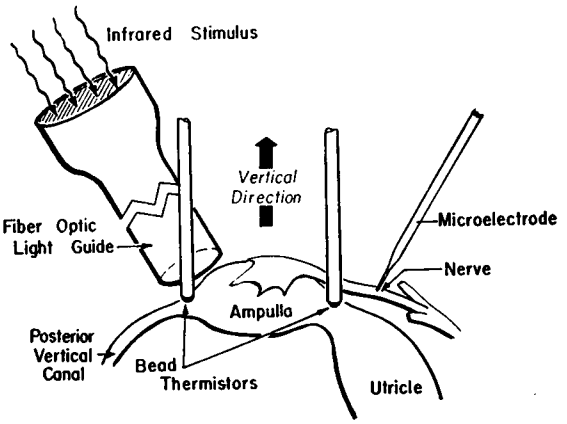

FIGURE 2

old set to exclude noise. The discriminator output pulses were integrated by a ratemeter with a time constant of 1.6 sec and the ratemeter output was recorded on moving film. The filmed records of single cells were calibrated in impulses per second by driving the ratemeter with regular pulse trains of known frequencies. Interspike interval histograms of single cell records were plotted with a Nuclear Data Enhancetron 1024 (Nuclear Data, Inc., Palatine, Ill.).

RESULTS

Fig. 3 shows the afferent multicell responses that were observed in a fiber bundle as a result of heating each side of the ampulla of the posterior vertical canal from above. It is representative of the responses observed in 11 other preparations. The heated side of the ampulla is indicated in the inset above each set of three successive responses. The maximum temperature changes that were recorded during a stimulus were a 2°C increase from the thermistor on the heated side and also a 2°C differential between the two thermistors positioned as indicated in Fig. 2. These values are upper bounds because the stimulus probably caused some direct heating of the thermistor on the heated side.

An increase of activity (Fig. 3 *a*) or a decrease (Fig. 3 *b*) was observed depending on whether the heat was applied to the canal or utricular sides of the ampulla, respectively. On the basis of the accepted functional polarization of this receptor, these results indicate that the mass flux J_m was directed toward the warmer side of the ampulla. The electrokinetic model (Fig. 1 *f*) predicts that thermo-osmosis should cause flow toward the warmer side of the ampulla; whereas the displacement model (Fig. 1 *e*) predicts that thermal expansion should cause flow toward the cooler side of the ampulla. Therefore, the results shown in Fig. 3 exclude the displacement model and establish evidence for the electrokinetic model. Before this conclusion can be accepted, however, it is necessary to exclude experimentally several alternative explanations for the responses of Fig. 3.

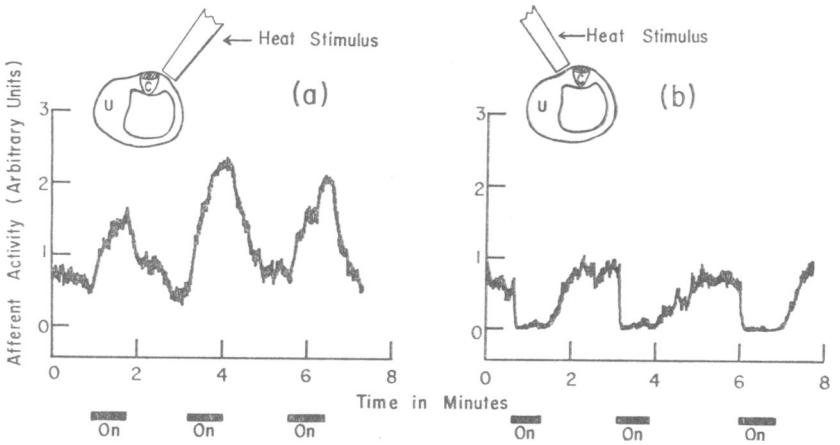

FIGURE 3 Multicell responses to heating each side of the ampulla of the posterior vertical canal from above. Zero on the ordinate corresponds to the discriminator threshold; all activity and noise less than threshold are represented as zero. The increase in (*a*) and the decrease in (*b*) both imply that J_m was directed toward the side being heated which is shown in the insets.

