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INTRACELLULAR STUDIES OF HAIR CELLS IN THE MAMMALIAN COCHLEA

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SUMMARY

1. Intracellular recordings were made from inner hair cells in the first turn of the guinea-pig cochlea, the recording sites being confirmed by the injection of Procion yellow dye and subsequent histology.

2. The receptor potential, in response to a pure tone burst, consisted of an AC response which followed the wave form of the stimulus and was analogous to the extracellularly recorded cochlear microphonic and a depolarizing DC response which followed the envelope of the tone burst and was analogous to the extracellularly recorded summating potential.

3. The DC response was broadly tuned at high sound pressure having a maximal amplitude of 27 mV at a sound pressure level of *ca.* 100 db; however the bandwidth of the response was reduced at lower sound pressure level. Isoamplitude curves for the DC response were indistinguishable from the threshold curves for auditory nerve fibres.

4. The AC response was tuned in a similar fashion to the DC response except that it was attenuated at 6–9 db/octave with respect to the DC response. It is suggested that this difference was due to the effect of membrane capacitance and resistance on the AC response. In contrast the extracellularly recorded AC component was not subject to this attenuation.

5. The total resistance and capacitance in three cells were found to be 46–61 MΩ and 7·8–15·8 μF respectively.

6. Intracellular resistance changes were measured during sound stimulation, the resistance change being proportional to the DC receptor potential, indicating constant current flow through the hair cell. The current varied between 0·37 and 0·81 nA between cells. The time constant for seven cells was found to lie between 0·31 and 0·76 msec.

7. A map of the basilar membrane showing position of hair cells against characteristic frequency corresponded to the cut-off frequencies of the basilar membrane mechanical measurements and the innervation sites of spiral ganglion cells.

INTRODUCTION

The frequency selectivity of the cochlea, which represents its ability to resolve the components of a complex sound, is a subject of controversy. Mechanical measurements of basilar membrane vibration to pure tones show that the amplitude of

vibration varies along the length of the basilar membrane, with the maximum depending in a graded way upon the frequency of the tone (von Békésy, 1960; Johnstone & Boyle, 1967; Rhode, 1971; Wilson & Johnstone, 1975). The basal turn responds maximally to high frequencies, the apex to low frequencies. The mechanical frequency tuning curves (f.t.c.s; the relationship between the sound pressure level required to produce an arbitrarily determined displacement and frequency at a single point on the basilar membrane) are broadly tuned. In contrast, the neural f.t.c.s (the relationship between threshold response and frequency) of the single nerve fibres which innervate the individual hair cells that are distributed in the organ of Corti along the cochlear partition, are sharply tuned (Kiang, Watanabe, Thomas & Clark, 1965; Evans, 1972).

There has been considerable speculation about the process responsible for the sharpened neural f.t.c.s and the existence has been proposed of a second filter in addition to the broad mechanical tuning of the basilar membrane (Evans, 1972). This filter has been shown to be physiologically vulnerable, as the neural f.t.c.s become detuned and resemble the mechanical tuning of the basilar membrane when the cochlea is made anoxic or treated with metabolic inhibitors and ototoxic drugs (Robertson & Manley, 1974; Evans & Klinke, 1974). There is evidence that the proposed second filter is private to each nerve fibre and located peripheral to it (Evans & Wilson, 1973). In the mammalian cochlea more than 95 % of the auditory nerve fibres innervate inner hair cells (Spoendlin, 1972) and it is probably from this population that the neural f.t.c.s have been derived, thus the inner hair cells are candidates for the site of the neural sharpening process.

Until recently the only measurements of hair cell activity were the extracellularly recorded cochlear microphonic and summating potentials. These have been recorded from single-ended electrodes on the round window (Adrian, 1931), spiral lamina (Yates & Johnstone, 1976) and in the scala media (Honrubia & Ward, 1968) or from differential electrodes placed either side of the cochlear partition (Dallos, 1973). Interpretation of these data has been difficult because the recordings are essentially field potentials from a large number of generators, dominated by the responses of the outer hair cells, that have different spatial and temporal relationships to each other. This is further complicated by the complexity of the resistance network of the cochlear partition and the relationship between its adjacent turns.

We have avoided the difficulties inherent in complex field potential analysis by making intracellular recordings of the AC and DC components of the receptor potential (homologues of the extracellularly recorded cochlear microphonic potential (c.m.) and summating potential (s.p.) respectively) in the first turn of the guinea pig cochlea in an attempt to examine their tuning properties and to compare them with neural and mechanical f.t.c.s.

A preliminary account of this work has been reported elsewhere (Russell & Sellick, 1977*a*, *b*).

METHODS

Guinea-pigs weighing 120–200 g were maintained throughout the experiment under pentobarbitone-phenoperidine anaesthesia and were paralysed with Flaxedil (gallamine triethiodide) and artificially ventilated (method of E. F. Evans, personal communication). The cochlea was exposed by displacing the mandible and opening the bulla tympanica ventrolaterally. The first turn of the cochlea was illuminated from behind, on the scala vestibuli side, by placing a 1 mm

diameter fibre optic light source against it. This permitted a view of blood vessels in the bone of the first turn during exposure of the basilar membrane and facilitated the positioning of the micro-electrode. The cochlea was opened by thinning the bone over the scala tympani with a No. 15 scalpel which had been ground to a chisel point with a width of about 1 mm. Care was taken to avoid blood vessels in the bone and the opening was made by hooking out bone fragments with etched stainless steel hooks. The threshold (within an accuracy of 10 db) of the compound action potential (a.p.) evoked by a pure tone burst of 1 msec rise time was monitored during exposure of the basilar membrane by a fine Teflon insulated silver wire positioned close to the round window and secured to the skull with cyanoacrylic cement. Maintenance of the a.p. threshold to tones above 10 kHz during exposure of the basilar membrane depended upon the amount of bleeding from the bone which occurred during the exposure. It was possible to expose the basilar membrane while preserving the original a.p. threshold. A fall in a.p. threshold to tones of frequencies below 10 kHz indicated that the general physiological condition of the animal was low or that damage had occurred to the tympanic membrane or ossicular chain.

The sound system was a driven half-inch condenser microphone (B&K 4133) in a hollow ear bar which was inserted in the tympanic ring and secured to the temporal bone and adjacent bones with dental cement. A brass bar was then cemented between the temporal bone and the rigid head holder to ensure maximum stability of the preparation. A distortion compensation network was used to reduce the second and third harmonic distortion from the sound system to at least 50 db below the fundamental. It was necessary to momentarily drain the scala tympani so that the electrode could be accurately placed on the basilar membrane. This was achieved by placing a fine wick of cellulose material with long parallel fibres (Boots Nappy Liner) through the round window after the round window membrane had been removed, and applying suction to it. The optimum position for the end of the wick was on the spiral lamina adjacent to the recording site. The perilymph returned after suction was turned off so that recording was done while the scala tympani was flooded. Occasionally the a.p. threshold was reduced by as much as 40 db during drainage when the wick was particularly effective as was observed by Robertson (1974). At the end of the experiment the sound system was calibrated with a calibrated probe tube attached to a $\frac{1}{2}''$ microphone. All sound pressure levels are corrected for the sound system and are relative to 0 db re 2×10^{-5} N/m^2.

