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LOW-FREQUENCY CHARACTERISTICS OF INTRACELLULARLY RECORDED RECEPTOR POTENTIALS IN GUINEA-PIG COCHLEAR HAIR CELLS

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SUMMARY

1. Intracellular receptor potentials were recorded from inner and outer hair cells in response to low-frequency tones, from the basal, high-frequency region of the guinea-pig cochlea.

2. The receptor potentials recorded from inner hair cells are asymmetrical about the resting membrane potential with the depolarizing phase, which corresponds to rarefaction in sound pressure, exceeding the phase of hyperpolarization by a factor of about 3. It was found that the relationship between the peak-to-peak voltage responses and sound pressure level could be described by rectangular hyperbolae.

3. When the frequency of the sound stimulus was progressively increased from 100 Hz to 4 kHz, the 'periodic' (a.c.) component of the receptor potential was attenuated with respect to the 'continuous' (d.c.) component. The characteristics of the inner hair cells could be described by two stages of low-pass filtering, with one of the filters having the same corner frequency as the electrical time constants which varied in different cells between 178 and 840 Hz.

4. Receptor potentials recorded intracellularly from two morphologically identified outer hair cells were symmetrical about the resting membrane potential (about −65–70 mV) and had a maximal amplitude of only 5 mV at frequencies and intensities which yield 20–30 mV voltage responses from inner hair cells. No d.c. component receptor potentials were recorded in response to high-frequency tones.

5. Phase and amplitude measurements were made from receptor potentials from inner hair cells, and from 'cochlear microphonic potentials' which were recorded from the organ of Corti and scala tympani. The phase of depolarization in both potentials was associated with displacement of the basilar membrane towards the scala vestibuli. The phase of the intracellular receptor potentials leads the cochlear microphonic by about 90° and the sound pressure by about 180° at frequencies below 100 Hz. Above this frequency the phase lead progressively declines and at higher frequencies becomes a phase lag. These phase relationships indicate that inner hair cells respond to the velocity of the basilar membrane at frequencies below 200–600 Hz, and to its displacement above this, and that the voltage responses of the inner hair

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cells are limited by their membrane time constants. It is suggested that outer hair cells respond to basilar membrane displacement throughout their frequency range.

6. It is shown that, with respect to frequency, the different growth rates of the cochlear microphonic potentials and inner hair cell receptor potentials, and the dominance of cochlear microphonic potentials in the organ of Corti, result in an effective electrical interaction between inner hair cells and cochlear microphonic potentials.

INTRODUCTION

Inner hair cells in the basal turn of the guinea-pig cochlea respond maximally to high-frequency tones, between about 14 and 40 kHz (Russell & Sellick, 1977), and their frequency tuning curves resemble those of fibres in the auditory nerve (Russell & Sellick, 1978). In response to high-frequency tones, the receptor potentials, which are generated by the inner hair cells, consist of a 'continuous' (d.c.) component, which is a maintained depolarization of the membrane potential lasting for the duration of the tone, and a 'periodic' (a.c.) component which resembles the wave form of the acoustic stimulus a.c. The relative contributions of these two components to the receptor potential change with the frequency of the acoustic stimulus, so that in the voltage response to high-frequency tones the d.c. component predominates, and at low frequencies the a.c. component is the dominant potential.

In the first part of this paper we describe the transfer characteristics of the voltage responses of inner hair cells. These were measured at frequencies below the cut-off frequencies determined by the membrane time constants of the hair cells. A comparison was also made between the cut-off frequency of the low-pass filter equivalent to the membrane filtering and that of the ratio of the a.c. and d.c. components of the receptor potential to see if the passive electrical properties of the hair cell membrane were responsible for the attenuation of the a.c. component with increasing frequency.

Measurements of extracellular receptor potentials from normal cochleas and those in which outer hair cells had been destroyed led Dallos (1973) to proposed that the cochlear microphonic potential originated from outer hair cells while the summating potential was produced from inner hair cells. In support of Dallos' proposal we have already shown a close correspondence between the summating potential and the d.c. component of receptor potentials recorded from inner hair cells (Russell & Sellick, 1978). In this paper we provide supporting evidence for his proposal that the cochlear microphonic potential originates from outer hair cells.

The second part of this paper is concerned with how inner and outer hair cells are mechanically coupled to the tectorial membrane. It is generally accepted that hair cells in the acoustico-lateralis system are depolarized when their stereocilia are displaced towards the kinocilium, or the basal body, if the kinocilium is absent as in the cochleas of adult mammals (Flock, Kimura, Lundquist & Wersall, 1962). This mode of excitation has been firmly established by direct experimental observation in the hair cells of the frog sacculus (Hudspeth & Corey, 1977; Hudspeth & Jacobs, 1979). In the mammalian cochlea, displacement of the stereocilia is believed to be caused by relative movements between the basilar and tectorial membranes. From the position of the basal body and the geometry of the organ of Corti, it has been

proposed that cochlear hair cells are depolarized by movements of the basilar membrane towards the scala vestibuli (Davis, 1965). The ultrastructural observations of Kimura (1966), Engström & Engström (1978) and Lim (1972) reveal that the tips of stereocilia of outer hair cells are firmly embedded in the underside of the tectorial membrane, while those of inner hair cells are free-standing. These morphological observations imply that the stereocilia of outer hair cells will be moved directly by the displacements of the tectorial membrane in relation to the basilar membrane, but the stereocilia of inner hair cells will be moved by viscous drag of fluid streaming around them. Thus, the displacement of the free-standing cilia of inner hair cells will be proportional to basilar membrane velocity (Billone, 1972). In this paper we attempt to test this hypothesis by making comparisons of the amplitude and phase of intracellularly recorded receptor potentials from inner hair cells with extracellularly recorded microphonic potential and the sound pressure recorded at the tympanic membrane. Some of the findings which are reported in this paper have been presented as preliminary reports elsewhere (Sellick & Russell, 1980; Russell & Sellick, 1981).

METHODS

Preparation

The experiments were performed on young dark-eyed guinea-pigs from our own colony weighing 180–300 g. These animals were chosen because the stria vascularis is pigmented and easy to identify during dissection of the cochlea. They were anaesthetized with phenobarbitone, Operidine (Jansons) and Droleptan (Jansons) according to an anaesthetic regime devised by Evans (1979). Throughout surgery the animals respired naturally and their heart beat and withdrawal reflexes were monitored, to ensure that the former remained constant and the latter did not occur. The first turn of the cochlea was exposed through ventral and lateral openings in the temporal bone (Russell & Sellick, 1978; Sellick & Russell, 1978). The basilar membrane was exposed through a small opening into the scala tympani and it was illuminated by a fibre-optic light guide inserted into the ventral opening in the temporal bone. The sensitivity of the cochlea was monitored before and after exposure of the basilar membrane by measuring the compound action potential with a silver electrode on the round window in response to pure tones. In the experiments reported here, the response thresholds remained unchanged after exposure of the basilar membrane, and were typically 20–30 dB sound pressure level (s.p.l.) at 17 kHz. At the completion of surgery, the animals were paralysed with an intramuscular injection of Flaxedil (Gallamine triethiodide; 5–10 mg/kg) and artificially respired. Care was taken to monitor heart rate continuously and to administer the anaesthetic regime. This period of the experiment lasted 1–2 hr and the animal was then given an overdose of barbiturate.

Sound stimulation

Tone stimuli were delivered through a closed sound system similar in design to that described by Evans (1979) but equipped with two sound sources. High-frequency tones (1–30 kHz) were delivered to the hollow ear bar from a $\frac{1}{2}''$ capacitance microphone (B & K 3134). These were used to test the sensitivity of the cochlea during the experiment and to stimulate hair cells in the basal turn of the cochlea at their characteristic frequencies (14–20 kHz). Low-frequency tones (17–4000 Hz) were delivered from a Beyer DT48 dynamic earphone which was coupled to the ear bar through a 4 mm plastic tube which terminated in a short 1 mm probe tube inserted into the wall of the hollow ear bar.

The electrical driving signals to the dynamic earphone and capacitance microphone were provided by gated tone burst generators. In the latter case 1 msec rise and fall times of the tone burst envelope were shaped to reduce the harmonic distortion caused by the onset and offset transients of the tone burst. Distortion compensation was not applied to the driving voltage delivered to the capacitance microphone because this microphone was not used to deliver tone bursts

above 50 dB s.p.l., at which level the distortion is small. The distortion of this sound source was less than 48 dB below the fundamental over the intensity range 0–50 dB s.p.l. and for the dynamic earphone it was better than 54 dB over 0–120 dB s.p.l. All intensities are in sound pressure levels in decibels relative to 2×10^{-5} Pa (dB s.p.l.).

The sound pressure level in the hollow ear bar was monitored continuously at the level of the tympanic membrane by a B & K 4134 $\frac{1}{2}''$ condensor microphone equipped with a damped 1 mm probe tube. This was calibrated in the sound system against a B & K $\frac{1}{4}''$ 4135 microphone which was placed in the position normally occupied by the tympanic membrane. The probe microphone was calibrated over the range 10–30,000 Hz and corrections were made for the low-frequency phase leads introduced by the B & K 2619 preamplifier, the B & K 2503 measuring amplifier, and the static pressure equalizing system, relative to the sound pressure level measured at the tympanic membrane. The combined phase lead from these sources is about 42° at 10 Hz, disappearing to zero at 180 Hz. Above this, the measured sound pressure progressively lags that at the tympanic membrane due to the low-pass filter characteristics of the probe tube. The characteristics of the calibrated probe microphone resemble those described by Crawford & Fettiplace (1981) for a similar microphone used in their experiments, and the characteristics of the higher-frequency sound source ($\frac{1}{2}''$ B & K microphone) are similar to those published by Evans (1979).

Recording

Intracellular recordings were made from micropipettes drawn from 1 mm fibre-filled borosilicate tubing (Clarke Electromedical) and pulled on an air-blast puller (Brown & Flaming, 1977). These were back-filled with either 4 M-potassium acetate, 6% Procion Yellow, or 3% Lucifer Yellow (Stewart, 1978) and had resistances of about 100–200 MΩ, 400 MΩ and 400 MΩ respectively. The electrodes were positioned on the basilar membrane with the aid of a hydraulic microdrive, similar in design to a device described by Evans (1979). When the fibre-optic light guide was adjusted, so that the basilar membrane appeared as a dark field, the electrode could be accurately located without draining the scala tympani. The cochlear microphonic potential was recorded in scala tympani with an insulated silver wire placed close to the position of the micro-electrode.