Convection

Convective fluid displacements might be an alternative explanation for the responses of Fig. 3. Ledoux (1958) found evidence that convection effects occurred in the canals of isolated heads of frogs in response to flowing warm or cool water on the tympanum — a stimulus analogous to that used clinically in the caloric test. Ledoux's stimulus technique would cause fluid in one limb of the canal to become warmer and less dense than fluid in the other limb. The resulting hydrostatic imbalance would then cause a convection current. Ledoux's criterion for the direction of net fluid movement was the polarity of the afferent responses, and his results indicated that fluid in the warmer limb of the canal was displaced upward toward the ampulla as would be expected from convection.

In order to test the effects of convection, about 1 mm of the canal or utricle adjacent to the ampulla was heated with a focused light beam. Fig. 4 *a* shows the biphasic responses of a single afferent cell to a light beam focused on half the ampulla and the adjacent portion of the utricle. These biphasic responses are representative of those observed in other preparations under similar stimulus conditions, and they imply that J_m reversed in direction. The initial decrease implies flow toward the warmer side in agreement with the responses of Fig. 3, and the later increase implies flow toward the cooler side in agreement with Ledoux's results. In Fig. 4 *b*, the first response shows a similar initial decrease; at the arrow, however, the light beam was moved further away from the ampulla toward the utricle. This caused a rapid increase compatible with flow toward the cooler side. Successive responses (Fig. 4 *b*)

to the light beam at the new position show only the increase. When the beam was focused on a segment of the canal adjacent to the opposite side of the ampulla (Fig. 4 *c*), the same cell showed a decrease in mean rate indicating flow toward the cooler side.

These results indicate that the focused light beam caused responses in agreement with Ledoux's evidence for convection only when the heated fluid was located initially below the level of the ampulla and that convection responses (Fig. 4 *b*, *c*) were of the opposite polarities of those in Fig. 3. The responses of Fig. 3 occurred when the local heating was applied to the ampulla oriented at the top (Fig. 2) such that convective flow caused by hydrostatic pressure differences would have been minimized. Hence, convective mass flow is not a valid alternative explanation for the responses of Fig. 3.

Local convection currents might have occurred in the heated side of the ampulla that could have caused flow toward the warmer side. But solutions to the Benàrd problem of classical hydrodynamics (Chandresekhar, 1961) showed that a critical adverse temperature gradient must be present in a layer of fluid heated from below before thermal instability occurs in the form of convection cells. Because the ampulla was heated from above, rather than from below, it is unlikely that convection cells resulted.

