

The application of a capacitive probe technique for direct observation of electromechanical processes in the guinea pig cochlea

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The demonstration of evoked mechanical responses of the outer hair cells in the mammalian cochlea by indirect measurements introduces a new range of problems into direct mechanical measurements. Direct and indirect measurements indicate that the frequency spectra of evoked electromechanical responses may extend well into the range of audio frequencies, revealing a need to develop terminology and protocols for distinguishing evoked mechanical responses from the traditional traveling wave when both are apparently superimposed on the motion of the basilar membrane in the normally functioning cochlea. Details are presented of a frequency-modulation capacitive probe technique for measurement of vibrating structures of the guinea pig ear. Considerations include the design of the transducer, calibration, sensitivity, linearity, and sources of noise, as well as the influence of the technique upon the animal preparation, and in particular the issues associated with draining scala tympani for the measurement. Relative advantages and disadvantages of the technique are compared with salient features of other techniques currently available. In view of the apparent complexity of cochlear mechanics some preliminary experiments are required to elucidate some of the key questions about reverse-transduction processes in general. A "simple" first experiment is to test existence of any rectifying or motile response.

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INTRODUCTION

The indirect demonstration of active electromechanical processes in the mammalian cochlea has significantly modified objectives of direct mechanical measurements of the motion of the basilar membrane. Among the more significant related findings are those which have implicated an active electromechanical role for the outer hair cells (OHC) in normal cochlear processing. The first of these results showed that the mechanics of the cochlear partition could be altered by stimulation of the crossed olivo-cochlear bundle (COCB) (Mountain, 1980; Siegel and Kim, 1982). Since the COCB synapses with the OHCs, electrical properties of the OHC membrane will be altered by stimulation and by reverse transduction, mechanical properties such as compliance and damping are altered to produce the changes observed in the ear canal.

Most relevant to the present study are the observations of Brownell and Kachar (1986), who showed that isolated guinea pig OHCs in culture respond to pulsed electric currents with rapid length changes of the cell body of up to 0.6 μm , or 1% of their length. Flock *et al.* (1986) and Zenner (1986) have reported OHCs may undergo very large decreases in length in the presence of adenosine triphosphate (ATP) and micromolar concentrations of Ca^{2+} .

If the length changes due to electrical stimuli and also the calcium-triggered changes are indicative of normal pro-

cesses in the cochlea, *in vivo* mechanical measurements may show some form of electromechanical behavior. The OHC bodies are firmly attached between the basilar membrane and the reticular membrane. On the other hand, the reticular and tectorial membranes are separated by an array of rigid links formed by the OHC stereocilia (Lim, 1980). Depending on the mechanical loading of the OHC *in situ*, "dc" movements of all three membranes may amount to several hundreds of nanometers or possibly more. Vibratory movements of the basilar membrane appear to be 0.4 nm at threshold (Sellick *et al.*, 1982) and extend upward to 30 nm. Therefore, any active movement due to the OHCs constitutes a potentially significant second mode of movement of the cochlear partition in response to the traveling wave. Cochlear potentials exhibit strong rectification characteristics but, as yet, analogous behavior has not been studied at length in the mechanics due to difficulties in carrying out such measurements.

Direct measurements of basilar membrane vibratory motion have been carried out by a variety of methods (Johnstone and Boyle, 1967; Khanna *et al.*, 1968; Rhode, 1971; Köhllöffel, 1972; Wilson, 1973; Wilson and Johnstone, 1975; LePage and Johnstone, 1980; Sellick *et al.*, 1982; Robles *et al.*, 1985). Each method appears to have significant advantages over the others in certain respects; however, all have significant disadvantages. The velocity-sensitive techniques (Mössbauer and laser-Doppler methods) do not transduce the mean position of the basilar membrane and have been used to produce frequency response characteris-

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tics only for the vibratory component of the motion. Capacitive probe techniques have been used previously in hearing research by von Békésy (1960) and Wilson and Johnstone (1975). The technique offers the advantage of being relatively straightforward to apply to the measurements of the basilar membrane. The advantage of the capacitive probe technique for the present study (LePage, 1987) is its high sensitivity to displacement and short averaging times, coupled with a sufficiently wide dynamic range over which the electrical output is essentially linear. These characteristics appear to be essential for a complete description of basilar membrane motion.

An early set of measurements of the basilar membrane of the guinea pig using the capacitive probe described here (LePage and Johnstone, 1980; LePage, 1981) suggested that, in addition to the traveling wave, the basilar membrane may exhibit another low-frequency or dc component. This appeared as both a frequency- and level-dependent shift in the position of the basilar membrane in response to clicks, which appeared to correlate with significant nonlinear compression at the characteristic frequency at the place being measured. Notably, both the nonlinear compression and level dependence in the low-frequency component were absent in guinea pig preparations treated with kanamycin and shown to lack OHCs (LePage *et al.*, 1980).

The objective of this paper is to consider the relevant features of the capacitive probe technique and introduce some key issues in making direct measurements of electromechanical behavior of the cochlear partition. For a discussion of methodologies which depend upon preliminary results, the reader is referred to LePage (1987).

I. PREPARATION OF THE GUINEA PIG

A. Mounting the preparation

Pigmented guinea pigs weighing 200–250 g are anesthetized using the neuroleptanesthesia approach of Evans (1979), beginning with a dose (25 mg/kg, IP) of sodium pentobarbitone and followed by Innovar Vet (0.35 cc/kg/h, IM; equivalent to 0.14-mg fentanyl, 7-mg droperidol per dose). Following anesthetization, the preparation is paralyzed with Flaxedil and is artificially respired. Paralyzing the preparation is necessary to eliminate periodic contractions of the tensor tympani which visibly move the tympanum. The animal's head is clamped rigidly between two horizontal earbars and a snout clamp. The body is positioned prone on a specially designed animal table so as to minimize the effect of body respiration and heartbeat movements on the head.

B. Opening of the bulla

The bulla is opened using the ventrolateral approach (Wilson and Johnstone, 1975), which avoids the extensive surgery of the ventral approach. Access to the bulla is obtained by removing the thin bone adjacent to and posterior to the tympanic membrane, revealing the schematic view shown in Fig. 1. The N_1 electrode was placed on the bony curtain adjacent to the round window. A reference electrode is placed in the neck muscles for recording the compound

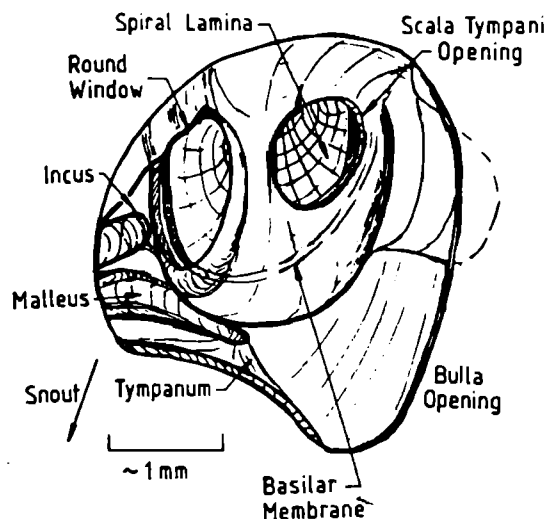


FIG. 1. A "wide-angle" view looking at the left guinea pig cochlea and ossicles through the bulla opening via the ventrolateral approach. The opening for mechanical recording must be larger than for spiral ganglion cell recording due to the need to place the permanent wick. The opening here is for access to the 15-kHz point.

action potential (N_1) and for single-unit recording. At this time, an N_1 audiogram is obtained for reference purposes. This is obtained by visual detection of the presence of the N_1 response on an oscilloscope screen in response to gated, 25-ms tone bursts with 1-ms rise times.