Signals from the micro-electrodes were led into a high-input impedance preamplifier with capacity compensation. The amplifier also incorporated a bridge circuit for injecting constant current through the micro-electrode and for balancing out the voltage developed across the electrode's resistance. The frequency characteristics of the micro-electrodes were calibrated *in situ* in the organ of Corti, after the electrodes had penetrated the basilar membrane. A current pulse (*ca.* 10^{-10} A) was injected through the micro-electrode and the capacitance was adjusted until the pulse appeared relatively square without 'ringing' on the rising and falling phases. The phase and amplitude characteristics of the electrode were then measured by passing sinusoidal current through the electrodes and measuring the modulus and phase of the voltage change with a pair of Brookdeal 9503-SC phase-lock amplifiers and 5042 Omniphase (Sellick & Russell, 1979). The potassium acetate-filled micro-electrodes which were used for phase and amplitude measurements of the intracellular responses had flat amplitude and phase characteristics over the range of 17–4000 Hz for which these measurements were made. The frequency responses of the micro-electrodes were also compared in the scala media with those of the silver–silver chloride wire which was used to measure the extracellular potentials of the cochlea. The ear was stimulated with tones, and the recordings of the cochlear microphonic and the summating potential by the two electrodes were compared and found to be very similar.

On the basis of these calibrations no phase or amplitude compensation due to the electrode characteristics was applied to the intracellular recordings.

The electrodes were advanced into the hair cells by causing the amplifier circuit to ring by operating a switch which caused momentary capacitance over-compensation without disturbing the original settings.

The electrical time constants of the hair cells were determined by the method of Pinto & Pak (1974). Sinusoidal currents $0{\cdot}5 \times 10^{-11}$ A in amplitude and 130 Hz in frequency were injected through the recording electrode by means of the bridge circuit. The time constants of the hair cells and their impedance changes during acoustic stimulation were derived from the in-phase and 90° out-of-phase voltage changes across the cell membrane produced by the injection of current.

The membrane time constants were calculated from the relationship derived by Pinto & Pak (1974)

$$\frac{\Delta Re\,[Z(jw)]}{\Delta Im\,[X(jw)]} = \frac{1}{2}\left[\frac{1}{\omega RC} - \omega RC\right],$$

where $\Delta Re\ [Z(jw)]$ and $\Delta Im\ [Z(jw)]$ represent the changes in the real and imaginary components of membrane impedance as a result of a change in total membrane resistance. RC is the membrane time constant, and ω is the angular frequency of the measuring current.

Intracellular and extracellular potentials, sound pressure and trigger pulses were stored on the four channels of an FM tape recorder for future averaging on a Biomac 1000 signal averager, and phase and amplitude measurements were made from the averaged wave forms.

Histology

Dye marked cells were observed in 10 μm sections of epoxy-embedded cochleas with the aid of a fluorescence microscope.

RESULTS

1. Response from inner hair cells

In a previous study (Russell & Sellick, 1978) we marked intracellularly only those cells which produced large d.c. component receptor potentials in response to tone bursts at frequencies close to their characteristic frequency (16–18 kHz). Eleven dye-marked cells were recovered; they were all inner hair cells. In this current study we have added a further ten morphologically identified inner hair cells to this population and a further forty-five which share their response characteristics and are assumed to be inner hair cells. Electrode penetrations at other locations across the width of the basilar membrane failed to reveal any other cell types which generated large continuous component receptor potentials.

Examples of the voltage responses of an inner hair cell to low-frequency tones are shown in Fig. 1*A*. The responses are asymmetrical about the membrane potentials with the depolarizing phase of the receptor potential exceeding the hyperpolarizing phase. This is illustrated in Fig. 1*B*, where the peak amplitudes of the receptor potentials in the depolarizing and hyperpolarizing directions about the resting membrane potential are plotted against the peak rarefactions and compression phases of the stimulus tones respectively. In order to make these comparisons it is necessary to adjust the phases of the receptor potential and sound pressure changes so that they coincide. These phase differences exist because the basilar membrane displacement responds to stapes velocity at low frequencies (Wilson & Johnstone, 1975) and the inner hair cells respond to basilar membrane velocity (see below). The responses of the hair cells were measured at frequencies below the cut-off frequencies of their membranes in order to avoid any phase and amplitude changes which would otherwise be introduced by the passive electrical properties of the membranes, if higher frequencies were used.

The input–output characteristics of a wide variety of acoustico-lateralis organs have been described by rectangular hyperbolae (Boston, 1980; Crawford & Fettiplace, 1981). An attempt was made to see if this functon fitted the relationship between the peak amplitude of the receptor potentials recorded from inner hair cells and the peak stimulus intensity. The smooth curve through the data points in Fig. 1*B* is composed of two rectangular hyperbolae whose equations are given below:

$$V_{\mathrm{D}} = V_{\mathrm{max,\,D}} \frac{P}{\bar{P}_{\mathrm{D}} + P} \quad \text{for} \quad P \geqslant 0, \tag{1A}$$

$$V_{\mathrm{H}} = V_{\mathrm{max,\,H}} \frac{P}{\bar{P}_{\mathrm{H}} - P} \quad \text{for} \quad P \leqslant 0, \tag{1B}$$

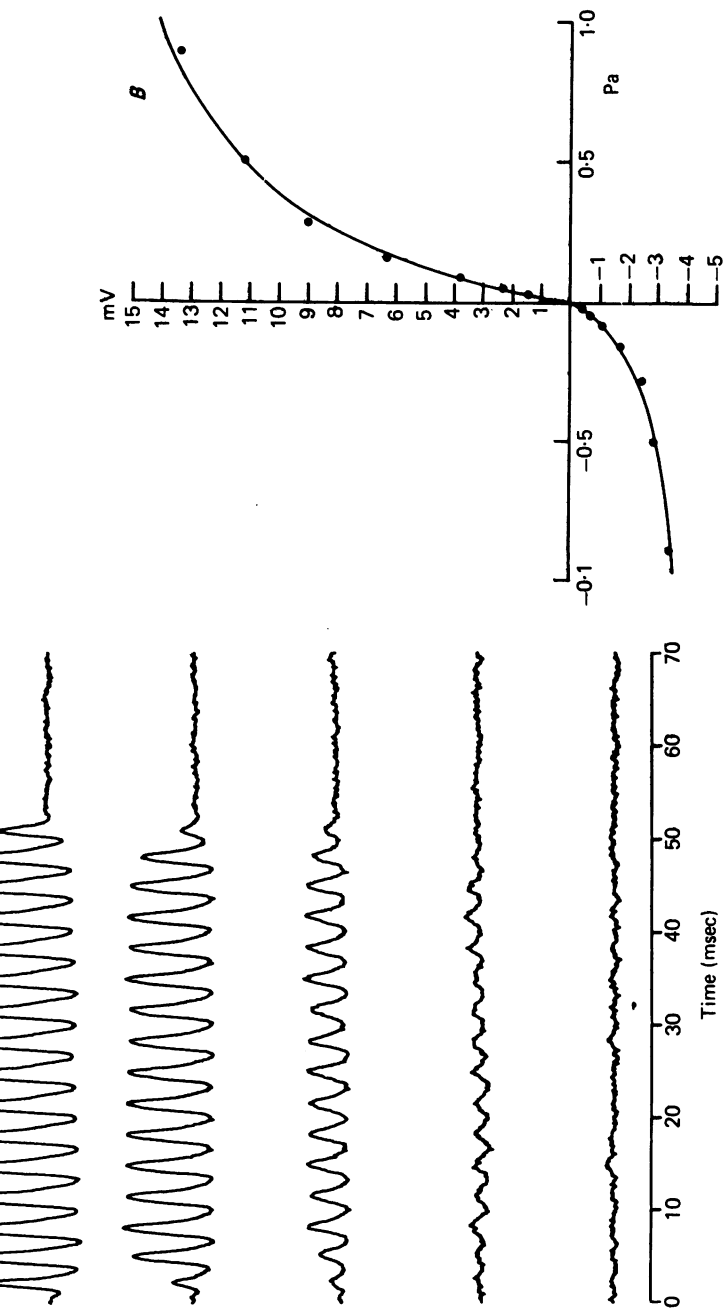

Fig. 1. *A*, voltage responses from an inner hair cell to a 300 Hz tone burst at the intensities indicated besides each trace. The sound pressure is given in dB relative to 20 μPa. *B*, amplitude–intensity relationship of averaged responses of a cell whose single responses are illustrated in Fig. 1*B*. The peak depolarizing (positive) and hyperpolarizing (negative) voltage responses about the membrane potential are plotted against the peak rarefaction and compression phases of the sound pressure respectively. The continuous line through the points is composed of two rectangular hyperbolae calculated according to eqn. (2).

where V is the peak receptor potential, p is the peak sound pressure, V_{max} is the saturation level of the receptor potential, and $\bar{P}$ is a constant equal to the sound pressure that produces half a maximum response. The subscripts D and H refer to the depolarization and hyperpolarization of the membrane potential. A positive sign has been assigned to rarefaction which is associated with depolarizing voltage responses and a negative sign has been given to compression which is associated with hyperpolarizing voltage responses in hair cells.

TABLE 1. Inner hair cell input–output characteristics. Columns 2 and 3 were measured from the data, $V_{max,D}$; $V_{max,H}$; $\bar{P}_D$ and $\bar{P}_H$ were derived from the intercepts and slopes of the relationship given by eqn. (2), and r_D and r_H are the correlation coefficients of the data to the best fit to eqn. (2)

Cell no.	Ratio of depol./hyperpol.	P-P amplitude at 90 dB s.p.l.	$V_{max,D}$ (mV)	$V_{max,H}$ (mV)	$\bar{P}_D$ (Pa)	$\bar{P}_H$ (Pa)	Correlation coefficients r_D	r_H
34-3	4·01	16·8	18·7	−4·7	0·333	−0·296	0·988	0·984
32-1	2·428	11·9	9·9	−4·1	0·2	−0·143	0·981	1·0
36-1	3·233	10·3	8·9	−2·8	0·185	−0·167	0·992	0·997
34-2	2·106	23·3	16·3	−7·7	0·109	−0·103	0·964	0·984
25-1	2·778	28·3	26·6	−9·6	0·108	−0·098	0·961	0·98

It was not technically possible to make intracellular recordings from inner hair cells when their voltage responses were saturated. This is because the basilar membrane motion displaced the intracellular electrode during the presentation of very loud sounds. V_{max} and $\bar{p}$ were derived from the linear transformation of the Michaelis–Menten equation described by Eadie (1942). This is achieved by rearranging eqn. (1) to yield:

$$\frac{V}{V_{max}} = P - \bar{P}\frac{V}{V_{max}P}. \tag{2}$$

Plotting V as a function of V/P gives a straight line with a slope of $\bar{P}$ and an intercept of V_{max}. This linear transformation has the advantage that it is suitable for evaluating the goodness of fit of the Michaelis–Menten equation to the data. The constants V_{max} and $\bar{P}$ have been calculated for five cells and are given in Table 1. The last two columns contain the correlation coefficients for the data to a best fit of a Michaelis–Menten equation as determined by a least-squares method (Cornish-Bowden, 1976) where a value of unity indicates a perfect fit.