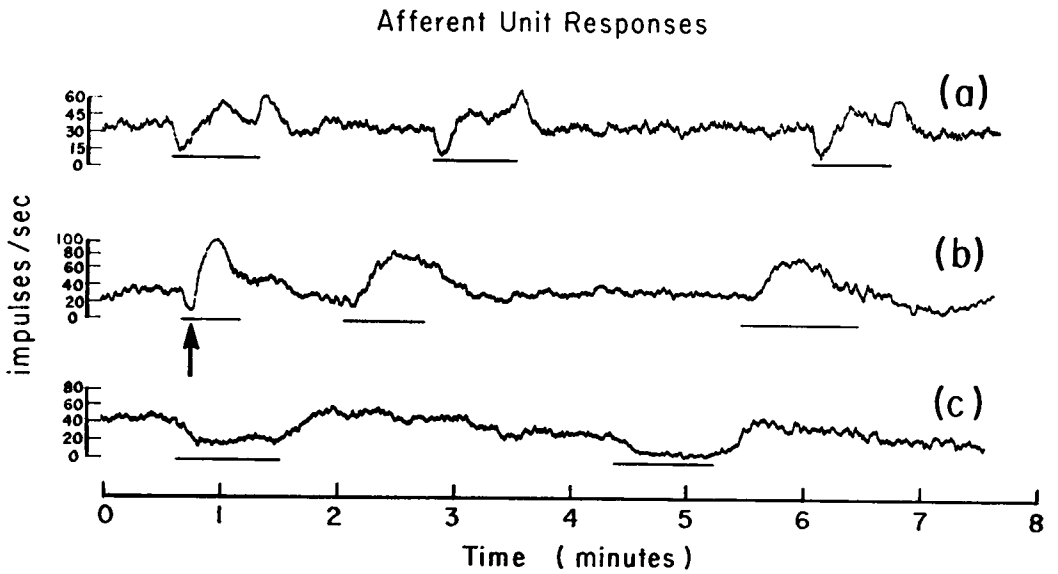

FIGURE 4 Afferent unit responses to a focused infrared beam. Bars indicate stimulus is on. (*a*) Biphasic responses caused by a beam focused on half the ampulla and an adjacent region of the utricle. (*b*) Same stimulus as (*a*), but at the arrow the beam was moved further toward the utricle (away from the ampulla) and left there. (*c*) Same single unit as in (*b*), but the beam was focused on the canal adjacent to the opposite side of the ampulla.

Temperature Reception

Another explanation is that temperature changes of the ampulla that were unrelated to transduction might have caused the responses of Fig. 3. But this is unlikely because the responses reversed in polarity when the opposite side of the ampulla was heated. If the responses had been caused by temperature changes, the direction of the spatial gradient of temperature would have had to determine the polarities of the responses. Murray (1962), in a review of physiological temperature receptors, concluded that the spatial gradient direction appeared to be of no importance, even for altering the responses of the same polarity, compared with the actual temperature change. Hence, a temperature receptor effect also is an unlikely alternative explanation for the responses of Fig. 3.

The Isolated Head

Another possibility is that isolating the head could have resulted in anomalous afferent activity that would not have occurred in the intact animal. However, Ledoux (1958), after extensive investigations, concluded that no differences in vestibular afferent activity were discernible between intact and isolated preparations of *Rana pipiens*. This agrees with the conclusion of Gualtierotti and Alltucker (1966) that afferent activity recorded from isolated preparations "correspond to the true physiological firing characteristics."

DISCUSSION

The polarity of the main effect described above (e.g. Fig. 3) is opposite to that predicted by the displacement model of semicircular canal transduction. This result implies that the receptor may in fact be sensitive to thermal effects that resemble the thermo-osmosis of liquids in charged membranes.

Thermo-osmosis, an electro-osmotic phenomenon attributed to the volume forces caused by the electric field that occurs inside a charged membrane (Carr and Sollner, 1962; Kobatake and Fujita, 1964), may occur as discussed in detail above in the gelatinous cupula in a manner analogous to that of liquids in gelatin membranes (Lippmann, 1907). Individual cilia on the hair cells appear to extend into channels in the cupula (Iurato and de Petris, 1967). It appears warranted to speculate that the cilia could have interacted with an electric field that occurred during thermo-osmosis in the cupula and this interaction could have been transduced by the hair cells.

The magnitudes of fields occurring during thermo-osmosis can be estimated from studies on charged membranes. Carr and Sollner (1962), for example, considered that thermo-osmosis in membranes 30–50 μ thick resulted from thermopotentials of about 1 mv, i.e. field strengths of about 0.2 v/cm, or less. This field magnitude is small relative to those which usually influence membrane depolarization; specialized electroreceptors, however, are sensitive to fields as weak as 1 μv/cm (Murray,

1967). This suggests that, even after the occurrence of dissipative energy losses, fields resulting from electrokinetic effects in the cupula could be sufficiently large to be detected by the hair cells in the semicircular canal, especially if the latter preserve the high electrosensitivity of lateral line organs.