Nuttall and LaRouère (1980) have shown that opening the bulla may cause a substantial drop in cochlear temperature over the period of an experiment. This may explain the findings of LePage (1981) in which a rectal temperature of 38.5 °C was necessary to avoid early loss of hearing sensitivity. Cochlear temperature was not monitored in these experiments and may be a factor in the long-term deterioration of the preparation. The N_1 threshold, however, serves as a blanket measure of animal condition and neural threshold. As such, retention of low N_1 thresholds tends to indicate absence of deterioration of cochlear function.

C. Opening the wall of scala tympani

The wall of scala tympani is opened at about 3 mm from the base. This is carried out as noiselessly as possible by first thinning the bone with a blade. Neonatal guinea pigs are used since the cochlear bone becomes exceedingly hard and brittle in the adult animal. Entering the fluid-filled cavity always produces an efflux of perilymph under pressure which is absorbed by a wick prepositioned beside the opening to remove any blood the instant it is released into the perilymph. This wick is replaced periodically to limit any accumulation of fluid in the middle ear cavity.

Figure 1 depicts an opening so as to provide access to the basilar membrane. The dissection procedure is aimed at producing the smallest hole which allows simultaneous insertion of the capacitive probe and a wick for maintaining scala tympani drained during the mechanical measurement. Disruption of the spiral ligament damages the preparation permanently. A large vein runs inside the wall of scala tympani at about 2.5 mm from the basal hook. In most cases, this vein must be removed to gain access for the mechanical measure-

ment, but in the process it discharges blood into the perilymph, which coincides with a loss of neural sensitivity. It has not been possible to determine whether this loss is due to interference with the vasculature of the basal turn or whether the formation of potent clotting factors results in loss of sensitivity (Johnstone and Humphrey, 1978). It was observed, however, that noise due to fast suction of the blood-containing perilymph using a pipette causes a loss of sensitivity, so this procedure was abandoned and the blood was taken out by gentle capillary action using hand-held wicks.

D. Placing the permanent wick

Once the scala tympani is drained, the next step is to insert a permanent wick. A strip of diaper-liner material is precut about 300 μm wide and about 10 mm long in the direction of the parallel fibers. One end of this is inserted through the wall opening, bridging the basilar membrane 500 μm above, and is laid along the spiral lamina bone beside the basilar membrane. Since the spiral lamina is much more rigid than the wick, the procedure of placing the wick does not of itself produce a significant movement of the basilar membrane or consequent loss of N_1 sensitivity, provided that the spiral ligament is not dislodged. Once the wick is in position, the rest of the wick is laid along the bone outside the cochlea, to which it adheres through surface tension. The free end pokes out of the bulla. During mechanical recording a small suction pipette is brought into contact with the free end of the wick and provides very effective draining of the fluid over the basilar membrane. The draining can be very gentle, taking up to 20 s to drain the basal turn, while still reducing the fluid covering the basilar membrane to no more than 5 μm in depth. This being completed, the capacitive probe is positioned over the basilar membrane ready for the first recording, as shown in Fig. 2. The suction is removed and scala tympani is allowed to refill. In the fresh preparation refilling takes less than 1 min.

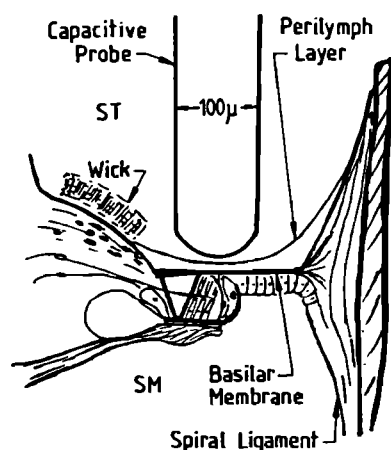


FIG. 2. Cross section of guinea pig basal turn as opened with scala tympani (ST) at the top and the capacitive probe in the recording position. The view shows approximate dimensions of the structures and the normal working level of the fluid with the suction applied via the wick resting on the spiral lamina adjacent to the basilar membrane.

E. Checks on hearing sensitivity

An N_1 threshold audiogram is again obtained while scala tympani is full. The N_1 is checked with tone pips of 10-ms duration and 1-ms rise time. All instances where a single N_1 threshold is quoted hold for a stimulus frequency corresponding to the probe position according to the frequency-place relation (Robertson and Johnstone, 1979) for the guinea pig first turn of

$$f = 45 \times e^{-0.277x}, \quad (1)$$

where f is the frequency (kHz) and x is the distance (mm) from the hook at the base of the cochlea. All instances where a threshold is quoted for a particular measurement (LePage, 1987) are obtained from a level-dependent series of measurements, interspersed with the frequency series.

Mechanical recordings begin typically after 45–60 min of preparation. Substantial threshold losses may have occurred by this time. Since this amount of threshold loss did not accompany single-neuron spiral ganglion experiments (LePage, 1981), the poorer yield (33%–50%) would appear to reflect increased trauma to the mechanical preparation in making the larger wall opening. Figure 3 shows an N_1 audiogram series typical of earlier capacitive probe experi-

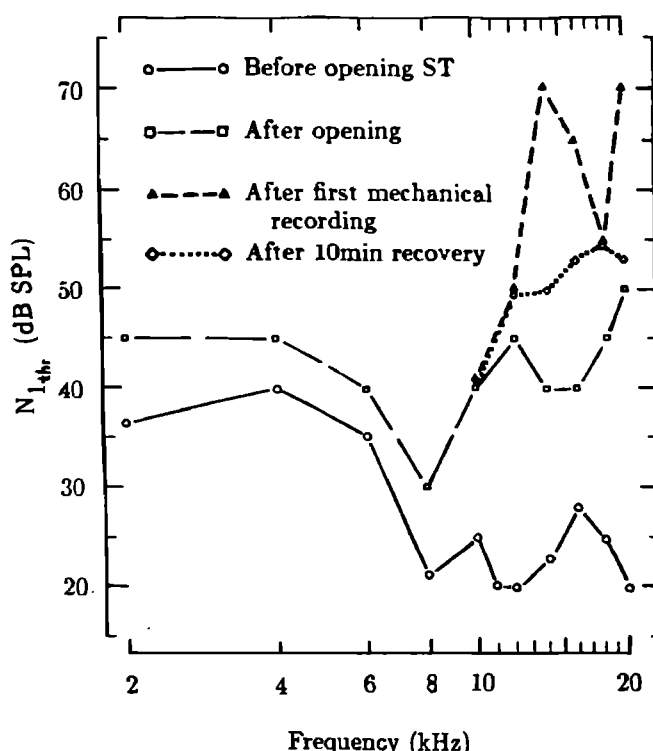


FIG. 3. An example of the use of the compound action potential (N_1) threshold audiograms for monitoring condition of the preparation throughout the capacitive probe experiments. While techniques to limit the loss upon opening have improved since these early data were collected, comparison of the circles and squares shows the type of loss which may occur to damage the preparation upon opening the wall of scala tympani wide enough for mechanical recording. The curve with triangles shows the relatively severe, but partially reversible losses which may occur as a result of mechanical recording. If sound levels exceeding 80 dB are used, the total threshold loss can amount to 70 dB, suggesting mutually potentiating effects of draining with loud sound.

ments (LePage and Johnstone, 1980). The curve with circles represents a typical low-threshold preparation. Opening scala tympani and placing the wick can result in losses of 20–30 dB (squares). Mechanical recording itself appears to produce further substantial losses (triangles) which may recover slightly (diamonds). Later experimental procedures reduced the losses to 20 dB. These later procedures include limiting the sound levels delivered while the scala tympani is drained, since draining and loud sound appear to have mutually potentiating effects on sensitivity loss (Alder, 1978).