Micro-electrodes were drawn from 1 mm o.d. fibre filled borosilicate glass tubing (Clark–Electromedical) on a Livingstone type electrode puller. They had initial resistance of about 700 MΩ and 200 MΩ when filled with 6 % aqueous solution of Procion yellow and 4 M-potassium acetate respectively. Their resistances were reduced to half these values by bevelling the electrode tips on a grinding wheel to facilitate penetration of the basilar membrane (Brown & Flaming, 1975). A hydraulic microdrive was used to advance the electrode through the basilar membrane. A Bioelectric P1 probe equipped with capacitance compensation was used to measure intracellular receptor potentials. Optimal compensation of the probe produced an amplification of signals between 1 and 20 kHz of 5–6 times depending on the degree of capacitance compensation and the electrode resistance. The magnitude of c.m. is, therefore, subject to a small unsystematic error.

The AC component of the receptor potential was measured with potassium acetate electrodes and two Brookdeal 9503SC lock-in amplifiers in quadrature. The modulus of the signal was obtained from a Brookdeal Omniphase, displayed on a chart recorder, and stored on an FM tape recorder for future analysis. Some of the analysis was performed with a small special purpose computer (Biomac 1000). During electrode penetration a search stimulus consisting of a pure tone swept between 1 and 23 kHz with a total duration of 10 sec was gated to produce tone bursts 80 msec in duration, with rise and fall times of 10 msec and approximate sound pressure level (s.p.l.) of 80 db. The spacing of the tone bursts was arranged so that successive tone bursts were 500 Hz higher in frequency than the preceding ones. Upon entering a cell successive sweeps were attenuated in 10 db steps until the response disappeared. Intensity functions corrected for s.p.l. were plotted for individual frequencies from this data and isoamplitude curves were derived from these.

Resistance measurements were made from hair cells during sound stimulation and simultaneous recording of the DC component of the receptor potential. The measurements were made according to the method of Pinto & Pak (1974). Sinusoidal currents ($1{\cdot}5 \times 10^{-11}$ A) with frequencies of either 40, 100 or 150 Hz were passed through the recording electrode via a bridge

circuit into an impaled hair cell. Those recording electrodes with good electrical characteristics were chosen for this experiment. Voltage changes due to the electrode's intrinsic impedance were minimized with the amplifier bridge circuit and voltage changes due to changes in the resistance of the hair cell were measured with the lock-in amplifiers, one set in phase and one 90° out of phase. Thus the real and imaginary impedances of the hair cell could be observed and the time constant of the hair cell calculated. Measurement of the real and imaginary impedance changes at different frequencies indicated that the changes in impedance were due to pure resistance changes.

Inner hair cells were dye marked by ionophoretic injection of Procion yellow after the DC receptor potential was measured at different frequencies and s.p.l.s. Only one cell was marked in each animal to avoid confusion between the electrical response and the dye mark. The cochlea was quickly dissected out of the animal and immersed in 2·5 % glutaraldehyde buffered to pH 4 with 1 mM-potassium hydrogen pthalate. The cochlea was fixed overnight, decalcified for a further day in 8 % EDTA at ph 4, embedded in Epon, sectioned at 10 μm and examined with a fluorescence microscope for the dye-marked hair cell.

The tonotopic distribution of hair cells (other than those dye marked) was mapped by placing a small ink mark on the spiral lamina adjacent to the recording site. The cochlea was then dissected out to expose the basal extreme or hook region of the basilar membrane. Two photographs were taken through the dissecting microscope, one of the hook region and the other of the recording site. Care was taken to ensure that the basilar membrane was as much in the plane of the microscope objective as possible. Distance from the hook to the recording site was then measured from the two photographs.