As discussed above, an electric field could result also, by the mechanoelectric effect (equation 3), from the conditions imposed by a physiological rotational stimulus. The generation and transduction of a mechanoelectric field was represented in the theory section as the electrokinetic model. The sensitivity of this model would be limited theoretically by the effects of thermal agitation in the initial mechanoelectric transduction described by equation 3. A quantitative estimate of the phenomenological coefficients in equation 3 would require knowledge of the boundary and initial conditions; specific physical models could be based on displacement potentials, streaming potentials or solid-state effects (Naftalin, 1965). Qualitatively, however, the electrokinetic model does not require macroscopic movements of the cupula or the cilia; hence dissipative energy losses from frictional forces opposing these movements need not occur. Indeed, after a field was established initially by the mechanoelectric effect, the only dissipative energy losses would be current shunts and dielectric losses unrelated to the transduction mechanism.

Hence, it is of interest to consider what physical process might account for the detection of weak fields by the hair cell. The membranes of the cilia and the upper surface of hair cells appear to be impermeable to ions (Dohlman, 1960), so the detection of fields would appear to be due to electrostatic forces as opposed to ionic currents. Dissipative energy loss of an electric field in a dielectric (dielectric loss) is generally associated with movements of charge carriers and the effect of these movements in an electric field is called polarization (Jackson, 1962). The average molecular dipole moment $\langle \mathbf{P}_{mol} \rangle$ in a heterogeneous system is the vector sum of contributions from various sources (van Beek, 1967): (*a*) induced (electronic) polarization resulting from the relative displacement of electrons and nuclei, (*b*) dipolar polarization resulting from the partial alignment in the direction of the field of molecules with permanent dipole moments, (*c*) interfacial (Maxwell-Wagner) polarization occurring at boundaries between the components of a heterogeneous system. $\langle \mathbf{P}_{mol} \rangle$ is related to the macroscopic polarization $\mathbf{P}$ (electric dipole moment per unit volume) and the macroscopic electric field $\mathbf{E}$ as follows (Jackson, 1962):

$$\mathbf{P} = N\langle \mathbf{P}_{mol} \rangle = \chi_e \mathbf{E}, \tag{6}$$

where N is the number of molecules per unit volume and χ_e is the electric susceptibility.

A weak electric field in the cupula might be detected in the hair cell by the polarization it induces in the long-chain filaments of polyatomic molecules in the cilia. At least two types of induced polarization could occur. (*a*) Studies of dielectric behavior of colloidal solutions, as cited by van Beek (1967), show evidence that particles such

as polystyrene are frequently surrounded by electric double layers when they are dispersed in dilute KCl solutions. Low frequency electric fields then polarize the molecules by inducing dipole moments in the double layers. (*b*) Frohlich (1958) noted that large molecules can have CH_3, C=O, or OH groups that are in themselves dipolar, but that the vector sum of the individual dipole moments can cause the total dipole moment of the molecules to be zero. These molecules then behave like nonpolar molecules in that their polarizations are of the induced (electronic) type with resonant frequencies in the optical range. A rigorous description of induced polarization requires the use of quantum mechanical perturbation techniques. A semiclassical description however based on a distributed charge Ze that is harmonically bound in a molecule yields the same expression for the induced dipole moment as would the quantum mechanical derivation. As pointed out by Jackson (1962), each charge e that is displaced a distance x can be considered bound by a restoring force

$$\mathbf{F} = -m\omega_o^2\mathbf{x}, \tag{7}$$

where m is the mass of the charge and ω_o is the (optical range) frequency of harmonic oscillation. The action of a field E causes the charge to be displaced a distance x from its equilibrium position such that

$$e\mathbf{E} = m\omega_o^2\mathbf{x}. \tag{8}$$

The induced dipole moment is then defined as

$$\mathbf{P}_{\text{ind}} = e\mathbf{x} = \frac{e^2\mathbf{E}}{m\omega_o^2} \tag{9}$$

for one electron. If there are Z electrons per molecule with f_j of them bound by a restoring force $-m\omega_j^2\mathbf{x}$, then the induced dipole moment is

$$\mathbf{P}_{\text{ind}} = \frac{e^2}{m}\sum_j \frac{f_j}{\omega_j^2}\mathbf{E}, \tag{10}$$

where $Z = \sum_j f_j$.