Considerable variation exists in the amplitudes of the inner hair cell voltage responses to low-frequency tones at high sound pressure levels. This variation may be due to differences in the quality of the electrode penetration. However, it is possible to control for these differences by comparing the magnitude of receptor potentials at low frequencies of auditory stimulation with those at frequencies close to the characteristic frequencies of the hair cells. When this was done, it was found that three cells out of a population of forty-five produced very small responses to low-frequency tones, but responses which fell within the normal range of the population at their characteristic frequency. For example, cells 407-1 and 407-4 from the basal turn of the same cochlea had resting potentials of 30 and 25 mV respectively and each produced 17 mV continuous potentials in response to 16 kHz tones at

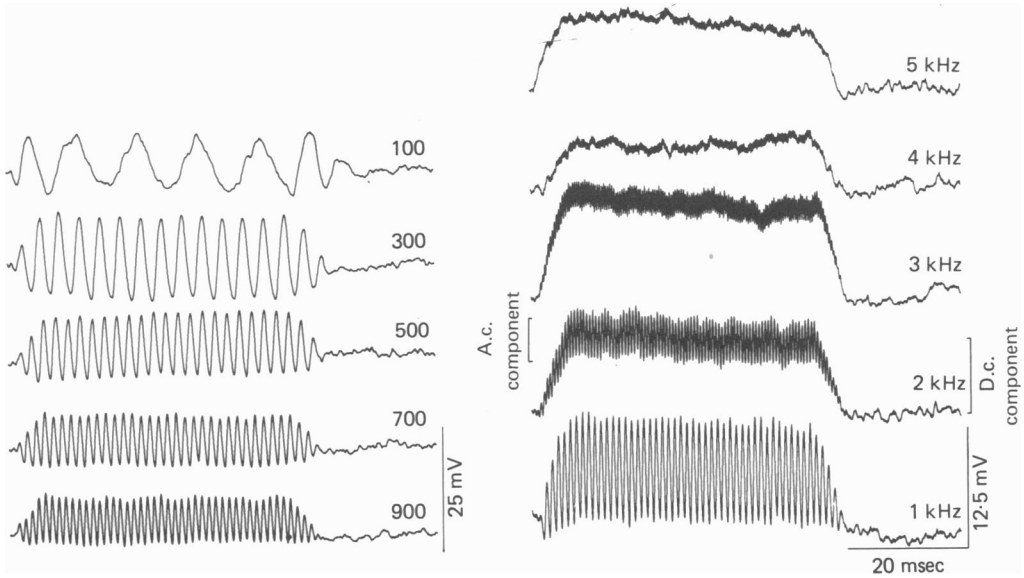

Fig. 2. Intracellular receptor potentials recorded from an inner hair cell in response to 80 dB s.p.l. tones at the frequencies indicated by the side of each trace. Notice that relative to the d.c. component, the a.c. component of the receptor potential is reduced as the frequency is increased, and that above 1 kHz the response is dominated by the d.c. component.

80 dB s.p.l. However, their responses to tones at 300 Hz and 100 dB s.p.l. were quite different from each other. Cell 407-1 produced a 3 mV voltage response while that from 407-4 was 24 mV. The basis for this variation is unknown, but may represent differences in the coupling of cells to the viscous drag of fluid in the subtectorial space.

When the frequency of the tone is successively increased, for example in increments of 200 Hz from 100 Hz as shown in Fig. 2, it can be seen that above a particular frequency, the peak-to-peak amplitude of the inner hair cell voltage response progressively decreases. The period of each cycle of the stimulus wave form, for which the hair cell is hyperpolarized below the resting potential also decreases, so that for tones above about 1 kHz the hair cell is depolarized throughout the tone burst. This type of frequency-dependent transformation occurs to an asymmetrical, periodic current when it passes through a low-pass electrical filter. It was previously proposed (Russell & Sellick, 1978) that the membrane filter of the inner hair cells attenuates the phasic components of the receptor current so that the voltage response of inner hair cells to high-frequency tones is the residual d.c. component of the receptor potential. To test this hypothesis that, in inner hair cells, the d.c. component of their receptor potentials arises from the integration of their voltage responses by their membrane filter, the ratio of the a.c. component to the d.c. component was plotted against frequency, and the corner frequencies of this relationship (-3 dB points) were compared with those of the membrane filter.

The d.c. component was calculated from the difference between the resting potential of the cell and the level of the half peak-to-peak a.c. component. This corresponds closely to the d.c. component derived from the difference in areas of the

depolarizing and hyperpolarizing phases of the receptor potential, provided that the wave form is sinusoidal with low distortion (Fig. 3*A*). The resulting curve resembles that from a low-pass filter, but only in four instances out of a total of eleven cells does the filter appear to have a single R–C filter stage which might be attributed to the electrical time constant of the hair cell membrane (e.g. Fig. 3*A* and *D*). In the other examples (3*B* and *C*) the filter appears to have at least two stages. Theoretical curves have been fitted to the data, with one pole corresponding to the corner frequency of the membrane filter properties and another with a higher corner frequency. These curves are given by the equation:

$$\frac{V_o}{V_i} = \frac{1}{(1+\omega^2/\omega_0^2)^{\frac{1}{2}}} \frac{1}{(1+\omega^2/\omega_1^2)^{\frac{1}{2}}}, \tag{3A}$$

where V_i is the input signal to the filter, V_o is its output, ω_0 is the corner frequency of the membrane filter, and ω_1 is the corner frequency of the second filter stage and ω is the angular frequency at which the measurements were made ($\omega = 2\pi f$).

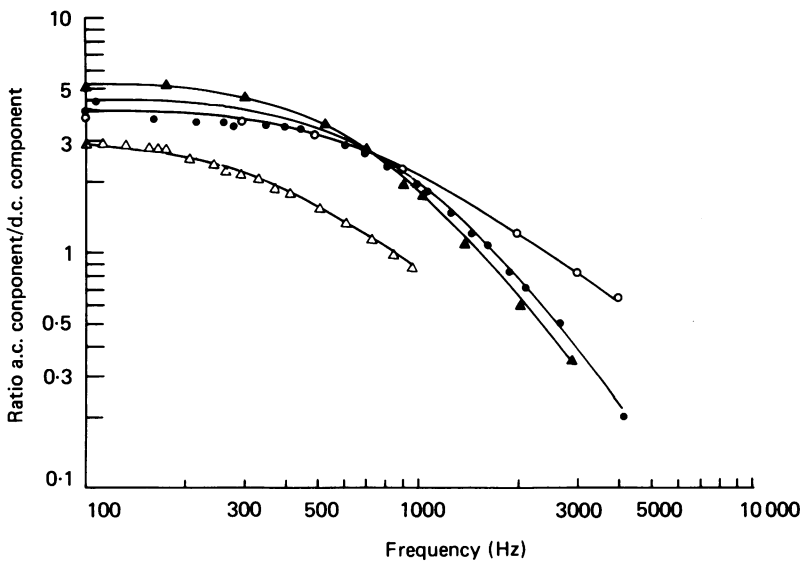

Fig. 3. The ratio of the a.c. and d.c. components of the inner hair cell receptor potential in four hair cells. ○ and ▲: d.c. component calculated from the differences between half the peak-to-peak a.c. component and the resting potential; ● and △: calculated from the integral of the wave form. The continuous curves are for theoretical single (open symbols) and double-pole filters (filled symbols) with one pole determined by the electrical time constant of the hair cell and the other determined by eye. The corner frequencies of these filters in Hz are as follows, with that of the electrical time constant given first: ○, 600; ●, 720,1200; ▲, 550,1100; △, 280.

The origin of this second filter stage is not known and may represent a change in the band width of the recording system caused when the recording electrode entered the cell or a change in the a.c./d.c. component ratio with increasing frequency which is not due to the low pass electrical characteristics of the hair cell membrane. Table 2 includes cells whose a.c./d.c. component relationships could be fitted with a single stage R–C filter with a corner frequency corresponding to that of the measured

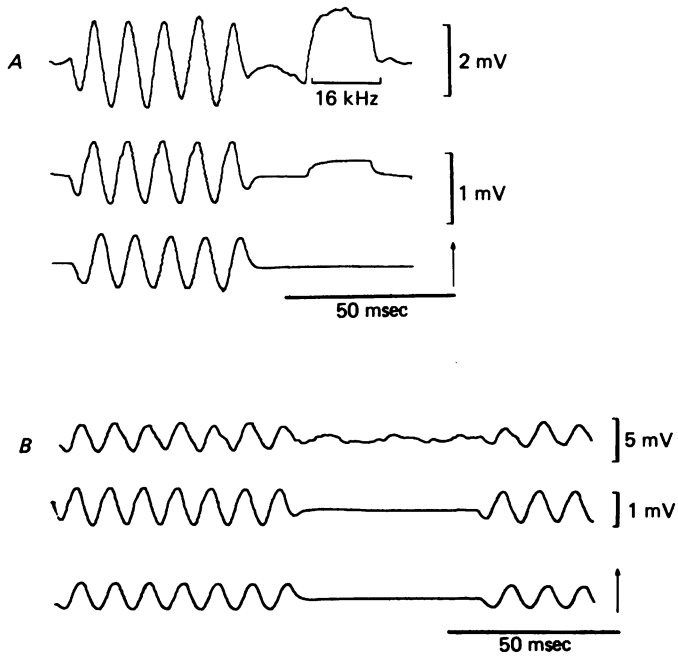

Fig. 4. Intracellular voltage responses recorded from the organ of Corti. *A*, potentials recorded from an identified inner pillar cell in response to a low-frequency tone burst (100 Hz at 90 dB s.p.l.) followed by a response to a high-frequency tone (16 kHz at 80 dB s.p.l.). Upper trace: response from inner pillar cell, membrane potential −80 mV; middle trace: cochlear microphonic and sound pressure recorded from scala tympani; lower trace: sound pressure recorded at tympanic membrane. *B*, upper trace: intracellular receptor potential from an outer hair cell in the middle row in response to a low-frequency stimulus (100 Hz at 90 dB s.p.l.), membrane potential −70 mV. Middle and lower traces: as in *A*. Arrows indicate direction of rarefaction. All traces are computer averages of sixteen sweeps.

membrane filter. Thus in these cells at least, it appears that the attenuation of the a.c. component of the receptor potential with increasing frequency can be attributed to the electrical impedance of the hair cell membrane.