II. THE CAPACITIVE PROBE

A. Design and implementation

1. Electronic circuit diagram

The capacitive probe transduces the vibrational displacement of the basilar membrane in real time. It was constructed from a prototype of a frequency modulation (fm) design by Yates (1976). The displacements of the moving surface cause changes in the frequency of a modified Colpitts (Clapp) oscillator operating at 56 MHz. The output is heterodyned with a 51.5-MHz crystal oscillator to 4.55 MHz, where an audio signal is produced by a standard commercial fm detector. The schematic circuit diagram of the transducer is shown in Fig. 4. The changes from Yates' design include an increase in the frequency of the carrier from 43.5 MHz plus the inclusion of a variable capacitance diode for remote fine tuning of the probe oscillator for maximal sensitivity. The head-stage oscillator is mounted in a copper shell 1 in. in diameter and 1.75 in. long. It is connected to the demodulator housing by a three-wire shielded cable. All other electrical connections to the transducer are attached to

the demodulator. This head stage is mounted to a micromanipulator with fine movement controls in line with the probe and perpendicular to the table on which the animal is mounted.

2. Probe tips

The tip used for basilar membrane motion measurements in the first turn of the guinea pig cochlea is 100 μm in diameter and has a ground flattened hemispherical tip. The width of the basilar membrane in the region between 2 and 4 mm from the base is approximately 150 μm . The actual tip is a piece of stainless steel wire 6 mm long and is bent into a configuration normal to the surface of the basilar membrane, while the probe shaft attached to the head stage is normally about 20° to the horizontal. For middle ear measurements the probe tips used are somewhat larger, between 1 and 2 mm², so that the smaller movements of the ossicles yield a larger signal, increased in proportion to the area. Tips larger than the standard size require a heater element, supplied by dc current from a battery, to prevent condensation on the tip.

3. Methods of reduction of condensate on probe tip

Unlike the amplitude-modulation probe of Wilson (1973), which requires remedial attention in the event of the probe tip contacting the perilymph, no special care was required for this unshielded probe tip. To prevent condensate from building up on the probe, it was found that fabrication of the probe tip with a metal of low thermal conductivity such as stainless steel reduced the heat-sinking effect to an acceptable level. Any residual tendency to fluid accumula-

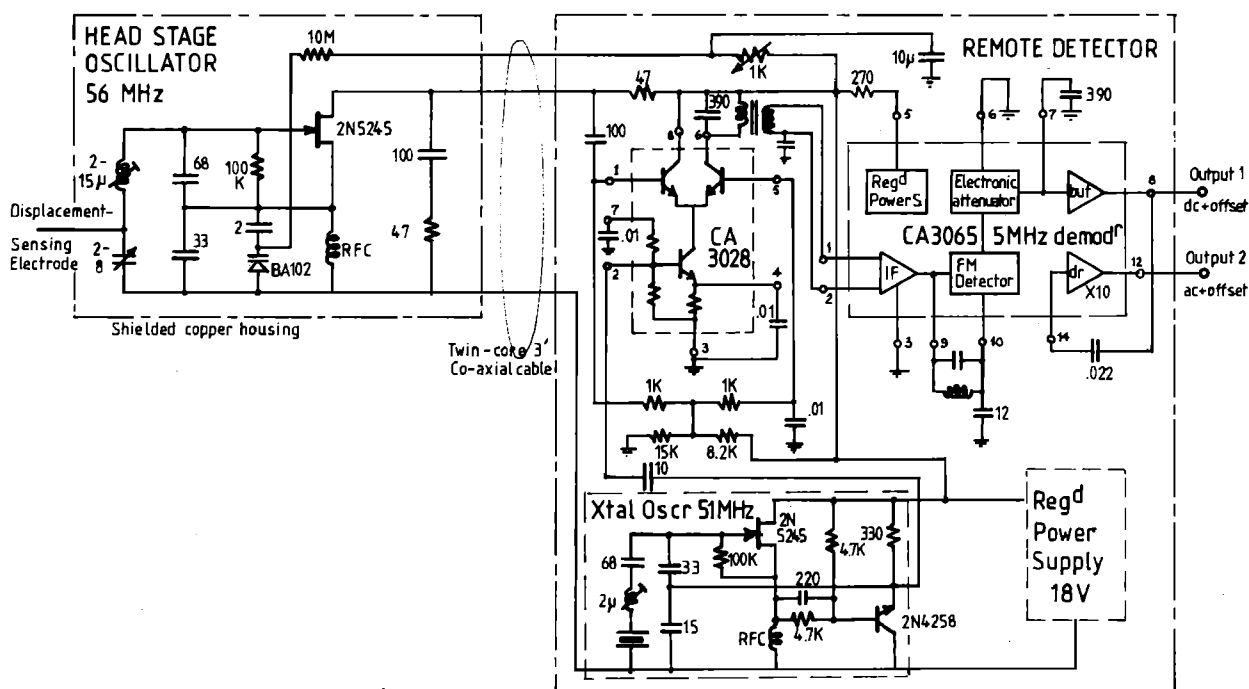


FIG. 4. Electronic circuit schematic diagram of the fm capacitive probe. The dc output is fed into a specially designed amplifier to remove the offset introduced by the demodulator plus any rf content in the output signal. The transducer outputs an electrical signal proportional to the displacement of the moving object. The head stage is held in a micromanipulator and may be easily positioned. Units are multiples of ohms, farads, and henrys as appropriate.

tion was prevented by coating the tip with a thin film of high-temperature grease. When scala tympani was drained for a measurement it was necessary to break the meniscus surrounding the probe tip, but there was no further tendency for the fluid to adhere to the tip.

4. Conditioning of the capacitive probe output signal

The capacitive probe output is connected to a specially designed low-drift amplifier having a gain of 800 and a three-pole active antialiasing filter with a low-pass breakpoint of 25 kHz. The low-frequency characteristics of the amplifier are selectable—it may be dc or ac coupled (12 dB/oct RC filter) with a high-pass breakpoint of 4 Hz (time constant of 40 ms).

5. Probe polarity

Displacement of the vibrating object toward the tip of the probe makes the dc output from the device go negative. The signal-conditioning amplifier is noninverting, so in the basilar membrane records shown (LePage, 1987), displacement toward *scala vestibuli* is *positive going* and displacement toward *scala tympani* is *negative going*.

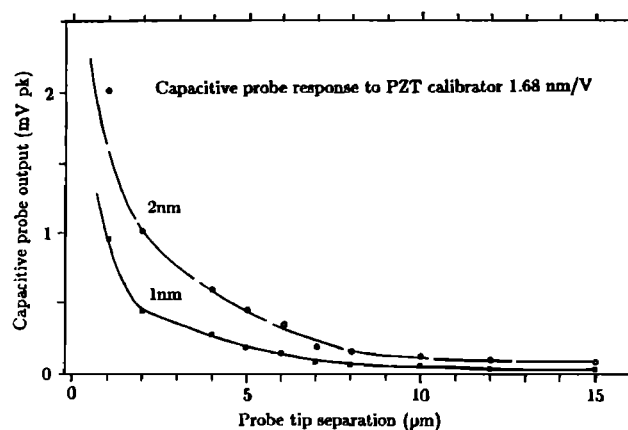


FIG. 5. The sensitivity of the capacitive probe to two fixed vibration levels of a piezoelectric (PZT) rod at 3.87 kHz as a function of probe tip separation—determined by stepping the probe micromanipulator increasing in steps from a condition with the tips touching. There is clearly error in the determination of the point of touching and hence the greater errors in the determination of the characteristic for small distances. Calibration of the PZT rod determined by the supplier at two different times was 1.68 and 1.77 nm/V. The calibration figure for the rod obtained by this procedure is of the same order. For an estimated working distance of 2–3 μm the probe output is 1 mV for 2-nm movement.