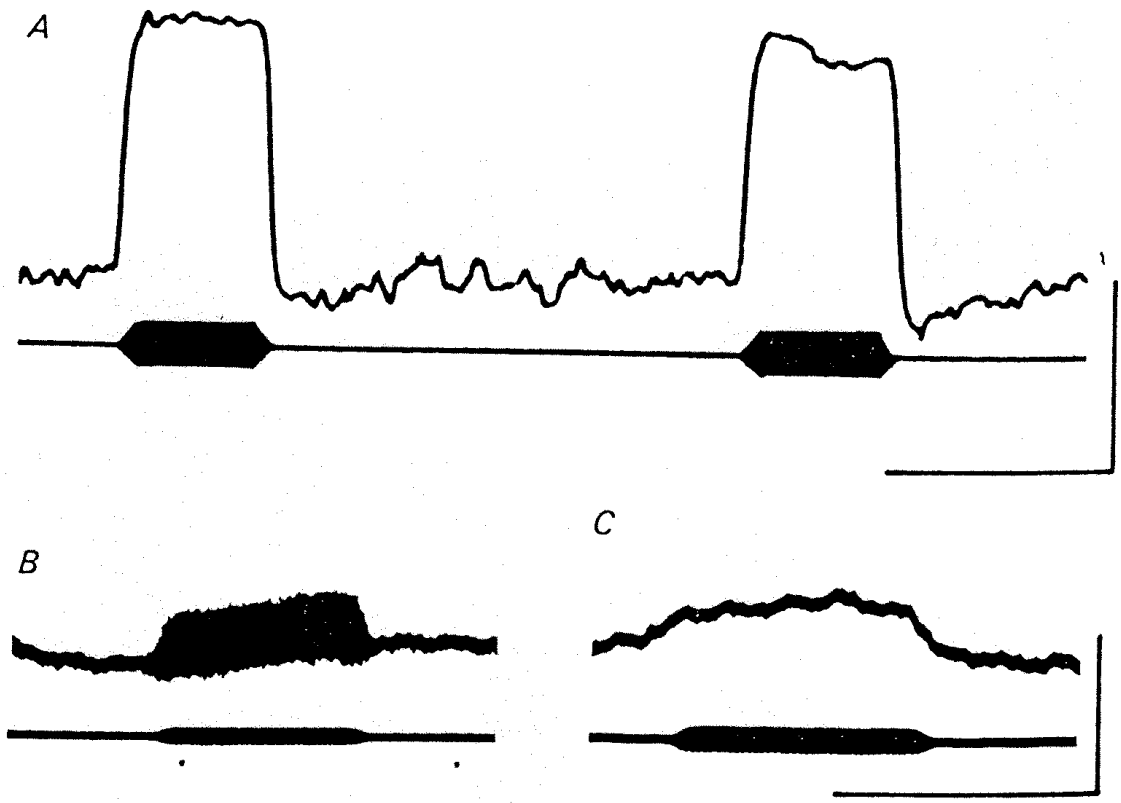

Fig. 1. *A*, DC component of receptor potential recorded intracellularly from an identified inner hair cell in response to a tone burst at its c.f. (17 kHz) and s.p.l. of 80 db. Horizontal bar 100 msec, vertical bar 10 mV. *B*, AC component of receptor potential recorded intracellularly from an inner hair cell with a c.f. of 16 kHz in response to a 2 kHz tone burst at s.p.l. of 80 db. Horizontal bar 100 msec; vertical bar 5 mV. *C*, Depolarizing potential recorded from a supporting cell in response to a 17 kHz tone burst at 80 db, corresponding to the c.f. of adjacent hair cells. Horizontal bar 100 msec; vertical bar 5 mV. Lower trace in all records shows the tone burst envelope.

RESULTS

1. *Receptor potentials from morphologically identified inner hair cells*

The first object of the experiments was to record receptor potentials from inner hair cells in the organ of Corti. This was achieved by aiming Procion filled microelectrodes in the direction of the inner hair cells and marking those cells which produced a large electrical response to sound stimulation. It was possible to record only the DC component of the receptor potential because of the poor frequency response of the high impedance Procion yellow electrodes. The DC component always consisted of a depolarizing potential of the order of 5–17 mV in response to a tone of

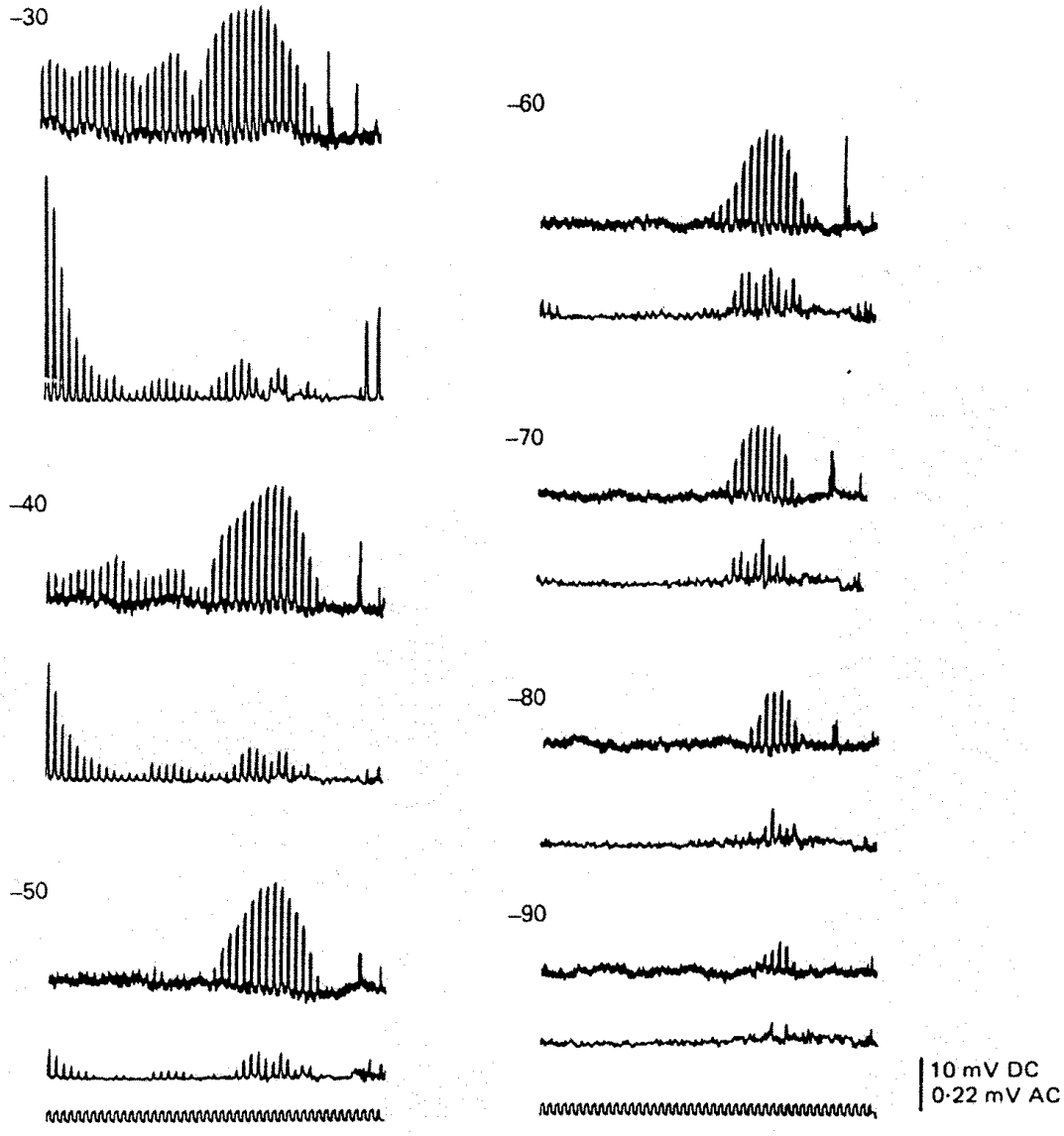

Fig. 2. DC (upper trace) and AC (lower trace) receptor potentials to 80 msec tone bursts from a presumed inner hair cell. In each pair of traces the upper trace is the output from the probe amplifier and the lower trace is the modulus output from the lock-in amplifiers. The numbers by the side of each pair of traces indicate db attenuation of the sound system. The lower traces show the trigger pulses for individual tone bursts at 500 kHz intervals; the frequency scale is linear from 1 to 23 kHz.

about 80 db at characteristic frequency (c.f.) (Fig. 1*A*). Procion dye was injected into twenty nine cells from which potentials of this magnitude were recorded; 11 were found histologically and all were identified as inner hair cells. These cells could be held with stable resting potentials of 25–45 mV for as long as 30 min but the majority were lost within a few minutes of penetration. This was considered satisfactory evidence that it was possible to record selectively from inner hair cells and the Procion dye-filled electrodes were abandoned for the lower resistance potassium acetate electrodes which, with their higher frequency response and with capacitance compensation, made it possible to record the AC component of the receptor potential (Fig. 1*B*).