2. Responses from outer hair cells and supporting cells

Receptor potentials were recorded from two cells in the middle row of outer hair cells which were identified after being filled with fluorescent dye. They were much smaller than those recorded from inner hair cells, yielding maximum potentials of about 5 mV (Fig. 4*B*) but the resting membrane potentials were larger at −65 and −70 mV. They did not produce large d.c. component potentials in response to high-frequency tone bursts (16–18 kHz). Moreover, their receptor potentials, in response to triangular and sinusoidal sound pressure waves at frequencies below 200 Hz, were symmetrical, and twice as large as the cochlear microphonic potential (which they resemble) recorded from the extracellular space of the organ of Corti, and intracellularly from supporting cells (Fig. 4*A*). These limited observations agree with those of Tanaka, Asanamure & Yanagisawa (1980) and Dallos, Santos-Sachi & Flock (1982).

3. Inner hair cell receptor potentials and their relationship to basilar membrane displacement

The wave forms illustrated in Fig. 5 *A* and *B* show that for sinusoidal and triangular sound pressure changes, the cochlear microphonic potential is approximately proportional to the time derivative of sound pressure, and the inner hair cell receptor potential is approximately equal to its second derivative. Wilson & Johnstone (1975) have shown that for frequencies below 400 Hz, the displacement of the basilar membrane in the basal turn of the guinea-pig is proportional to the velocity of the

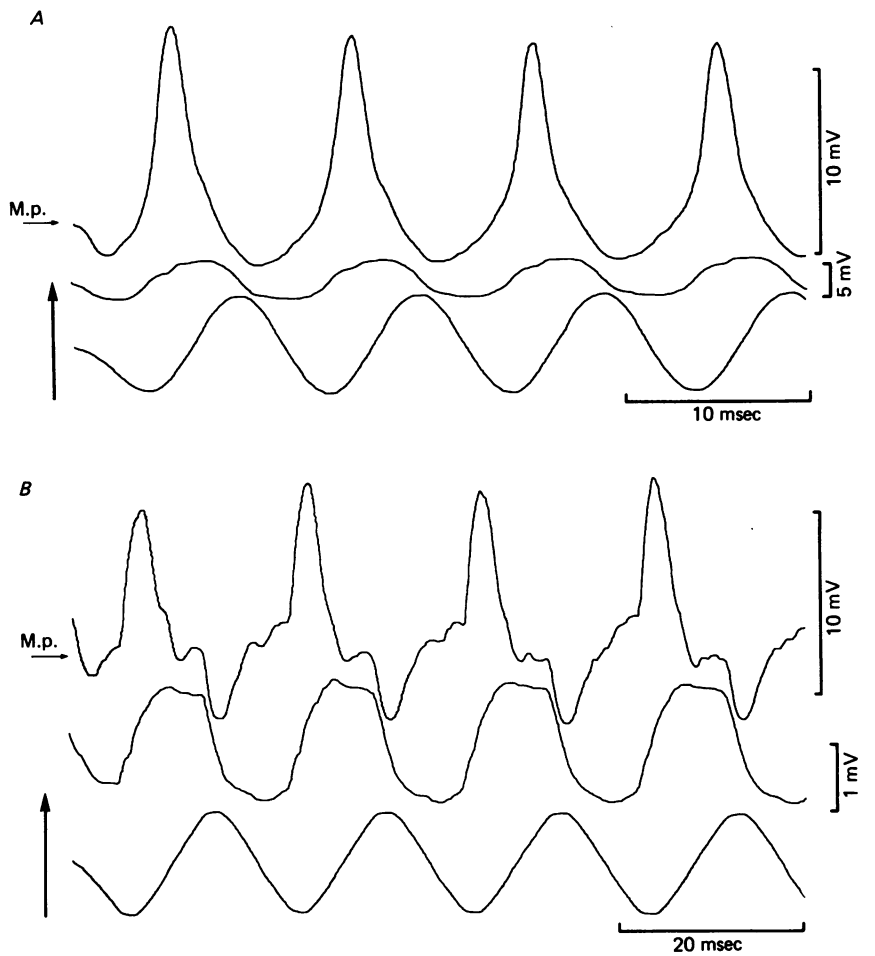

Fig. 5. Receptor potentials recorded intracellularly from inner hair cell in the basal turn in response to: *A*, a 102 Hz sinusoidal acoustic stimulus at 80 dB s.p.l.; *B*, a 52 Hz triangular acoustic stimulus at 100 dB s.p.l. Note the triangular wave form has been low-pass filtered at 1 kHz to reduce 'frequency splatter' at the apices. In both records, upper trace: receptor potential; middle trace: cochlear microphonic potential recorded adjacent to the intracellular recording site; lower trace: sound pressure recorded at tympanic membrane. Membrane potential (m.p.): *A*, −30 mV; *B*, −40 mV. Arrow in bottom trace indicates direction of rarefaction. Each trace is a computer average of eight sweeps.

stapes. Thus the wave forms illustrated in Fig. 6 indicate that the cochlear microphonic potential, which is dominated by the responses of outer hair cells (Dallos & Cheatham, 1976), is proportional to basilar membrane displacement, while the inner hair cell receptor potentials are proportional to its velocity.

A more complete description of the relationship between the inner hair cell receptor potential and basilar membrane motion was obtained over the frequency range 20–4000 Hz by observing the phase and amplitude characteristics of the receptor

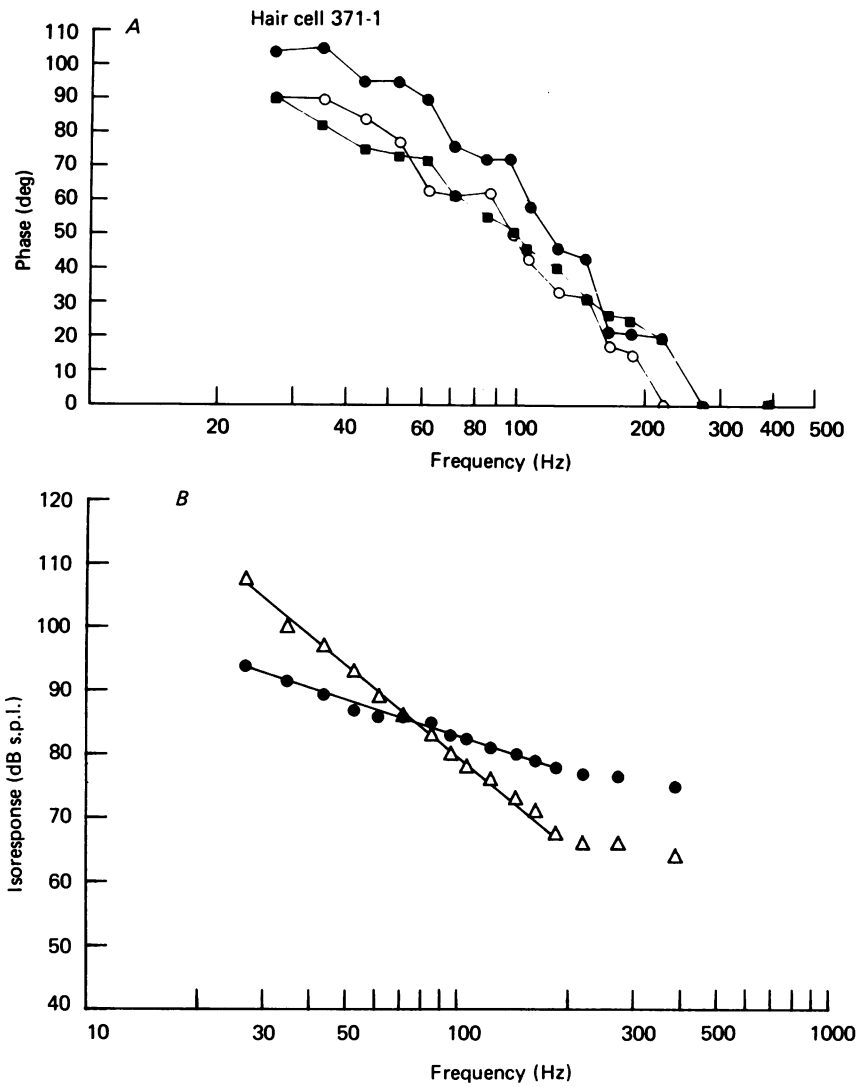

Fig. 6. *A*, phase of inner hair cell receptor potential with respect to the extracellularly recorded microphonic. The three curves are for three driver voltages to the Beyer speaker which produced 80 dB s.p.l.(○), 90 dB s.p.l.(●) and 100 dB s.p.l.(■) at frequencies of 100 Hz. *B*, isoresponse curves for the receptor potential at 2 mV (Δ) and for the cochlear microphonic potential at 0·04 mV (●). The lines indicate slopes of 12 dB/octave for the receptor potential and 6 dB/octave for the cochlear microphonic potential.

potential with respect to cochlear microphonic potential and sound pressure. Phase measurements, with respect to sound pressure, were restricted to frequencies below 400 Hz because of uncertainty about the middle ear transfer characteristics at frequencies above this (Wilson & Johnstone, 1975). If inner hair cells respond to basilar membrane velocity at low frequencies, then the isoresponse characteristics of their receptor potentials at these frequencies might be expected to have a slope of 12 dB/octave since these follow the second time derivative of sound pressure compared with the 6 dB/octave slope of the cochlear microphonic potential. Moreover, one decade below the electrical cut-off frequency (3 dB) of the hair cell, the receptor potential should phase lead cochlear microphonic potential by nearly 90°, reducing to 45° at the cut-off frequency. It is evident from Fig. 6 that these predictions are only partially met. Below 200 Hz the receptor potential isoresponse curve has a slope of 12 dB/octave, indicating that the inner hair cell's respond to the velocity of the basilar membrane; however, this slope is reduced above about 200 Hz. Below about 40 Hz the phase of the receptor potential leads the cochlear microphonic potential by 90° and sound pressure by about 180° but rapidly falls to zero between 200 and 300 Hz. At these low frequencies the voltage responses of some cells may be dominated by the electronic spread of the cochlear microphonic potential from the outer hair cells (Fig. 11). The isoresponse curves were derived from amplitude/intensity functions which were linear over the range of sound pressure levels employed and the phase data are plotted for three different driver voltages separated by 10 dB. At the highest attenuator setting the s.p.l. varied between 100 and 75 dB over the frequency range of the stimulus tones. However, it can be seen from Fig. 6 that the phase relationships are relatively independent of the stimulus intensity tones, which might be expected if their amplitude/intensity functions are linear. Table 1 shows that the time constants of hair cells vary between 0·19 and 0·89 msec which would produce cut-off frequencies between 937 and 178 Hz. However, the phase relationships between the receptor potential and cochlear microphonic potential change more rapidly from phase lead to zero phase difference than might be expected for a single pole, low-pass filter.