B. Performance characteristics

1. Calibration and sensitivity versus distance

A piezoelectric cylindrical rod (PZT), which vibrates in the direction of its axis, was used to calibrate the response of the capacitive probe. The tip of the probe was brought adjacent to the electrically grounded tip of the PZT. The manufacturer-supplied calibration for the rod for a 1-kHz voltage applied to the PZT is 1.68 nm/V. Figure 5 shows the output signal of the probe expressed in mV (pk) for two amplitudes of vibration of the PZT, 1 and 2 nm. The distance of separation was increased monotonically, so as to minimize backlash, from tips touching to 15 μm apart. For an estimated separation of 2 μm and a PZT movement of 2 nm, the capacitive probe outputs a 1-mV signal at 3.88 kHz. The figure shows that doubling of the calibrator amplitude doubles the output signal from the probe, indicating linearity of the transducer response for usual working distances.

2. Linearity

Figure 6 shows a further linearity check on the probe/PZT combination at three distances set 2 μm apart, with the closest approximately 2 μm from the calibrator. It shows that for levels above noise level, the curves grow essentially linearly. Here, the noise level of ca. 0.025 mV for 100 repetitions of the stimulus represents table vibration and electrical noise in the transducer equivalent to 0.5 nm without averaging and 0.5 \AA with averaging. The lower panel shows that the corresponding curves for the second harmonic are essentially at noise level for those frequencies, indicating that if baseline offsets are apparent, they are not likely to be due to distortion resulting from axial asymmetry in the probe itself. The dynamic range subtended by these points is determined by the maximum voltage available to drive the PZT.

3. Frequency response

A convenient check was to record the motion of a polarized Brüel and Kjær (B & K) type 4134 condenser microphone diaphragm as a function of distance of the probe from the diaphragm. The probe frequency response was flat up to 13 kHz. For frequencies above about 10 kHz, however, the response of the condenser earphone diaphragm is not flat because of the transition to higher modes of vibration than the fundamental mode (Wilson, 1973). For the frequencies of interest (< 20 kHz) the response of the combination is flat to within 10 dB.

4. Spatial localization

a. Location of measurement along cochlear partition. All distances in this study have been estimated on the basis of actual measurements taken (LePage, 1981), after marking the measurement place, removing the temporal bones, and measuring in 1-mm steps from the hook. Based on the figure of 2.5 mm/oct for the guinea pig from an extrapolated hook frequency of 45 kHz, these estimations were found to be within an error of ± 0.2 mm at the 2.5-mm point and within 300 μm at the 4-mm point, as determined from actual cutoff frequencies of these magnitude curves. The present series of measurements were all intended to have a common site of about 3.5–4 mm from the base. The probe was positioned according to the various landmarks established in the LePage (1981) study.

b. Radial location of probe tip across basilar membrane. It is possible to set the tip of the probe anywhere from about 25%–75% of the radial width of the basilar membrane at the 3.5-mm place. For the sake of consistency, the probe tip was set at a distance of $40\% \pm 10\%$ (visually judged) from the

Capacitive probe vs PZT calibration at 3.87 kHz

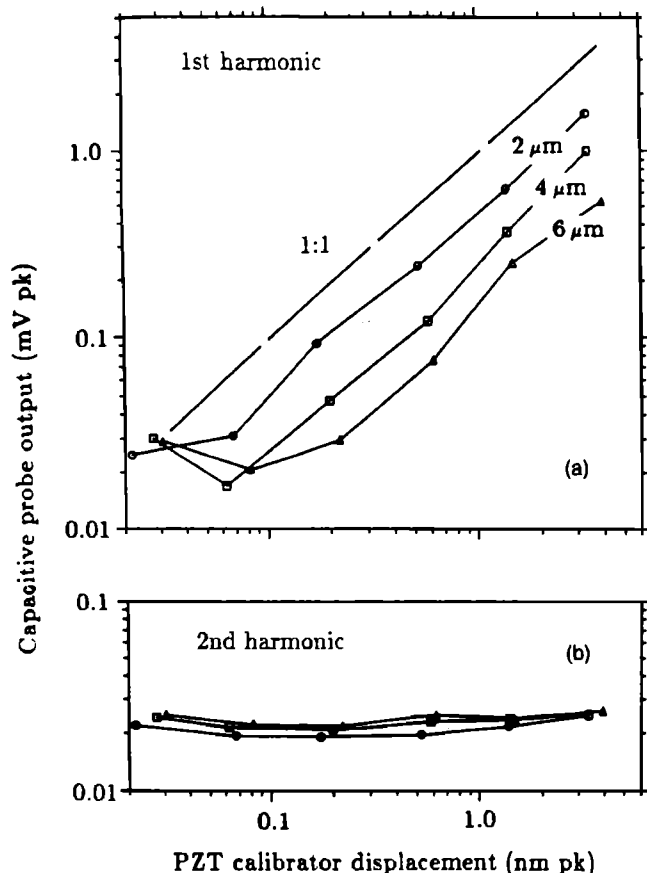


FIG. 6. Linearity check on the output of the capacitive probe/PZT rod calibrator combination. The top panel represents the output ac voltage values at 3.87 kHz from the capacitive probe for three different separations between the probe tip and PZT tip of 2- μ m steps. The curve representing the largest signal is for a separation of approximately 2 μ m while the signal drops as the separation is increased (4 and 6 μ m). The abscissa represents the ac amplitude of the PZT calibrator (nm), while the ordinates are the amplitude of the resulting ac signal from the dc output of the probe (mV). The dashed line represents a slope of 1 dB/dB, representing linear increase. The lower panel is the corresponding determinations for the second harmonic of the capacitive probe output signal.

edge of the basilar membrane on the spiral lamina (modiolar) side.

c. Extent of field of detection. Wilson (1973) found it desirable to shield the probe tip both to limit electrical interference and also to limit the field of spatial detection. Although shielding the probe tip was investigated in a variety of ways, it proved advantageous not to shield the probe tip. First, shielding contributed to the residual tip capacitance, limiting the sensitivity of the device. Second, by not shielding the probe, the tip could be bent so as to provide better orientation to the basilar membrane while limiting the size of the opening into scala tympani. Third, limiting the mass of the probe tip provided a way of limiting the heat-sinking effect of the tip and hence eliminating the need for a heater element to prevent condensate buildup. The smaller tip therefore provided better visualization using the binocular microscope.

Estimates of the field of detection suggest that shielding

is unnecessary for the purposes of limiting the field. Figure 5 may be used to give a first-order measure of the field of detection of the probe. The figure shows how the sensitivity of the probe falls off with distance. The probe tip can be retracted along a line *normal* to the basilar membrane by 5 μ m, for a halving of the measured displacement signal, i.e., a separation much less than the radius of the probe tip. By the time the tip is withdrawn 13 μ m, the sensitivity has dropped 20 dB. The capacitive probe is therefore most sensitive to detection of movements within a 5- μ m radial region surrounding the 50- μ m radius of the probe tip. For the probe tip located 2 μ m from the basilar membrane, the relative contribution to the total capacitance change at distances greater than 20 μ m is negligible for practical purposes. This sets the frequency-resolving ability of the technique to at least 1/25 oct. By comparison with cochlear potential measurements which integrate over several millimeters (Dallos, 1973), the mechanical measurements may be regarded as obtained from a length of basilar membrane subtended by up to ten hair cells.