Other cells encountered by the electrode before reaching the hair cells had resting potentials between 75 and 100 mV and, in response to a tone burst of *ca.* 80 db, small DC potentials, never more than 3 mV in amplitude, were recorded (Fig. 1*C*). These potentials were believed to be recorded from supporting cells and were the result of electrotonic spread of current from adjacent hair cells because they were not accompanied by resistance changes (cf. section 5).

2. *The variation of intensity function with frequency*

Only fourteen of the intracellularly recorded hair cells could be held for a period long enough (10–15 min) to explore the frequency and intensity characteristics of the DC and AC components of the receptor potential. At high s.p.l.s (100 db) all cells respond with a large receptor potential up to the characteristic frequency above which the receptor potential fell to very low values. Thus at high s.p.l.s the cells have a low pass characteristic. However as the s.p.l. is reduced they respond to a limited range of frequencies around their c.f. (Fig. 2). At very low s.p.l.s the DC and AC responses were reduced to the noise level of the recording system and the point at which this occurred varied between 0 db for very sensitive cells (Fig. 2) and 50 db for insensitive ones (Table 1). A correlation was found between threshold of the compound a.p. of the auditory nerve at c.f. and the sensitivity of the cells (Table 1).

Intensity functions (the relationship between the amplitude of the receptor potential and the s.p.l. at a fixed frequency) for the DC and AC components for very sensitive cells are illustrated in Fig. 3*A*, *B*, *C* and *D*. In general the intensity functions of the DC and AC components are similar in that they rise linearly with a slope close to 1 from the noise level of our measurements and saturate at higher s.p.l.s At frequencies above and below the c.f. of the hair cell the intensity functions begin to saturate at much higher s.p.l.s than at frequencies close to c.f. For example, in animal 222 the DC intensity function at c.f. (1 kHz) begins to saturate at s.p.l. of about 12 db whereas 2 kHz above and below c.f. saturation does not begin until 32 and 28 db respectively.

The intensity functions exhibit a second frequency dependency, namely that the saturation level of the receptor potential declines with increasing frequency, e.g. in Fig. 3*C* this level changes from above 20 mV at 12 kHz to below 4 mV at 19 kHz.

In addition to the frequency dependent characteristics described above, which they share with the DC intensity functions, the AC intensity functions are influenced by a further frequency dependent relationship, namely that the AC receptor

TABLE 1. Details of the response characteristics of the AC and DC components of the receptor potentials recorded presumably from inner hair cells

				DC f.t.c.[1]					AC f.t.c.				
Expt. no.	C.f. (kHz)	Resting potential (mV)	A.p. threshold s.p.l. (db)	$Q_{10\ db}$	Threshold s.p.l. (db)	H.f. slope db/octave	L.f. slope db/octave	Tip–Shoulder s.p.l. (db)	$Q_{10\ db}$	Threshold s.p.l. (db)	H.f. slope db/octave	L.f. slope db/octave	Tip–Shoulder s.p.l. (db)
105	23	35	15	7·7	21·5	500	253	26	—	—	—	—	—
222	17·5	30	0	6·0	−2	475	136	66	9	5	800	190	65[2]
223	17	20	0	8·5	8	340	104	79	7·4	12·5	400	135	59[3]
225	17	30	0	8·7	5	460	162	67	—	—	—	—	—
211	18	43	20	5·3	50	400	45	35	4·9	43	300	75	42[4]
210	15	35	30	6·0	42	650	98	29	—	—	—	—	—
218	17	40	10	9·4	7	425	130	69	8·5	8	460	144	62[5]
197	17	30	10	11·3	6	350	170	67	—	—	—	—	—

[1] Based on 2 mV f.t.c.
[2] Based on 0·2 mV f.t.c.
[3] Based on 0·4 mV f.t.c.
[4] Based on 0·06 mV f.t.c.
[5] Based on 0·04 mV f.t.c.

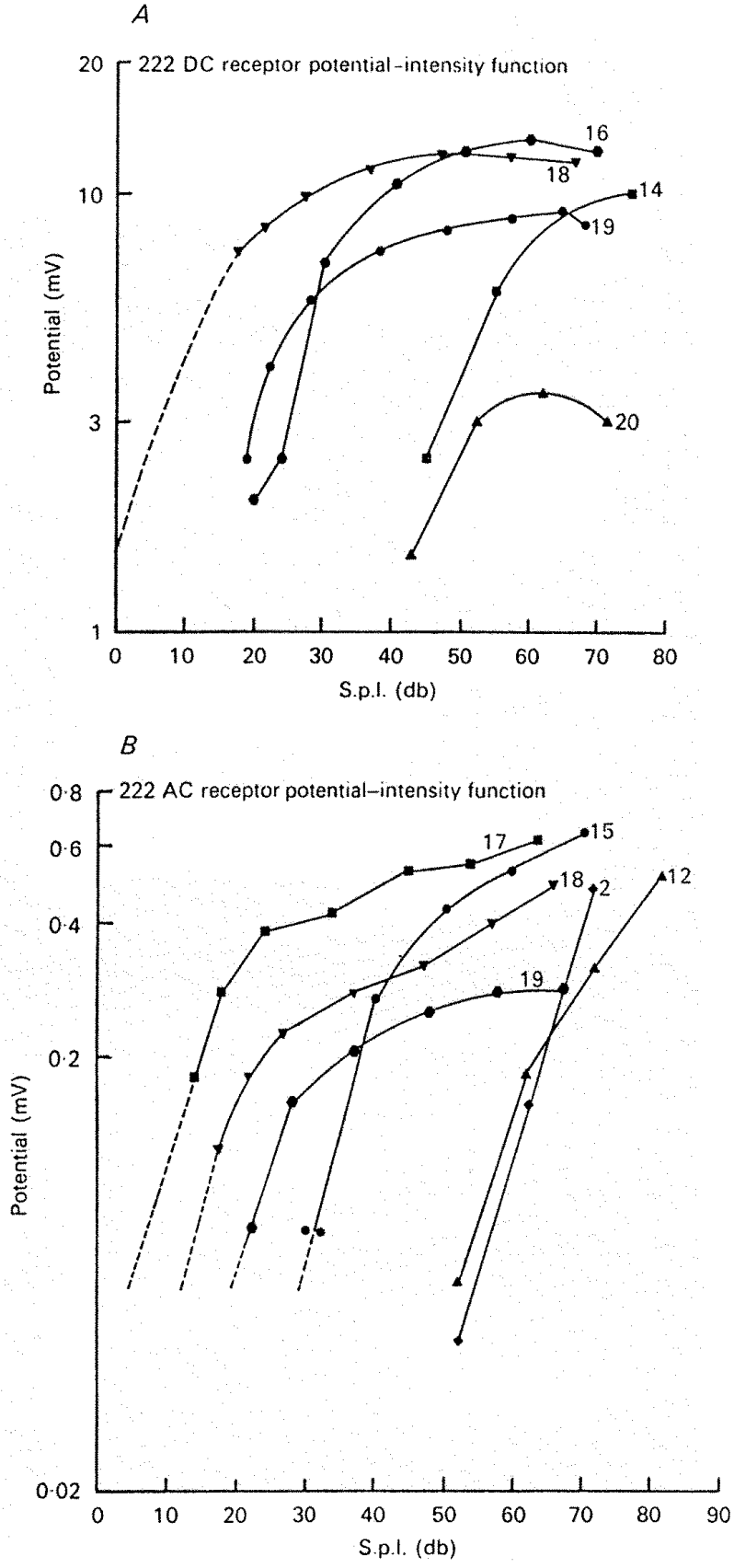

Fig. 3*A* and *B*. For legend see page 270.