Temperature is not a variable in equation 10 so the induced polarization would not be disrupted by thermal agitation (Jackson, 1962). The sensitivity of this effect for the detection of weak fields would be limited by quantum considerations as opposed to the classical limit of kT. These quantum limitations would be set by the magnitudes of the allowable shifts in energy levels of the molecules caused by the field, considered as a small perturbation, relative to the (degenerate) energy levels of the molecules in the absence of the field. The great sensitivity of a mechanism based on polarization can be estimated from the following argument. If the behavioral

threshold of stimulus energy is indeed close to 1 kT $\simeq 4 \times 10^{-14}$ ergs/molecule as suggested by de Vries (1949), the corresponding wave number $1/\lambda$ for an energy transition of kT

$$hc/\lambda = kT \tag{11}$$

would be $1/\lambda \simeq 200$ cm^{-1} if this energy were absorbed entirely by a single molecule. But, presumably, the threshold energy would be distributed among large numbers of molecules so the polarization of a single molecule would have to occur for energies much smaller than kT, i.e. for transitions of far less than 200 cm^{-1}. The splitting of energy levels by an electric field (the Stark effect) has been studied spectroscopically by observing the splitting of spectral lines (Herzberg, 1950). Splittings of about 10^{-3} cm^{-1} have been observed from diatomic molecules in field strengths in the range 10^{2}–10^{3} v/cm. However, smaller "hyperfine" splittings were predicted theoretically and can be observed by using high resolution spectrometers. So the occurrence of hyperfine splittings in the Stark effect suggests that a transductive mechanism based on polarization would be sufficiently sensitive for the detection of threshold stimuli. Transduction in the hair cell could then be viewed, in this hypothesis, as a quantum amplification process that is modulated by the average microscopic polarization of an ensemble of long-chain molecules associated with the cilia.

A physiological rotational stimulus would result in responses of the same polarities in both the electrokinetic and the displacement model (Fig. 1 *c* and *d*). The cupular transduction mechanism could then be sensitive both to electrokinetic effects and to mechanical displacements, and thereby have an extended range of sensitivity to rotational stimuli. The results in Fig. 3 imply that, if this were the case, the electrokinetic mechanisms would be more sensitive than the displacement one, at least for stimuli near threshold. Both mechanisms might be detected by a similar molecular effect in the hair cell because mechanical forces should also affect the microscopic polarization of long-chain molecules. Frohlich (1958) cited evidence that the experimental relaxation times of the polarization of dipolar molecules is dependent on the chain length of the molecules and pointed out that there is better agreement between theory and experimental data when the molecules are considered flexible rather than rigid dipoles. Hence, the bending or shearing of cilia that contain long-chain molecules could result in a change in the net dipole moment and should also be detected by a transduction mechanism that was sensitive to microscopic polarization.

Because all labyrinthine receptors (i.e. canal, utricle, saccule, and cochlea) have morphological similarities, electroreception might be a general property of the labyrinth. The evidence that some otoliths are strongly piezoelectric (Morris and Kittleman, 1967) or composed of calcite which is electrically anisotropic (Panofsky and Phillips, 1955) would suggest that electric fields in the macula could be of significance for transduction in the utricle and saccule. Naftalin (1965) pointed out possible electrical influences in transduction in the cochlea and emphasized that electrical

properties resembling those observed in crystalline solids might also occur in the tectorial membrane. The cupula, otolithic macula and the tectorial membrane show structural differences; these imply that electric fields, if important to transduction, would not necessarily be generated by the same mechanisms in all cases. However, it is characteristic of a field theory that the interaction of a field with a system of charges should not depend explicitly on the detailed nature of the field sources (Panofsky and Phillips, 1955). So although the sources of an electric field might differ in different receptors in order to match the dynamics of different adequate stimuli, it would still be physiologically meaningful to attempt to determine a transductive mechanism for the interaction of an electric field with the hair cells that could be operative in all hair cell receptors. The hypothesis of microscopic polarization discussed above could be one such mechanism.

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