5. Directional sensitivity

Since the method of detection is capacitive, the probe senses displacements normal to the closest point to the probe tip. By comparison, optical methods tend to sense displacements normal to the basilar membrane. Both experimental approaches, therefore, have advantages and disadvantages. In practice, the basilar membrane mean position is not fixed, but tends to move toward scala tympani during the course of an experiment. This may create alignment problems for optical methods because the angle of reflection can alter by approximately 30° over a period of time. Such alignment problems do not exist for the unshielded probe tip, which will record the full displacement and not the resolved part of the motion in the direction of the light beam.

6. Dynamic range

For basilar membrane recordings, the noise level of the probe signal, without filtering, is less than 5 μ V rms. The largest signals normally recorded are approximately 3.5 mV rms due to respiration and heartbeat movement. This defines a dynamic range of approximately 60 dB which may be increased by signal averaging. Averaging 64 or 400 repetitions of a tone burst signal increases this range by 18 and 26 dB, respectively, giving typical input noise levels *in vivo* of 0.5 and 0.2 μ V, respectively. The number of repetitions used per average is effectively a tradeoff between the desired input noise level and the time over which significant drifts in operating conditions may occur. Preparation stability is somewhat unpredictable, yet in better preparations one can count on periods of up to 10 min in which stable fluid drainage conditions can be maintained. Recording times of 2 min are typical for a set of trials of ten frequencies or intensity levels.

7. Displacement range

The capacitive probe has a wide displacement range over which it will operate linearly. While providing sensitivity to nanometer displacements, the transducer can move

simultaneously through $1\text{ }\mu\text{m}$ or more with typically a 6-dB change in the sensitivity (see Fig. 5). By comparison, the laser interferometer (Khanna *et al.*, 1968) is limited to maximum excursions of fractions of a wavelength of the light used. Movements of the structures measured, either sound produced or due to respiration movement exceeding 30 nm, may eliminate the usefulness of displacement waveform recording in real time, without resorting to fringe counting techniques and/or a means of moving the preparation and using peak detection (Drake and Leiner, 1984). Moving a guinea pig's or cat's head in such manner is impractical. One of the significant advantages of the capacitive probe is the ability to monitor displacement waveforms visually in real time on a cathode ray tube or aurally over headphones.

III. EXPERIMENTAL AND THEORETICAL CONSIDERATIONS

A. Sources of noise in measurement

Noise in capacitive probe measurements originates both from the preparation and the transducer. The sources of noise may include mechanical (building) vibrations, animal movements such as respiration and heartbeat, and electrical noise within the transducer, including radio-frequency (rf) interference. Also included in the category, uncorrelated with the stimulus, are thermal instabilities in the mechanical components and temperature-dependent electrical drifts. In the second category of noises which will not disappear with signal averaging are those synchronous with the stimulus. Into this category are the vibrations of other structures such as middle ear structures which are also being driven by the stimulus.

1. Uncorrelated noise

Uncorrelated noise will be made up of both electrical and mechanical noise. The electrical noise can be determined with the capacitive probe on the bench. Similarly, any mechanical noise due to building and other vibration can be distinguished or eliminated by vibration isolation. For the animal experiments the method adopted to determine uncorrelated noise is to leave the capacitive probe in place over the basilar membrane and remove the sound stimulus. This is effectively the situation shown in Fig. 6 for a very low-level stimulus. The noise level in that situation appears to be approximately 0.1 nm.

The capacitive probe output signal contains both ac and dc information. The probe sensitivity may therefore depend on the presence of any dc component of the basilar membrane motion itself if the size of the low-frequency movement is sufficient to modulate the sensitivity.

a. Vibration isolation. The experiments were carried out on a vibration isolated table. Using the capacitive probe as the check for effectiveness of the isolation, the additional mechanical isolation provided by a steel plate and rubber tube increased by 40–50 dB below 10 Hz by inflating the tube. The magnitude of the building vibration was significantly less than the respiration and heartbeat under the con-

ditions of measurement.

b. Respiration and heartbeat movement. In addition to low-frequency movements of the basilar membrane, other relative movements between the probe tip may be due to respiration and heartbeat movement. Figure 7 shows a capacitive probe recording of the movement of the basilar membrane caused by respiration and heartbeat. The respiration-produced movement is ca. 25 nm peak-to-peak (*p-p*) while that for the heartbeat is 10 nm *p-p*. This movement is reduced by 18–26 dB by averaging. The influence is further reduced by another 20 dB in the analysis to determine the dc component of basilar membrane motion (LePage, 1987).

c. Electric fields and probe shielding. All the measurements were carried out inside an rf-shielded sound attenuation booth. Since the probe itself is totally shielded apart from the tip itself, the remaining concern is electrical interference at the line frequency. For the most part, line frequency interference was no problem, given that all the metal structures on the table were grounded, and ground loops were eliminated.

d. Thermal stability—electrical and mechanical. The temperature coefficient of expansion of iron (representing the micromanipulator and animal supporting table) is $0.0000106/^{\circ}\text{C}$. Given a 10°C change from the beginning of the experiment, the probe tip could move ca. $10\text{ }\mu\text{m}$ over the warmup period; however, in the experimental situation, such movements are slow by comparison with the period of the measurement and at least two orders of magnitude smaller than animal movements due to respiration and heartbeat for any 2-min recording span.

The capacitive probe drift characteristics were plotted under similar circumstances to a guinea pig experiment. Typically, sound room lights would be turned on at the beginning of the animal preparation procedure to allow time for stabilization of the room temperature. Following turning on the capacitive probe, the drift in the dc conditions of the capacitive probe 45 min later were equivalent to about 0.5

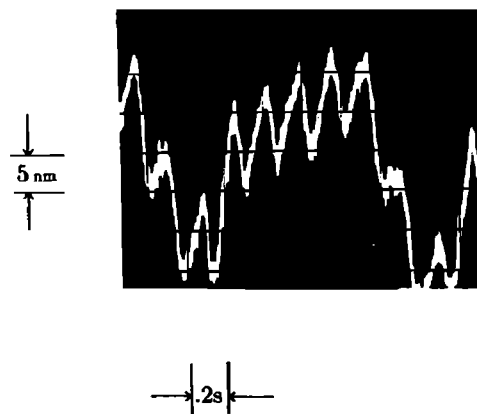


FIG. 7. Capacitive probe waveform recording of the basilar membrane movement produced by artificial respiration and heartbeat of the paralyzed guinea pig preparation. The techniques employed in mounting the preparation reduce the size of the heartbeat movement to 10 nm and the respiration movement to 25 nm. Averaging of the capacitive probe signal is therefore essential to extract the sound-produced motion of the basilar membrane.

V/h (Fig. 8). This is equivalent to a slow mechanical shift of about 0.3 nm/s. Practically, this limits the usefulness of the present transducer for long-term static position recording. Nevertheless, this does not limit its usefulness for looking for a dc shift in the position of the basilar membrane both before and during 12-ms tone bursts, with the amplifier input ac coupled with a 40-ms time constant, or for transducing velocities significantly greater than 0.3 nm/s.