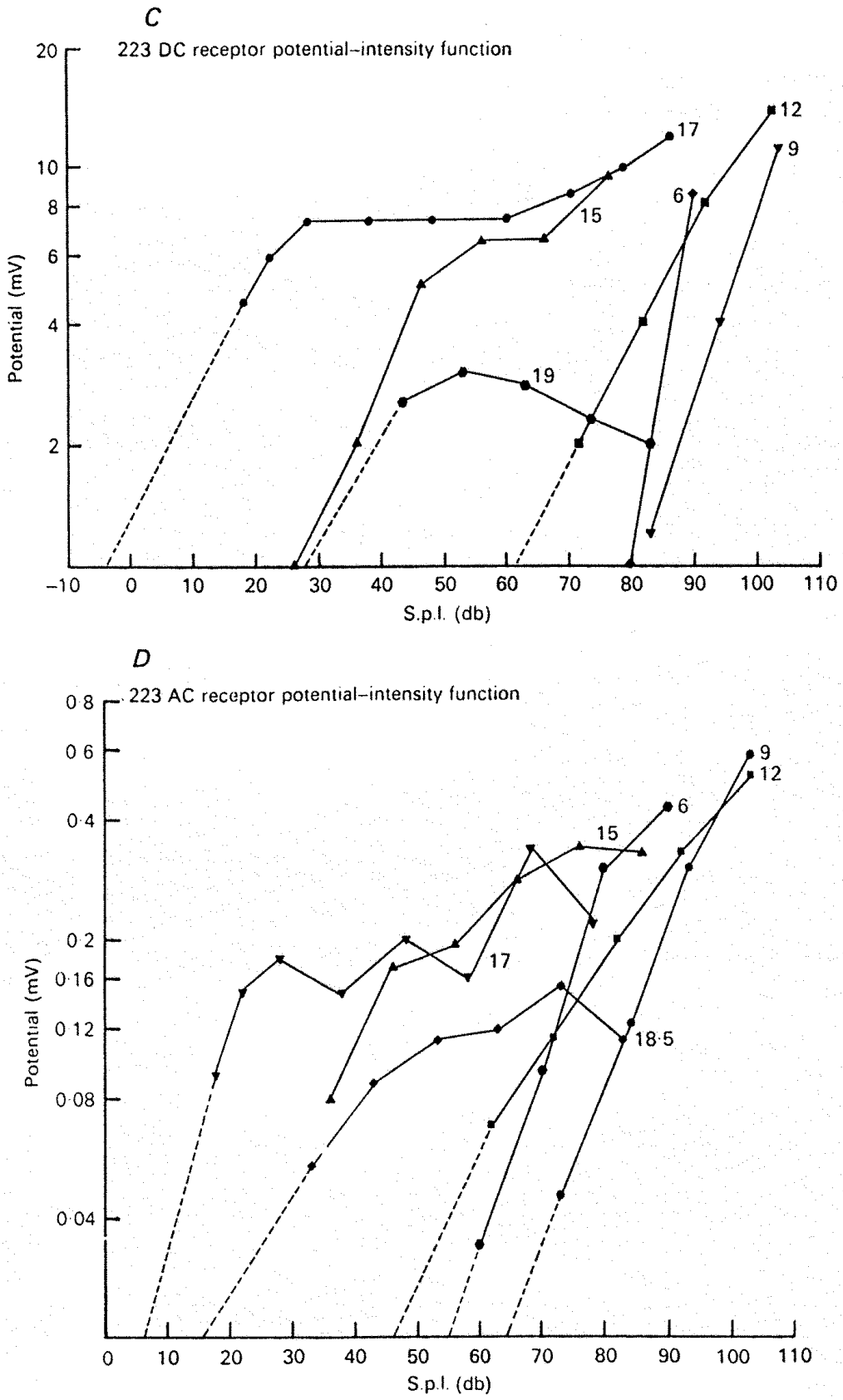

Fig. 3*C* and *D*. For legend see page 270.