The significance of this rapid decline in phase lead was examined in a series of experiments in which the phase and amplitude characteristics of receptor potentials were measured in hair cells whose electrical time constants had been determined. The amplitude characteristics were plotted as iso-sound pressure level responses, but were in fact derived from responses to constant driving voltage to the Beyer microphone and subsequently corrected for constant sound pressure level. This method was chosen because we needed to obtain measurements as rapidly as possible before the intracellular recording conditions became unstable. The method relies upon the fact that the relationship between receptor potential amplitude and stimulus intensity remain approximately linear over the range of sound pressure levels which were used to obtain these data. This requirement was fulfilled, in part because the sound was delivered through a small diameter probe tube which acted as a low-pass filter and attenuated the sound stimulus at a rate of 6 dB/octave above 100 Hz, which almost compensated for the frequency-dependent increase in sensitivity of the cochlear microphonic potential and inner hair cell receptor potentials.

The relationships between the amplitude of the a.c. component receptor potential

and frequency for constant s.p.l. are plotted in Fig. 7 for a single hair cell and for the cochlear microphonic potential recorded in the scala tympani adjacent to the intracellular recording electrode. The slopes of the curves rise at 12 dB/octave and 6 dB/octave respectively until about 300 Hz; at 700 Hz and above the amplitude of the receptor potential declines at a rate of about 12 dB/octave. Thus in the cell illustrated, at frequencies below 300 Hz, the receptor potential behaves as if it responds to basilar membrane velocity. These relationships become more apparent if the ratio of a.c. component receptor potential amplitude/cochlear microphonic potential is plotted with respect to frequency since the results are then standardized

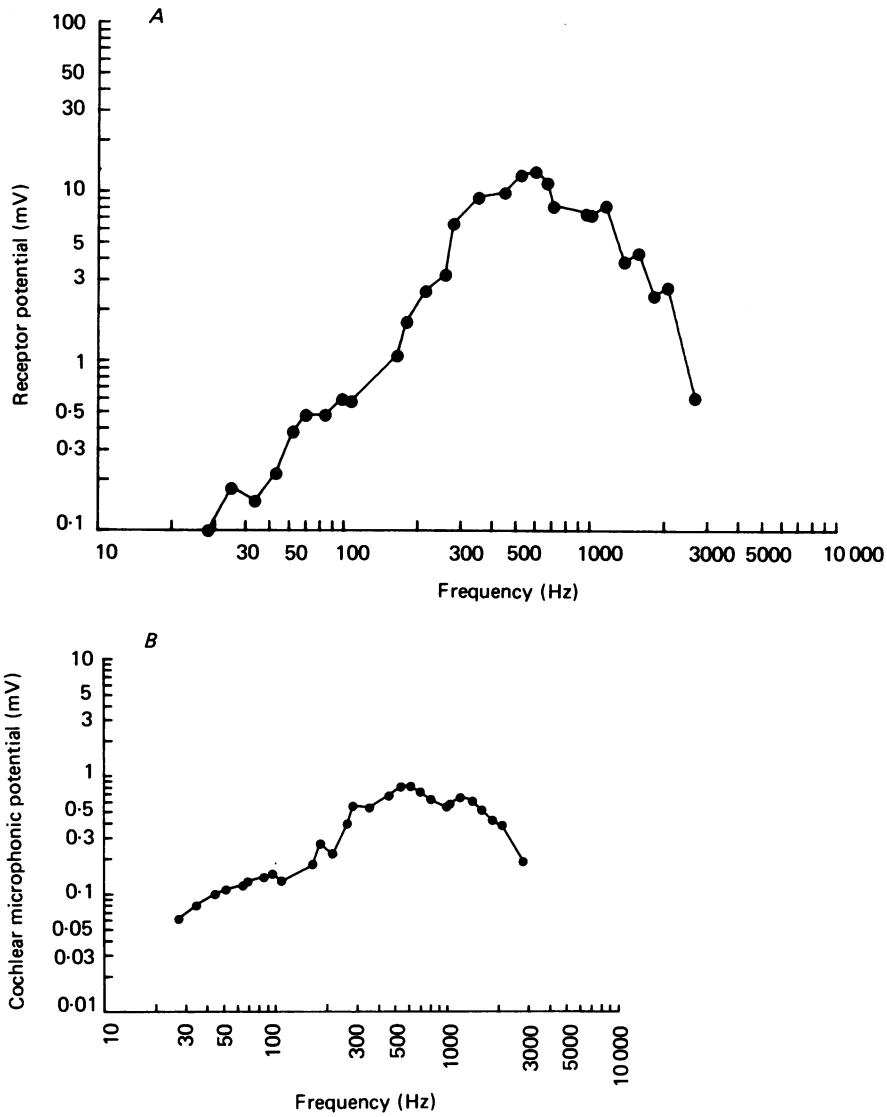

Fig. 7. The relationships between the amplitude of the inner hair cell receptor potential *A* and cochlear microphonic potential *B versus* frequency for constant s.p.l. at 70 dB s.p.l.

for constant basilar membrane displacement. This is illustrated in Fig. 8 where the relationship between the ratio of a.c. component receptor potential amplitude/cochlear microphonic potential *versus* frequency is shown for two cells.

The curves which have been drawn through the points are for a theoretical velocity detector which responds linearly to change in the a.c. component receptor potential amplitude/cochlear microphonic potential ratio with frequency, and whose characteristics are limited by two low-pass filters whose equations are given in (3A) above. One of these filters has a corner frequency determined by the electrical time constant of the hair cell; the other has been derived empirically. This second filter reflects an apparent change in the response of the inner hair cells from basilar membrane velocity to displacement and this change occurs at frequencies extending between about 200 and 600 Hz (Table 2).

This theoretical model of the inner hair cell response to low-frequency tones is supported by measurements of the phase of its voltage response relative to the sound pressure level, which at very low frequencies it predictably leads by 180°, and by 90° with respect to the cochlear microphonic potential (Fig. 9). The curves which have been drawn through the points are for a response to the velocity of basilar membrane motion which is limited by the presence of the two low-pass filters whose characteristics have been described above and whose phase relationships are given by:

$$\phi = \tan^{-1}\omega/\omega_0 + \tan^{-1}\omega/\omega_1. \qquad (3B)$$

Not all inner hair cells respond to basilar membrane velocity, and one cell (Table 2, cell 325-2) responded to displacement at frequencies below 30 Hz. This cell produced large d.c. component receptor potentials in response to high-frequency stimulation and its voltage responses at low frequencies were asymmetrical (Fig. 10*A*). It can be seen in Fig. 10*A* and *B* that at low frequencies the receptor potential is in phase with the cochlear microphonic potential, and the relationship between the amplitude of the receptor potential with frequency, at constant sound pressure level, has a slope of 6 dB/octave between 17 and 300 Hz, instead of the 12 dB/octave observed for velocity-dependent cells. The time constant of this cell was not measured, but the phase of the receptor potential lags the cochlear microphonic potential at high frequencies (Fig. 10*B*). The 45° point for this phase lag is 250 Hz, an indication that the time constant is probably about 0·6 msec.

4. Sensory interactions between inner hair cell receptor potentials and the cochlear microphonic potential

The release of transmitter from chemical synapses has been shown to be proportional to the potential difference across the presynaptic membrane (Katz & Miledi, 1967). In the organ of Corti, the potential difference across the presynaptic membranes of inner hair cells may be estimated by subtracting the extracellular potential recorded immediately outside them, from their intracellularly recorded receptor potentials. Examples of these measurements made on two hair cells, are shown in Fig. 11*A* and *B*. At the lowest stimulus frequency, 18 Hz, the intracellularly recorded receptor potential is symmetrical, in phase with the larger extracellularly recorded cochlear microphonic potential and probably represents the electrotonic spread of the extracellular receptor current into the cell. At this frequency it appears that the inner hair cell does not generate a receptor potential, or that it is too small for us to measure.

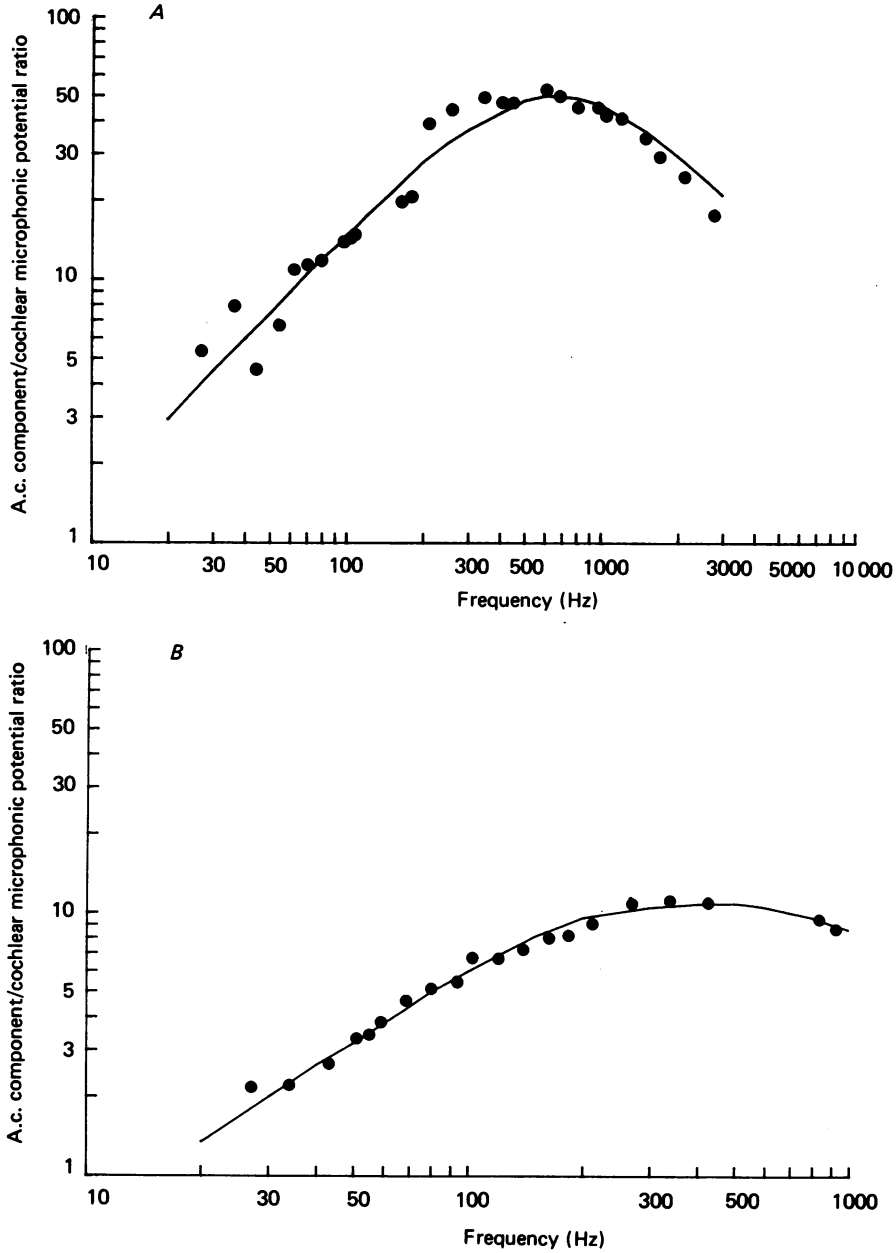

Fig. 8. The relationship between the ratio of the a.c. component of the inner hair cell receptor potential and cochlear microphonic potential (c.m.) *versus* frequency for the data illustrated in Fig. 7(*A*) and for another cell (*B*). The curves through the points are for a velocity detector which responds linearly to changes in the a.c. component/cochlear microphonic potential ratio with frequency but is limited by two single-pole low-pass filters with corner frequencies at, respectively, 720 and 600 Hz (*A*) and 840 and 300 (*B*). In each case the first filter corresponds to the hair cell's electrical time constant.