2. Noise correlated with the sound source

The method used to determine noise correlated with electric or magnetic fields due to the earphone or cables is to leave the probe in position but back it away about 20 μm . Alternately, measure the motion of an immobile object. Actual measurements of the spiral lamina and wall of scala tympani during sound stimulation yield noise levels.

The contribution to the capacitive probe output signal due to the unshielded probe registering movements of the middle ear structures can be estimated. Assuming again the situation for a parallel plate capacitor, the sensitivity of the probe due to movements of the basilar membrane will be proportional to the effective area divided by the distance of separation squared. The same situation will apply for the middle ear structures. The relative contributions will be the ratio. Based on an effective basilar membrane area of $2 \times 10^{-9} \text{ m}^2$ at a distance of 2 μm and effective area of the tympanum of 10 mm^2 , the ratio of the contribution to the capacitance due to the basilar membrane and the middle structures will be ca. 2000 (66 dB). This ratio may also be underestimated by between 10 and 20 dB due to the relative size of the movements of the tympanum and the basilar membrane. Hence, the contribution due to the synchronous movements of the tympanum and other structures may be considered negligible.

B. Disturbances to the normal condition of the cochlea

1. Effects of draining

a. The need to drain scala tympani. An inherent requirement of the capacitive probe technique is draining scala tym-

pani in the region of the probe tip. The fundamental limitation to using a capacitive probe under perilymph lies in the similarity of the dielectric constant of the perilymph and the basilar membrane itself. This makes it impractical to design a capacitive transducer which will operate under perilymph. In the present implementation, draining is necessary to prevent the perilymph from electrically short circuiting the probe capacitance. The most serious consequence is that draining may significantly alter the normal mechanical and electrical condition of the cochlear partition.¹ Robertson (1974) has shown that draining of scala tympani in the basal turn of guinea pig produces a reversible 40-dB loss of the sensitivity of single auditory spiral ganglion cells. Patuzzi *et al.* (1982) have shown that the sensitivity of the basilar membrane at the characteristic frequency (CF) may also be altered.

b. Draining and replenishment of perilymph. In a fresh preparation, draining scala tympani of perilymph by briefly inserting a small hand-held wick will not maintain scala tympani drained for more than a minute. Replacement occurs naturally as a result of flow under pressure from the cerebrospinal fluid (Salt and Stopp, 1979). Due to the rate of flow in the fresh preparation and the geometry of the basilar membrane in the basal turn, draining scala tympani in the region of the probe tip mostly entails draining the whole of the first turn. Considering the variability of perilymph flow, composition, and surface tension effects, the state of draining can be controlled quite well providing the basal turn is drained completely.

c. Approach to sensitivity control. Two experimental approaches have been used. The first, adopted by Wilson and Johnstone (1975), was to feedback control the position of the capacitive probe so as to keep constant the mean separation between the fluid surface covering the basilar membrane and the probe tip. They routinely tracked the fluid surface covering the basilar membrane over a range of 20 μm (Wilson, 1974; Wilson and Johnstone, 1975).

If the prime object of the experiment is to record faithfully the tuning curves of the vibratory component, feedback control will reduce variations in probe sensitivity and the probe signal will be a faithful representation of the ac magnitude for frequencies above the bandwidth of the feedback control signal. Indeed, the velocity-sensitive technique is obviously the technique of choice to use for purely ac mechanical measurements. However, with serious consideration now being given to an evoked mechanical response from the OHCs, it is necessary to re-evaluate the desirability of feedback control of probe sensitivity by maintaining the probe separation constant.

The problem concerns having no *a priori* knowledge of the behavior of any rectified component of the basilar membrane motion. If the "motile" response is essentially a slow response with a bandwidth not exceeding a kilohertz, feedback control of a massive structure such as the head stage of the capacitive probe may succeed in maintaining the gap constant and therefore probe sensitivity constant. If, on the other hand, the motile response has a bandwidth which extends through the spectrum of the traveling wave response, even for probe locations in the basal turn, feedback control of

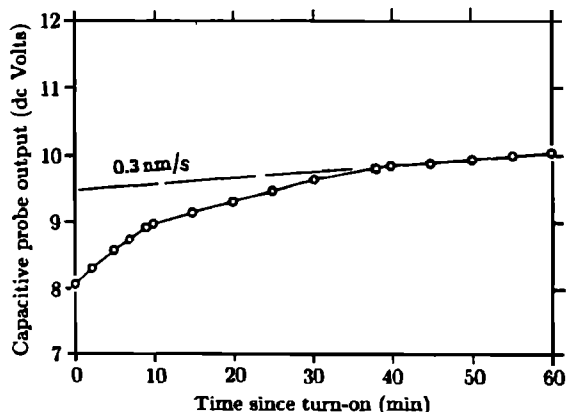


FIG. 8. Drift characteristics of the capacitive probe in a test situation in which the sound room lights and capacitive probe were switched on together. About 45 min later, the drift in the dc signal from the capacitive probe had stabilized to a value equivalent to 0.3 nm/s.

the position of the probe tip may be totally inapplicable because there is no way of distinguishing between a traveling wave response and a rectified response by filtering the output signal. Indeed, Mountain and Hubbard (1986) and Brownell and Kachar (1986) have suggested that the active response may be very rapid, maybe more rapid than most other examples of motile response studied. This could well mean that a motile response has a very low-frequency aspect to it, but its bandwidth may extend upward well into the audio-frequency range. Mounting the massive head stage on a voice coil as Wilson (1973) did may not track the baseline changes adequately. Hence, uncertainties will be introduced in the determination of the ac response, particularly if there are step changes or transients in the motile response as a frequency sweep occurs.

Until some preliminary measurements are made, it is not possible to predict the relative sizes of the traveling wave and any rectified component of basilar membrane motion, or indeed how to design an *in vivo* experiment to distinguish one component of the motion from the other. If the nonlinear compression exhibited in mechanical measurements is an indication of an active response influencing the sensitivity of the vibratory response (LePage, 1987), the magnitude of the latter may be quite secondary in importance to any baseline shift. The objectives of the measurement procedure therefore must be to look for a dc component of the basilar membrane motion. The experimental protocol needs to determine the magnitude, polarity, and time- and frequency-dependent characteristics of a baseline shift.

A first experimental objective is to test whether a dc component of the motion exists. For this experiment it is not of prime concern whether the sensitivity of the capacitive probe is being maintained constant simply in order to capture the magnitude of the ac component of the motion faithfully. In view of the possibility of a large rectified component, the second approach adopted in the series of experiments was that the transducer position be fixed and that the ac and dc components be treated as inseparable except at the stage of data analysis.

This approach carries with it some practical problems. The first is that means must be found to obviate low-frequency movements which will form part of the transduced signal, but constitute sources of noise. These problems are common to all displacement-sensitive techniques. A laser interferometer has the added problem that if these movements approach a quarter of the wavelength of the light, the technique will no longer be operating in its linear range. On the other hand, the capacitive probe has a wide displacement range, but it has the problem that fluid level variations need to be counteracted. Accordingly, the depth of the fluid covering the membrane should be maintained essentially zero for as short a time as necessary for the measurement. If scala tympani has to be drained at all, this is the only state that can be reproducibly achieved.

d. Influence of draining upon the macromechanics.

i. Drained region of scala tympani at zero pressure.