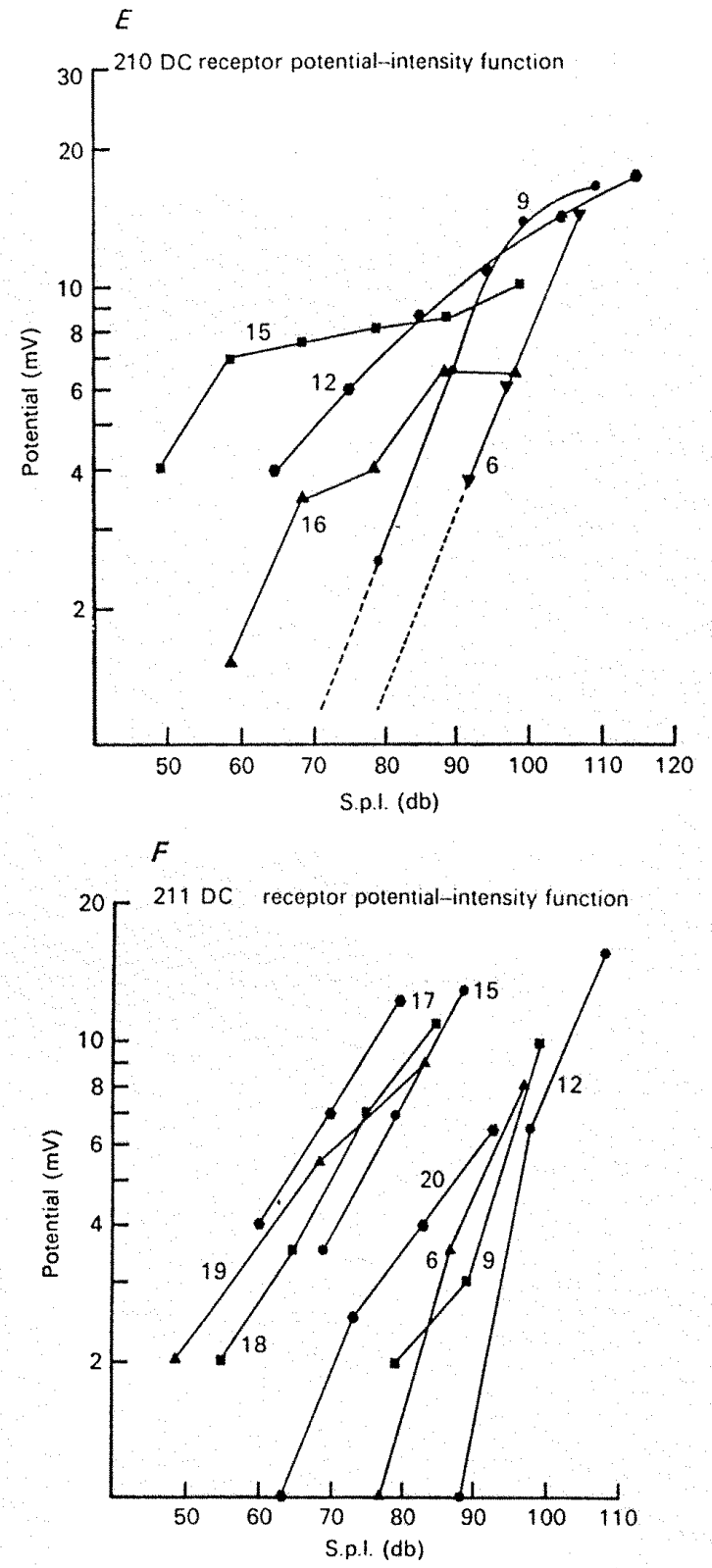

Fig. 3*E* and *F*.

Fig. 3. Intensity functions of the receptor potential. *A* and *C* are for the DC component, *B* and *D* for the AC component from sensitive cells; *E* and *F* are for the DC component from insensitive cells. The numbers by the side of each intensity function denote frequency.

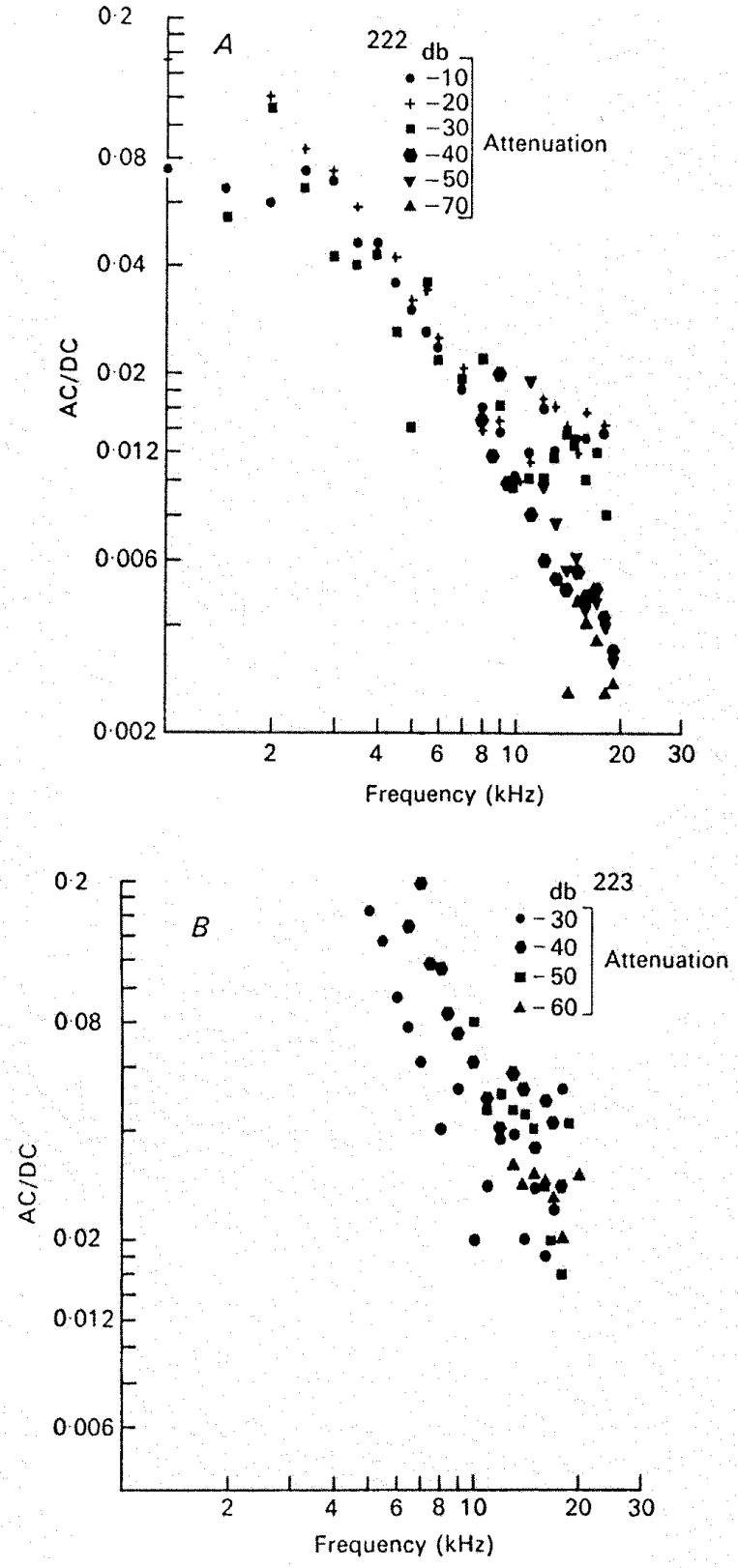

Fig. 4. *A* and *B*, the ratio of the AC and DC components of the receptor potential *vs.* frequency, showing the fall off from 6–9 db/octave of the AC component with respect to the DC component.

potential declines in amplitude with respect to the DC component at a rate of 6–9 db/octave as frequency of sound stimulation increases. Thus at 1 kHz the magnitude of the AC component is about 20 % of the DC component for cell 222 whereas at c.f. (17·5 kHz) it falls to 0·6 % of the DC component (Fig. 4). This would appear to cause the AC intensity functions to saturate at lower amplitudes at c.f. relative to frequencies below this, than the DC intensity functions.

Examples of intensity functions for the DC component from relatively insensitive cells are illustrated in Fig. 3*E* and *F*. The intensity functions of frequencies below c.f. are similar to those for sensitive cells; however for frequencies around c.f. the intensity functions are shifted to high s.p.l.s, and in some instances, e.g. cell 210, the shape of the intensity functions around c.f. may be different from those of sensitive cells and fail to saturate within the range of s.p.l.s used.