TABLE 2. Resting potentials, receptor currents and electrical time constants of inner hair cells. F_c is derived from T, column 6 is the frequency at which the phase of the receptor potential leads c.m. by 45°, and the last column is the frequency at which the hair cells change their response from velocity to displacement of the basilar membrane

Hair cell	Resting potential (mV)	Receptor current (mV/MΩ)	Time constant T (msec)	Cut-off frequency F_c (Hz) (3 dB point)	45° phase lead of r.p. with respect to c.m. (Hz)	45° phase lead corrected for T/F_c
402-2	35	0·35	0·89	178	—	—
403-1	20	0·90	0·22	723	170	270
403-2	25	—	0·26	612	195	350
403-3	35	0·27	0·28	576	180	720
403-6	40	0·62	0·62	252	160	325
403-7	35	0·10	0·31	515	160	360
403-8	25	0·13	0·38	418	160	420
403-9	30	0·64	0·48	328*	180	420
405-1	40	0·15	0·21	758	—	—
407-4	25	0·35	0·31	420*	180	315
407-8	45	0·42	0·56	283*	180	500
407-9	30	0·80	0·48	333	—	—
409-1	40	0·50	0·22	723	270	600
409-3	40	0·37	0·67	237	—	—
409-4	25	0·31	0·19	837	190	300
409-4	35	0·39	0·29	548	—	—
325-2	40	—	0·39	486	0	275
34A-2	45	—	0·21	600*	—	—

* Cells in which the relationship between the ratio of the a.c. to the d.c. component and frequency can be fitted with a single stage R–C filter whose corner frequency is the same as that of the membrane filter. Resting potential: r.p.; cochlear microphonic potential: c.m.

The extracellularly recorded cochlear microphonic potential is larger than the inner hair cell receptor potential for the responses shown in Fig. 10, viz. 18 Hz in *A* and 18 and 46 Hz in *B*. Thus for these frequencies and intensities, the potential difference across the presynaptic membrane of the synapse will be governed by the cochlear microphonic potential and it might be expected that neural excitation will be associated with the negative phase of this potential, when the basilar membrane is displaced towards the scala tympani. In this phase the inner hair cell will be depolarized relative to its electrical environment. At higher frequencies of auditory stimulation, when the inner hair cell receptor potential is larger than the extracellular cochlear microphonic potential, the microphonic potential still has an influence on the phase of the potential difference generated across the presynaptic membrane (Fig. 11*C*). From these phase relationships it can be predicted that neural excitation is associated with displacement of the basilar membrane towards the scala tympani at low frequencies, followed by a transition towards neural excitation associated with basilar membrane displacement towards scala vestibuli at higher frequencies.

DISCUSSION

1. Properties of the inner hair cell receptor potential

One of the most striking differences between the receptor potentials of inner and outer hair cells is in their symmetry about the resting membrane potential. Receptor potentials from inner hair cells resemble those which have been recorded from hair

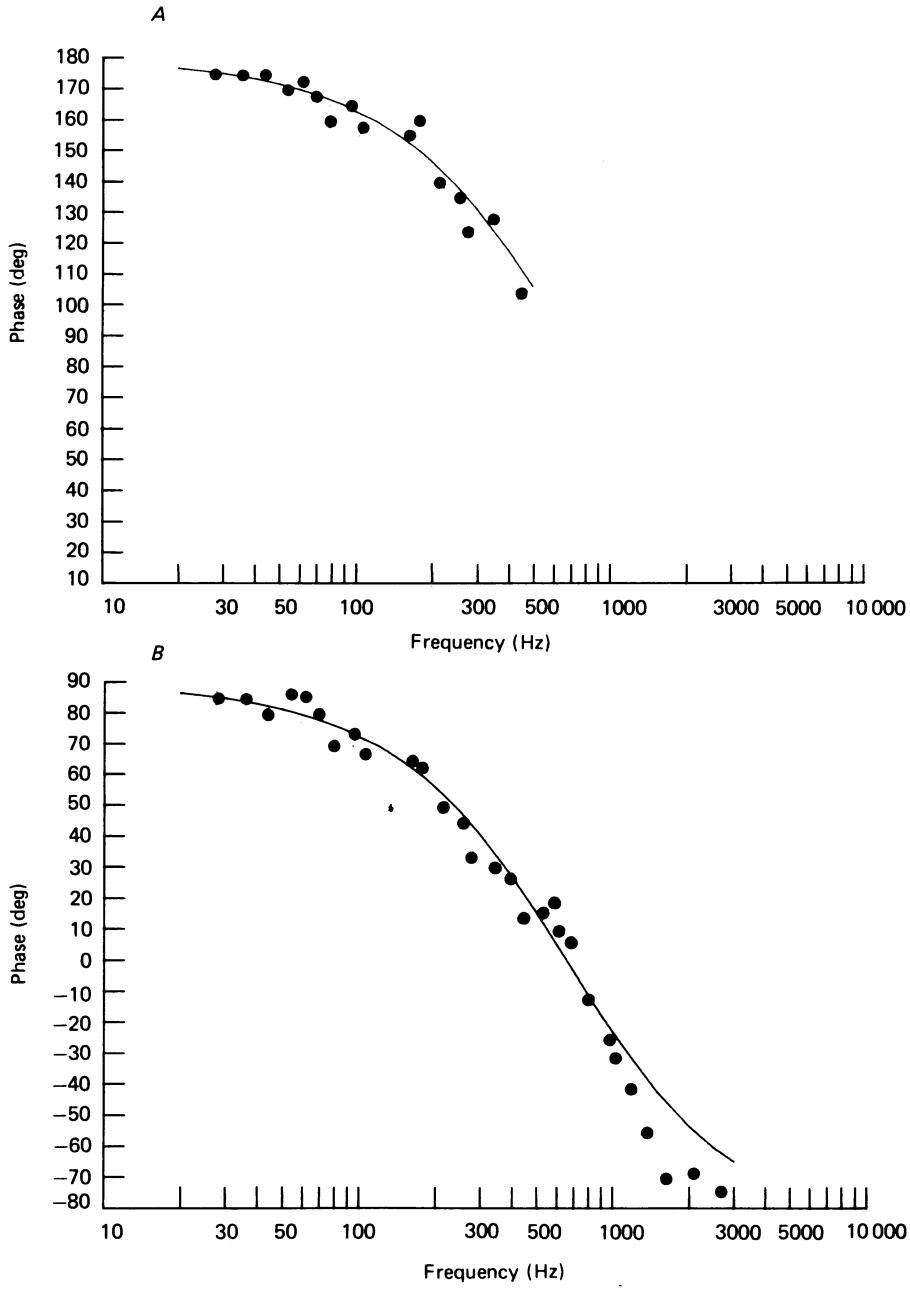

Fig. 9. For legend see facing page.

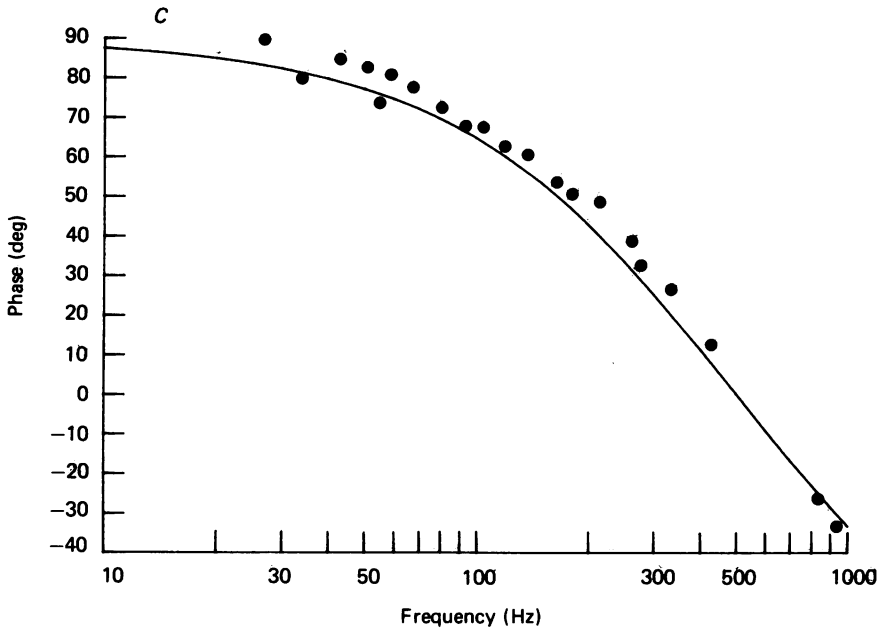

Fig. 9. The phase relationship of the cells illustrated in Fig. 10. *A*, for cell *A* with respect to sound pressure, *B* for cell *A* with respect to the cochlear microphonic potential, *C*, for cell *B* with respect to the cochlear microphonic potential. The theoretical curves are the phase relationships for the low-pass filters whose characteristics are described in Fig. 10.

cells in other acoustico lateralis receptors in that the peak amplitude of the depolarizing phase exceeds that of the hyperpolarizing phase by a factor of about 3 (Flock & Russell, 1976; Hudspeth & Corey, 1977; Crawford & Fettiplace, 1981). It is not known if the process responsible for the different symmetries of inner and outer hair cell receptor potentials is located before the conductance change associated with the receptor potential, for example as a property of the mechanics of the stereocilia, or if it is associated with the transduction process itself. This point has not yet been resolved in other preparations where it is possible to investigate the mechanical properties of the stereocilia by direct means (Hudspeth & Corey, 1977; Hudspeth & Jacobs, 1979).