Draining scala tympani of perilymph may well produce little effect on the mechanics of the traveling wave until the thickness of fluid is close to zero. Until the thickness approaches

zero it is possible that the pressure upon the basilar membrane may not be substantially different from normal until there is no fluid, due to surface tension effects. Due to the helicotrema, however, any pressure in scala vestibuli will also equalize.

ii. Effects of longitudinal mass reduction. Berkley and Lesser (1973) predicted that draining one side of their two-chamber model will produce an upward shift in the best frequency of a given place due to a halving of the effective mass of the fluid. Steele and Zais (1985) have investigated the effects of draining scala tympani in the guinea pig in two- and three-dimensional mathematical models. They modeled the situation in which the top wall of the upper chamber was removed. It was found that draining has very little effect on the frequency response characteristics of the basilar membrane except for the instance where the thickness of the fluid covering the membrane was less than 10% of the height of the chamber. Active or nonlinear behavior was not taken into account. Reducing the fluid thickness covering the basilar membrane to zero produced a 2.5-mm movement of the cutoff of a low-frequency tone (3.2 kHz) towards the apex in the two-dimensional model. For their 2-D model, they show a 2.5-mm shift, which for the guinea pig would be one full octave. Then, in the 3-D model representing parameters for the guinea pig, they conclude that the shift requires a half-octave (40%) increase.

iii. Effects of impedance discontinuity at the end of the drained region. The wick is placed on the spiral lamina and held there by surface tension. The suction applied externally produces capillary action within the wick, which is maintained while perilymph continues to return and the wick remains moist. The apical extent of the drained region undoubtedly varies from the beginning of the experiment to the end depending on the rates of suction and return. At the beginning of the experiment, the rate of return is quite high. The position of the wick always extends past the probe position to at least 4 mm from the base, so it can be safely assumed that at least 5 mm of the basal turn is well drained. One of the indicators of an aging preparation expected to have poor hearing thresholds is the poor rate of perilymph return, i.e., taking 15 min or more for scala tympani to refill. This may indicate a much longer region of draining. It is not possible to gauge the nature of any impedance discontinuity because it will vary. However, the measurements obtained from fresh preparations in which the perilymph is being continuously replenished are most representative of nonlinear mechanical behavior (LePage, 1981). In these cases it is more frequently observed that the perilymph thickness will increase in tapered fashion from the end of the wick. The important considerations will be the relative densities of the perilymph and the cochlear partition. It is quite possible that some reflections will occur for frequencies less than 1–2 oct below the characteristic frequency of the measurement site. Reflections occur in linear systems with impedance discontinuities, but these need not be responsible for nonlinear behavior such as a component evoked by a rectifier.

iv. Considerations of surface tension. The presence of a fluid surface different from the normal wall boundary introduces surface tension effects which may modify the normal

motion of the basilar membrane. They may be maximal for some finite thickness of fluid covering the membrane, and may decrease in importance relative to fluid mass as the height of the fluid increases. At the end of the drained region, surface tension sometimes plays a part in establishing a cross-sectional boundary, which, in fact, holds the fluid back until the surface tension is overcome, when filling ensues rapidly. The changes of surface tension could produce apparent saturation (second harmonic distortion) the mechanics. The key issue therefore is whether any dc component displays stimulus-dependent behavior.

e. Influence of draining upon the micromechanics. There may be several effects upon the preparation caused by draining. These may cause the preparation to become "damaged," or they may, in a more limited sense be responsible for modifying reversibly the behavior of the cochlear partition due to the introduction of shifts in the operating points of the hair cells (LePage, 1981). Two distinct influences of draining may be the introduction of a mechanical bias of the basilar membrane and a change in the electrical operating conditions of the hair cells due to modification of the electrical resistance of scala tympani.

i. Changes in basilar membrane position. Opening the cochlea immediately discharges any positive hydrostatic pressure which may exist normally. It seems quite likely that this will produce a transient movement of the basilar membrane in the direction of the pressure release (viz. toward scala tympani). Depending on the magnitude of gravitational and surface tension effects, draining may also result in a permanent displacement of the basilar membrane from its mean position. The potent effect of mechanical bias of the basilar membrane on cochlear sensitivity has been shown by a number of investigators (LePage, 1981; Schmiedt, 1982; Patuzzi and Sellick, 1984a,b). Loss of sensitivity appears to occur primarily with movement of the partition toward scala tympani. However, Patuzzi and Sellick (1984a) have also shown that neural sensitivity is reduced if a large enough bias exists toward scala vestibuli. It is therefore plausible that the effect of draining will produce a shift of the basilar membrane sufficient to reduce cochlear sensitivity. This could be an explanation of the reversible effect draining has upon the sensitivity and tuning of spiral ganglion cells (Robertson, 1974). It may also explain the lack of sharp basilar membrane tuning in many earlier capacitive recordings.

ii. Changes in electroanatomy. The second possible effect of draining is to alter the electrical environment of the hair cells. Models of hair cell transduction generally propose that quiescent current returns to the stria vascularis via scala tympani. In this case, draining scala tympani may change the resistance to the current flow. This will presumably introduce a change in the bias conditions of the hair cells and therefore influence the OHC processes.

All of the above effects may produce essentially static changes in cochlear parameters or function. However, the primary object of the first experiment is to test for a stimulus-produced shift of basilar membrane position. If there is a "dc" component of the motion, the existence of systematic polarity variations which are relatable to the electrophysiology will provide a strong internal control, showing that

draining of the cochlea has not damaged the mechanism responsible for the nonlinear behavior even if the behavior has been modified by the draining. The experiment must therefore obtain simultaneous measures of the electrical responses of the hair cells. The appropriate measure will be the summing potential, and probably a simple electrode on the round window will suffice for the first experiment.

2. Electric field due to probe

The influence of the oscillator voltage on the probe tip will have two effects. First, there is the possibility of an electrostatic force at twice the oscillator frequency, resulting in a net attractive force pulling the basilar membrane toward the tip of the probe. Second, there is the possibility that there will be gross cauterizing effects due to the rf signal or minor alternating current injection which may influence the hair cell behavior.

a. Electrostatic loading effects of the probe upon the basilar membrane. The electrostatic force of attraction on the basilar membrane may be estimated assuming the capacitance-separation relation for a parallel plate capacitor. For a probe tip to basilar membrane separation of $5\text{ }\mu\text{m}$ and an effective area of a circle $50\text{-}\mu\text{m}$ diam, the force to the ac voltage of 35 mV rms on the probe tip is 10^{-7} dynes. In practice, a worst case situation for the force due to the (56 MHz) voltage would be for an ac voltage of 100 mV rms, a distance of $1\text{ }\mu\text{m}$, and an effective area corresponding to a $100\text{-}\mu\text{m}$ -diam pickup area. This gives a force of 10^{-4} dynes. The force of attraction due to the ac voltage is a function of the inverse of the working distance squared, assuming the effective area is fixed. The effect of this is shown in Fig. 9. The voltage on the probe tip includes a dc bias voltage on the gate of the field effect transistor. The dc voltage on the probe tip is too small to be measured, and it is certainly less than the ac voltage on the tip of 0.1 V measured at the output of the head stage and is not important.

The force of attraction, as shown in Fig. 9, can grow as the probe tip nears the basilar membrane, pulling it toward scala tympani or in the inhibitory direction. For normal working distances of $2\text{--}3\text{ }\mu\text{m}$ the force of attraction may be neglected. If, on the other hand, the gap decreases significantly, it is clearly possible to reach a positive feedback situation where the force increases. The fluid surface pulls toward the probe tip, increasing the force still further, until eventually contact is made between the probe tip and the fluid. This situation occurs occasionally. When it does, the oscillator ceases to function. Cessation of recording immediately follows until the tip is separated, the fluid level returns to its normal height, the probe is repositioned, and another series of trials is queued. Normally the situation is continuously monitored and if the dc voltage appears to be monotonically dropping the probe is repositioned at a greater distance.