3. *Isoamplitude tuning curves for intracellular receptor potentials*

Our primary aim in these experiments was to make a comparison between the tuning properties of hair cells and auditory nerve fibres. The frequency response of single fibres in the auditory nerve has been described in terms of isothreshold or isorate contours which are referred to as neural frequency tuning curves (f.t.c.). They are derived by measuring the threshold of an arbitrarily determined increase in firing rate of the nerve fibre at different frequencies of sound stimulation. Hair cell f.t.c.s are isoamplitude contours derived from the isointensity functions and minor extrapolation and interpolation of these are made when necessary to fill gaps in the data. Therefore, before any comparison can be made between neural and hair cell f.t.c.s, it is essential to discover the amplitude of receptor potential which corresponds to a just noticeable increase in the background activity in an auditory nerve. This may be estimated from the threshold to pure tones of the compound a.p. of the cochlear nerve measured at the round window, which Johnstone (1977) has shown to approximate to the threshold of the spiral ganglion cells (cell bodies of the auditory nerve fibres). Our data suggest (Table 1) that a.p. threshold corresponds to about 2 mV for the DC component and about 0·01 mV for the AC component at a c.f. of 17 kHz.

Isoamplitude tuning curves for 2, 5 and 10 mV DC receptor potential are shown in Fig. 5. A measure of the sharpness of neural tuning curves is the $Q_{10\,db}$ (the characteristic frequency divided by the bandwidth 10 db below the peak). $Q_{10\,db}$ values for the curves shown in Fig. 5 and in Table 1 lie between 6 and 11·3. The $Q_{10\,db}$ for a given hair cell is relatively independent of the amplitude of the receptor potential until this exceeds a value around 10 mV when the tuning curve broadens. The detuned cells show this effect to a greater extent and also exhibit a shift of c.f. towards lower frequencies (Fig. 5*E* and *F*). This is a consequence of the intensity function saturating at lower amplitudes of receptor potential as the frequency of sound stimulation is increased. Other characteristics of the tuning curves, namely the high and low frequency slopes and the difference between the shoulder and the peak of the tuning curve are listed in Table 1.

Isoamplitude curves of the AC component of the receptor potential at convenient potential levels are illustrated in Fig. 6. These curves are similar to the isoamplitude curves of the DC component except that they reflect the 6–9 db/octave roll-off of the

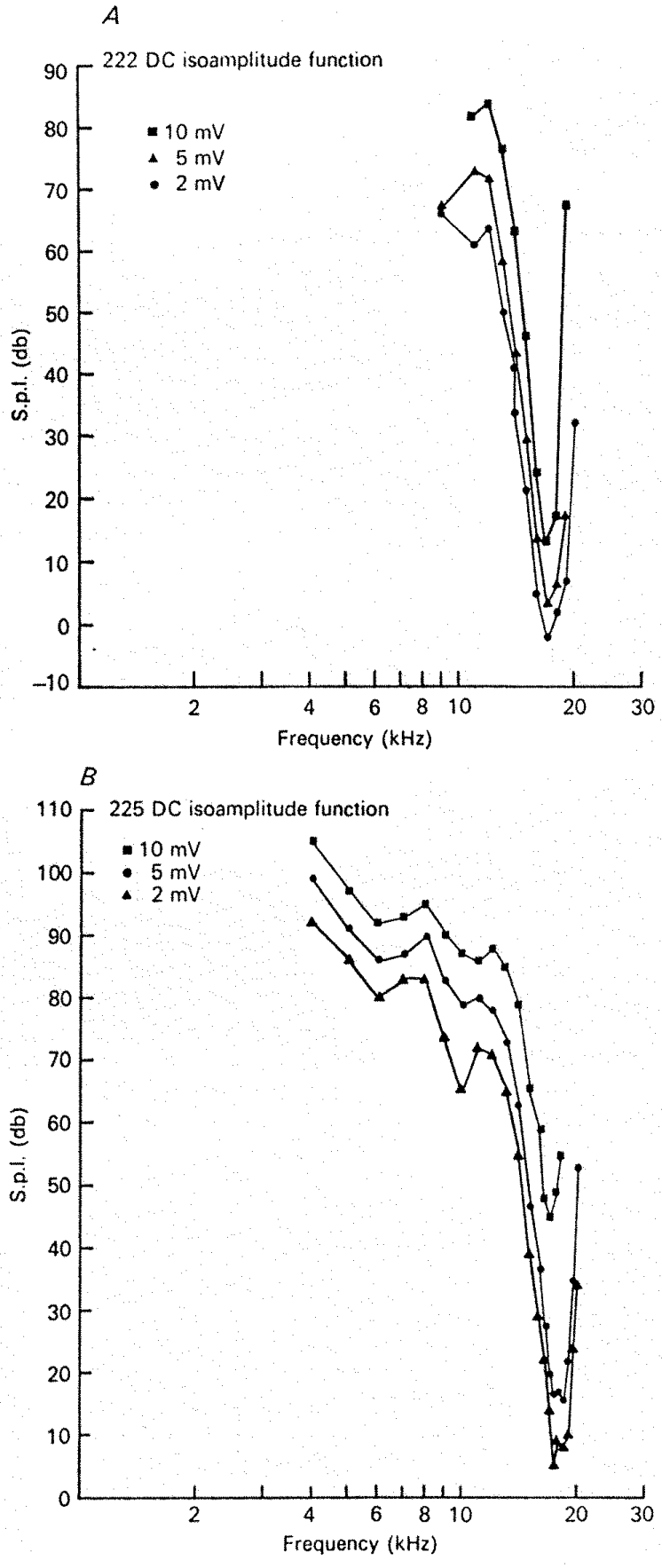

Fig. 5*A* and *B*. For legend see page 275.