The input–output characteristics of an inner hair cell, illustrated in Fig. 1*B*, have been fitted with reasonable agreement, by rectangular hyperbolae. This approximate fit to the input–output characteristics might be accepted with greater confidence if it had been possible to measure the saturation of the receptor potentials in the depolarizing and hyperpolarizing durations. However, this was not technically feasible. The discontinuity at the origin of these relationships has a functional significance in that at very low sound pressure levels the hair cell voltage response will contain a d.c. component, and this might be expected to grow at a constant rate from the point at which the voltage response could first be detected. This is in agreement with our earlier observations on amplitude–stimulus intensity relationships of the d.c. component of the receptor potential (Russell & Sellick, 1978). In response to high-frequency tones the a.c. component of the receptor potential,

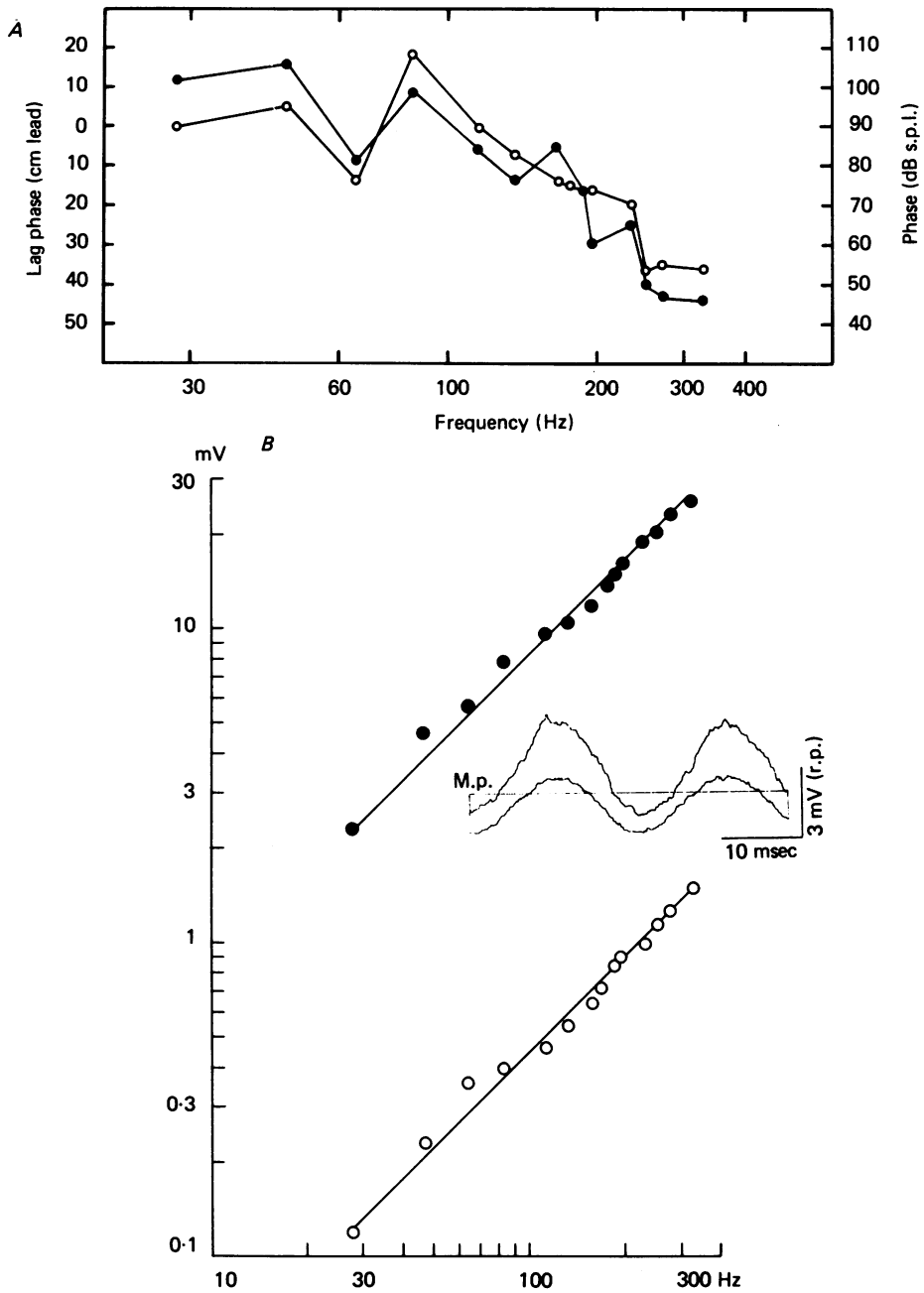

Fig. 10. *A*, the phase of the receptor potential in the displacement-sensitive cell 325-2 measured with respect to sound pressure (○) and cochlear microphonic (○). The time constant of this cell was not determined; however, the phase of the receptor potential lags the cochlear microphonic potential by 45° between 200 and 300 Hz, which indicates that the low-pass cut-off frequency of the cell is in this region. *B*, the relationship between the peak-to-peak amplitude of the receptor potential of a cell sensitive to basilar membrane displacement and stimulus frequency at constant 80 dB s.p.l.(●). ○ is the corresponding relationship for the cochlear microphonic potential measured in the scala tympani. The inset illustrates the receptor potential (r.p.) wave form (upper trace), and the cochlear microphonic potential (lower trace) in response to a 50 Hz tone at 80 dB s.p.l. M.p. indicates the membrane potential at −40 mV.

relative to the d.c. component, is very small, presumably because it is attenuated by the impedance of the hair cell membrane (Russell & Sellick, 1978). Our observations support this hypothesis in that the a.c. component of the inner hair cell receptor potential is progressively attenuated with respect to the d.c. component as the frequency of the stimulation tones is increased (Fig. 3). The low-pass filter characteristic described by this relationship can, in several instances, be defined by a single-pole, low-pass filter whose corner frequency coincides with the electrical time constant of the hair cell (Fig. 3*A* and *D* and Table 2). In the remaining examples the low-pass filter characteristics can be described by a two-pole filter with one of the poles having a corner frequency close to that of the membrane time constant. The other pole has a higher corner frequency and this filter might be due to the low-pass electrical characteristics of the micro-electrode which may have changed when it penetrated the cell and cannot be accurately accounted for. A further possibility is

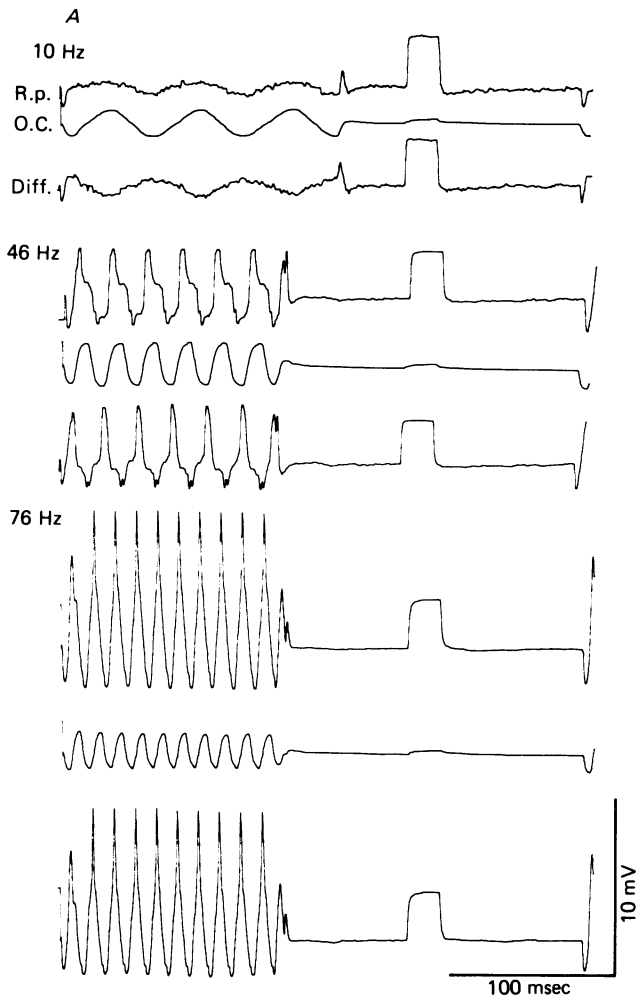

Fig. 11. For legend see p. 201.

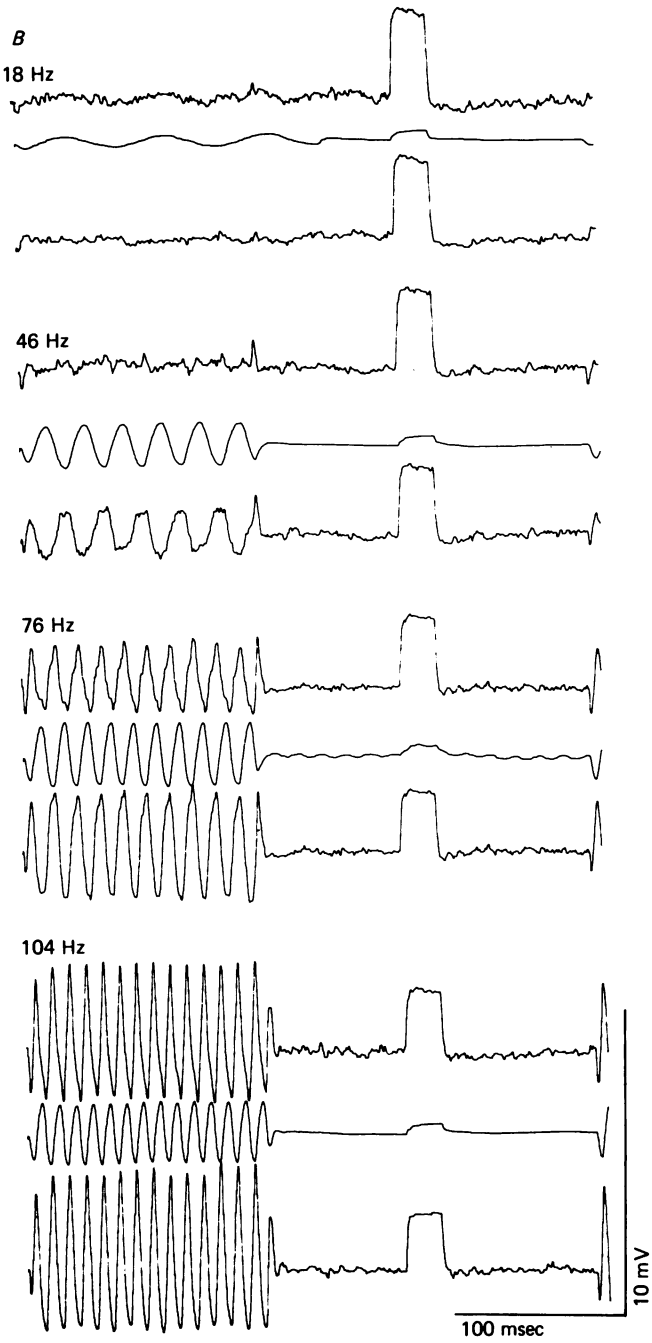

Fig. 11. For legend see facing page.