The forces exerted by the capacitive probe may be compared with the loading effect of other techniques such as the Mössbauer technique. The latter involves placing a relatively large mass on the cochlear partition. Palladium has 12 times the density of water and therefore, 12 times the effective

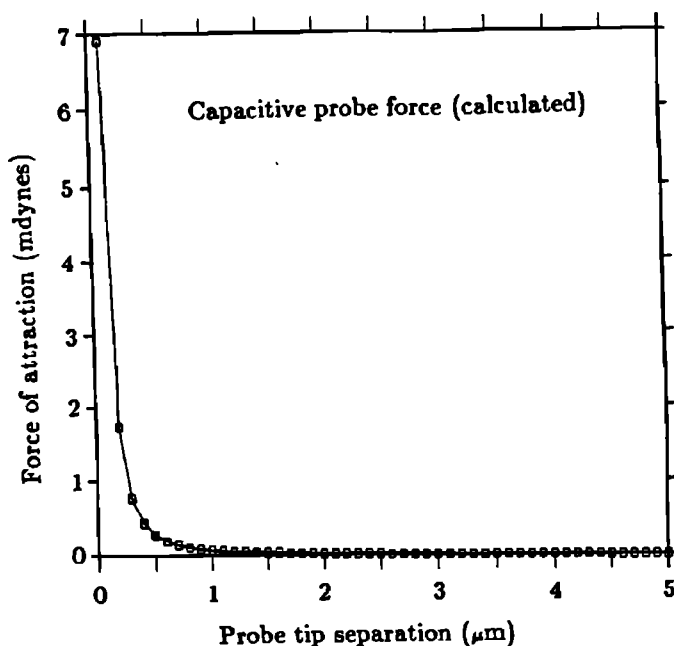


FIG. 9. A plot of the calculated force of attraction of the capacitive probe tip upon the surface of the fluid film covering the basilar membrane. The abscissa is the separation (μm), while the ordinate represents the force (mdynes) estimated as for a parallel plate capacitor as a function of separation of the plates. This suggests that for usual working distances of 2–3 μm the force of attraction is not significant.

tive density of the cochlear partition. Sellick *et al.* (1983) have shown that loading or inertial effects may occur, modifying the behavior of the cochlear partition in different ways depending on the radial location of the source across the basilar membrane. The gravitational force it exerts on the basilar membrane will depend on the size of the source. For the small and large sources quoted, the weight force will be approximately 70 and 300 dynes, respectively, toward scala vestibuli, or in the excitatory direction. Because of the marked effect of the static bias of the cochlear position upon neural sensitivity mentioned earlier, it is possible that the gravitational force predisposes the Mössbauer records to more sharply tuned basilar membrane records, whereas the capacitive probe recordings are predisposed to a bias toward scala tympani, which will reduce sensitivity and tuning.

b. Estimation of cauterizing effects of rf field. The second area for concern is that the rf signal may inject currents into the hair cells, modifying their behavior. This possibility is unlikely to have a major effect because the currents which can be delivered from the input of the field effect transistor (FET) are several orders of magnitude less than currents delivered directly into scala media, required to modify cochlear behavior (Hubbard and Mountain, 1983).

IV. DISCUSSION

A need to test specific models of electromechanical interaction

In carrying out direct mechanical measurements within the cochlea, problems arise because there may be two fast processes and possibly one slow process influencing the basi-

lar membrane at the same time, and this has led to a very complex picture of cochlear mechanics. Standard terminology as applied to the traveling wave may be entirely unsuited to the characterization of the second electromechanical process, in which biological energy is fed back as a component of the motion. Suppose, for example, that a frequency sweep is carried out while the displacement of the basilar membrane is being monitored at one point. As the tone reaches a certain frequency characteristic of that point, the OHCs become active, causing a change in the mean position of the basilar membrane in the process. Suppose further that as the frequency continues to rise, a second frequency is reached at which the hair cells go back to their original passive state. The timing of these evoked responses will be dependent on the rate of the frequency sweep, while the force generation process itself may be completely independent of frequency, but only requires that certain conditions be met to trigger active force generation. The dc signal from the capacitive probe will exhibit a change proportional to the change in mean position of the basilar membrane.

The purpose for the above hypothetical discussion is to illustrate that a first object of any subsequent experiments on the basilar membrane must be to test for any changes in the mean position of the basilar membrane. An obvious way of doing that is to carry out a mechanical experiment equivalent to the bioelectric potential measurements in the cochlea using gated tone bursts. The usefulness of the mechanical results will clearly be improved by simultaneous measurement of dc potential changes in the cochlea for comparison.

The capacitive probe provides waveform information, and therefore can provide responses to tone bursts. In particular, the technique offers the potential to characterize any analogous behavior to the time dependencies of cochlear potentials and any possible adaptive behavior at the macromechanical level. The technique may have the disadvantage of requiring draining of scala tympani, but on the other hand it is exquisitely sensitive to (Angstrom) changes in the mean position of the basilar membrane with the onset of a tone burst and any other long-term position change which may occur during a frequency sweep. The frequent monitoring of the N_1 threshold provides a good measure of the condition of the preparation, and indeed appears to be an accurate predictor of nonlinear compressive behavior. The technique, therefore, appears to be well suited for a preliminary examination of the possibility of dc shifts of the basilar membrane in live guinea pigs in response to various acoustic and electrical stimuli. Similar care is needed regarding the nature of the experiment given any other displacement-sensitive technique, particularly in regard to dynamic range and linearity. Capacitive probe recordings are quite specific for a given recording location, given the price paid for that specificity in terms of disturbance to the phenomena being measured.

Note added in proof: There is an uncertainty principle at work in invasive cochlear measurements. In the case of optical measurements of motion of the basilar membrane one can avoid the uncertainties associated with draining but then there is uncertainty about which surface(s) are reflecting and by how much, and the biological effects of the delivery of large light fluxes. Capacitive probe measurements inherent-

ly provide certainty about which surface is being observed (the conducting fluid film covering the basilar membrane constitutes a perfect "reflector"), and the spatial resolution can be made very high, but then there is uncertainty about cochlear function in the drained condition. A widespread view is that the technique produces damage to the preparation. An alternative explanation, consistent with the various mechanical and neural studies on draining is that its initial effect is not "damage" *per se*, but the introduction of an unwanted mechanical bias which influences cochlear sensitivity. As far as demonstrating active behavior is concerned, it is not important how much sensitivity is *lost*, but how much sensitivity is *retained*, since thresholds less than 65 dB SPL indicate the existence of nonlinear compression. The capacitive probe therefore offers basilar membrane specificity, displacement sensitivity, linearity, wide dynamic range, high sensitivity, low energy delivery, and good spatial resolution. Since it is possible to control for cochlear sensitivity with simultaneous electrical measurements, the uncertainties in the measurement can be minimized. Hence, the particular set of advantages of the capacitive probe technique for examining motile behavior inside the cochlea, particularly its wide dynamic range, appears currently to be unique.

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¹Consideration has been given to designing a capacitive transducer which will operate without draining scala tympani of perilymph or replacing perilymph with a nonconducting fluid. Even if such a device could be implemented, for example, by placing a piece of metal foil on the basilar membrane, uncertainties would be introduced by the incompressible fluid effects in the small gap between the basilar membrane and the probe tip. Second, a nonconducting fluid may alter the current paths necessary for normal hair cell operation (Johnstone *et al.*, 1975).

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