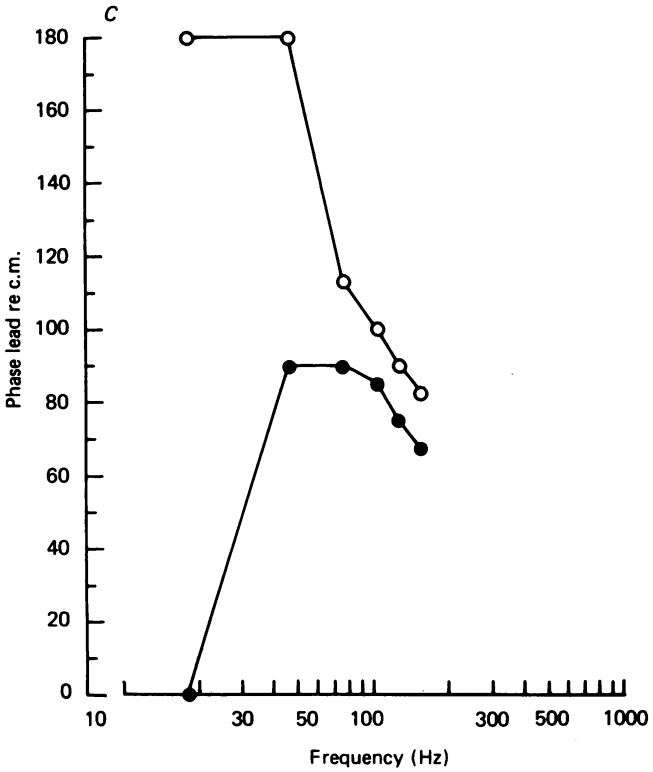

Fig. 11. *A* and *B*. Intracellular receptor potential (R.p.; upper trace), extracellular potentials recorded from the organ of Corti (O.C.; middle trace) and the difference between these two traces (Diff.; lower trace) for two cells in response to low-frequency tones at the different frequencies indicated against each trio of traces at 90 dB s.p.l. for *A* and 80 dB s.p.l. for *B*. In each trace the response to the low-frequency tone burst is followed by a response to a test tone at 16 kHz and 40 dB s.p.l. The inner hair cell voltage responses in *B* are very much distorted because of some non-linear behaviour of the cochlear partition which causes the cochlear microphonic potential to have a trapezoidal wave form in response to sinusoidal tones at high stimulus intensities. *C*, the phases of the inner hair cell receptor potential (●) and differential potential (○) *versus* cochlear microphonic potential for the cell illustrated in *B*.

that the response properties of the hair cells change with increasing frequencies. For example the stereocilia may become increasingly biased in the direction of excitation, and this would cause the hair cell responses to become increasingly asymmetrical and thereby increase the decline of the a.c. component relative to the d.c. component with increasing frequency. If transmitter release and, consequently, the excitation of auditory nerve fibres, is dependent upon potential changes across the presynaptic hair cell membrane, then it is important that the hair cell conductance is asymmetrical at all stimulus intensities so that a potential change can be produced in response to weak high-frequency tones.

It is not known what factors are responsible for the saturation of the voltage response. It is unlikely to be due to the reduction in driving voltage across the hair cell membrane, because the maximum depolarization of the hair cell which has yet been recorded (about +10 mV) is far below the presumed reversal potential of about

+80 mV (Johnstone & Sellick, 1972). In order to understand the basis for the saturation it is essential to have some knowledge about the conductance changes associated with the observed voltage changes, and the mechanical input to the inner hair cells. This is because the saturation can be attributed to either or both of these properties. The peak-to-peak amplitude of receptor potentials in response to low-frequency tones may reach maximum at around 6–30 mV above the resting membrane potential (Table 1), and the corresponding d.c. component would be about 2–10 mV. However, at frequencies close to the cells' characteristic frequency, the d.c. component may saturate at 30 mV. If the transfer function of the hair cell is independent of frequency, then the corresponding unattenuated a.c. component would be 120 mV, which is within the driving potential across the apical membranes of the hair cells. However, it may be that the stereocilia of the inner hair cells do become progressively biased with increasing frequency to yield proportionately larger d.c. components of the receptor potential.

Our results show a close correspondence between the inner hair cell voltage responses and the neural responses of fibres in the auditory nerve, which have been recorded elsewhere, in response to low frequency tones. In the majority of inner hair cells from which recordings were made, the membrane potential is modulated only in the depolarizing direction at frequencies above 1 kHz. This might form the foundation for an observation by E. Le Page (personal communication) who has recorded impulses from spiral ganglion cells in the basal turn of the guinea-pig cochlea. At frequencies above 1 kHz the afferent impulses are modulated only in the excitatory direction, and the a.c. component/d.c. component ratio of post-stimulus histograms of these impulses indicate that the ratio decreases with frequency in a manner predicted from our data. This decline in the a.c. component/d.c. component ratio of the receptor potential has a correlation in the decrease in phase locking of neural responses in auditory nerve fibres of 6 dB/octave above 1 kHz (Rose, Brugge, Anderson & Hind, 1968).

2. Receptor potentials from outer hair cells

In contrast to the receptor potentials of inner hair cells, those from outer hair cells are symmetrical and almost an order of magnitude smaller. Their small amplitude may be because they have a low electrical resistance or it may be that little receptor current flows through them. The fact that their voltage responses dominate the supporting cells and fluid spaces in the organ of Corti would support the view that the outer hair cells pass a large amount of current and that the small amplitude of their receptor potentials is due to their low access resistance. It should be kept in mind that the inner hair cells are completely surrounded by supporting cells whereas the lateral walls of outer hair cells are exposed to the extracellular spaces of Nuel. Hence it is conceivable that outer hair cells dominate the cochlear microphonic potential by passing large receptor currents into the extracellular space. The large intracellular responses which have been recorded from supporting cells may indicate strong electrical coupling between these cells and the extracellular spaces (low-input impedance) or with the outer hair cells, although there is no morphological evidence for the latter. The small size of intracellularly recorded cochlear microphonic potential from inner hair cells and the large size of their receptor potentials and input

resistance (Russell & Sellick, 1978) may indicate that this coupling does not extend to the inner hair cells.

The symmetrical receptor potentials of outer hair cells have an interesting implication for the functional limitations of these cells. At high frequencies of auditory stimulation they generate little or no d.c. component, and, therefore, will not excite afferent fibres at these frequencies, if it is assumed that neural transmission between the hair cells and their afferent innervation is a voltage-dependent process. Presynaptic bodies and vesicles are not commonly found in outer hair cells (Spoendlin, 1966), and the structure of the type II afferent fibres, which innervate the outer hair cells, lacks neurotubules, and are poorly myelinated (Spoendlin, 1978). Thus it is of considerable interest to discover if the type II hair cell neurones relay any sensory information to the central nervous system. If these afferent neurones do relay sensory information then they will carry information about the tonic displacement of the basilar membrane. Such information could be of considerable importance if it was fed back directly or indirectly to the efferent neurones which massively innervate the outer hair cells. The neurones are believed to exert post-synaptic effects on hair cells which may involve changing their mechanical properties and those of the cochlear partition (Mountain, 1980; Siegel & Kim, 1980).

3. The mechanical coupling of inner hair cells to basilar membrane motion

The idea that hair cells with free-standing stereocilia respond to basilar membrane velocity, proposed by Dallos, Billone, Durrant, Wang & Raynor (1972), is supported by our analysis of the phase and amplitude relationships of inner hair cells, which indicates that they respond to velocity at frequencies below 200–600 Hz and to basilar membrane displacement above this. Thus, in common with hair cells in other acoustico lateralis receptors, which respond to the motion of fluid in their environment, inner hair cells in the guinea-pig cochlea show transitional responses to this motion. For example, recordings of impulse activity in the sensory nerves, and extracellular receptor potentials in lateral line organs and the crista ampularis of the semi-circular canals (Kroese, van der Zalm & van den Berken, 1980; Melville Jones & Milsum, 1971), indicate that the responses of hair cells in these organs change from acceleration at very low frequencies of fluid motion, to velocity, and finally displacement at the highest frequencies.

On one occasion we encountered a presumed inner hair cell which responded to basilar membrane displacement throughout its low-frequency range (cell 325/2, Table 2). The response of this cell may have been a consequence of the experimental manipulation. For example the organ of Corti may have been displaced by the recording electrode so that the stereocilia of the inner hair cells were brought into contact with the undersides of the tectorial membrane. It may have been that the inner hair cell was one of a small population of naturally occurring cells with their stereocilia in contact with the tectorial membrane. Imprints of the stereocilia of inner hair cells have been observed in the tectorial membrane in the basal turn of kittens and cats, but not in guinea-pigs (Hoshino, 1974). However, the morphological relationship between hair cells and their accessory structures is not always a reflexion of their functional relationship. For example the free-standing stereocilia of hair cells in the basal region of the alligator lizard cochlea respond to basilar membrane

displacement and not velocity as might be expected from their morphology (Peake & Ling, 1980).

4. Interaction between inner hair cell receptor potentials and cochlear microphonic potentials

The different growth rates of the receptor potentials generated in inner and outer hair cells, with respect to frequency, and the dominating influence of receptor currents, originating in the outer hair cells, on the tissue of the organ of Corti, results in an effective electrical interaction between inner and outer hair cells. This interaction is probably small at high stimulus frequencies, because at these frequencies the receptor potentials from outer hair cells are much smaller than those from inner hair cells, and they do not generate a d.c. component receptor potential. Furthermore, their phasic receptor potentials will be greatly attenuated by the capacitative impedance of the tissue. However, this interaction is important at low frequencies of auditory stimulation when the cochlear microphonic potential is large compared with the inner hair cell receptor potentials and this has considerable significance for the mode of excitation of the auditory fibres which innervate the inner hair cells.

At very low frequencies of auditory stimulation the extracellular receptor potentials exceed those recorded intracellularly from the inner hair cells (Fig. 11 *A* and *B*) and this might be predicted to result in a decrease in transmitter release from the hair cell afferent synapse during the depolarizing phase of the cochlear microphonic potential, i.e. when the basilar membrane is displaced towards the scala vestibuli (Davis, 1965), causing a decrease in discharge rate of the auditory nerve fibres.

Sokolich (1977), Sokolich, Hamernik, Zwislocki & Schmeidt (1976) and Schmeidt, Zwislocki & Hamernik (1980) recorded the responses of single fibres in the auditory nerve of the gerbil to trapezoidal displacements of the basilar membrane caused by 40 Hz triangular sound pressure wave forms. They found the great majority of fibres were excited when the basilar membrane was displaced towards the scala tympani. This observation has recently been confirmed by Sellick, Pattuzzi & Johnstone (1983) in the guinea-pig cochlea. On the basis of our observations we would propose that the polarity of these responses was due to the relative depolarization of the inner hair cells with respect to their immediate environment. At frequencies above 40 Hz and 70 Hz for the afferent fibres innervating the cells illustrated in Fig. 11 *A* and *B* respectively, the polarity of excitation changes towards scala vestibuli.

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