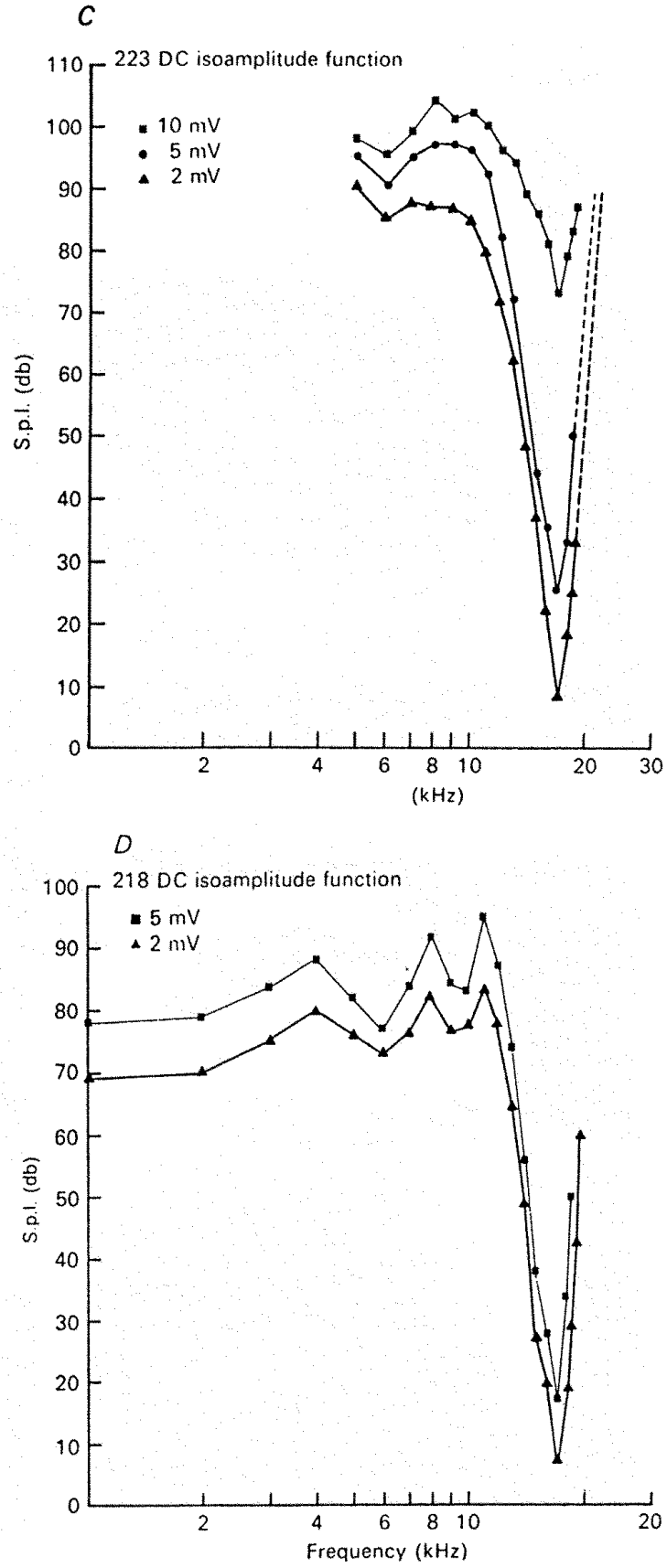

Fig. 5*C* and *D*. For legend see page 275.

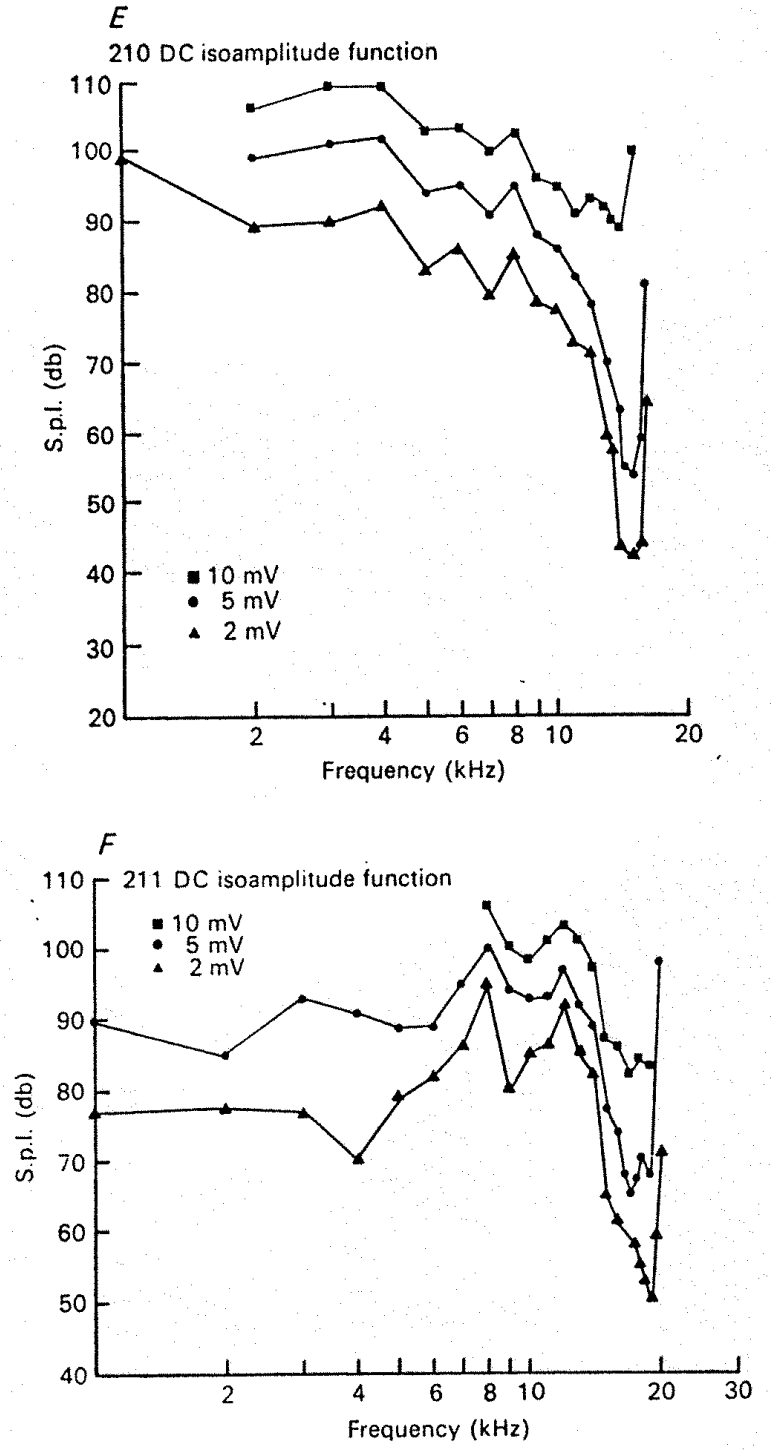

Fig. 5*E* and *F*.

Fig. 5. Isoamplitude curves for the DC component for four sensitive cells (*A–D*) and two insensitive cells (*E* and *F*).

AC component with respect to the DC component illustrated in Fig. 4. Other characteristics of these curves including those illustrated in Fig. 6 are listed in Table 1.

The frequency dependent attenuation of the AC component is due to the electrical time constant of the hair cell membrane rather than the other possible mechanisms

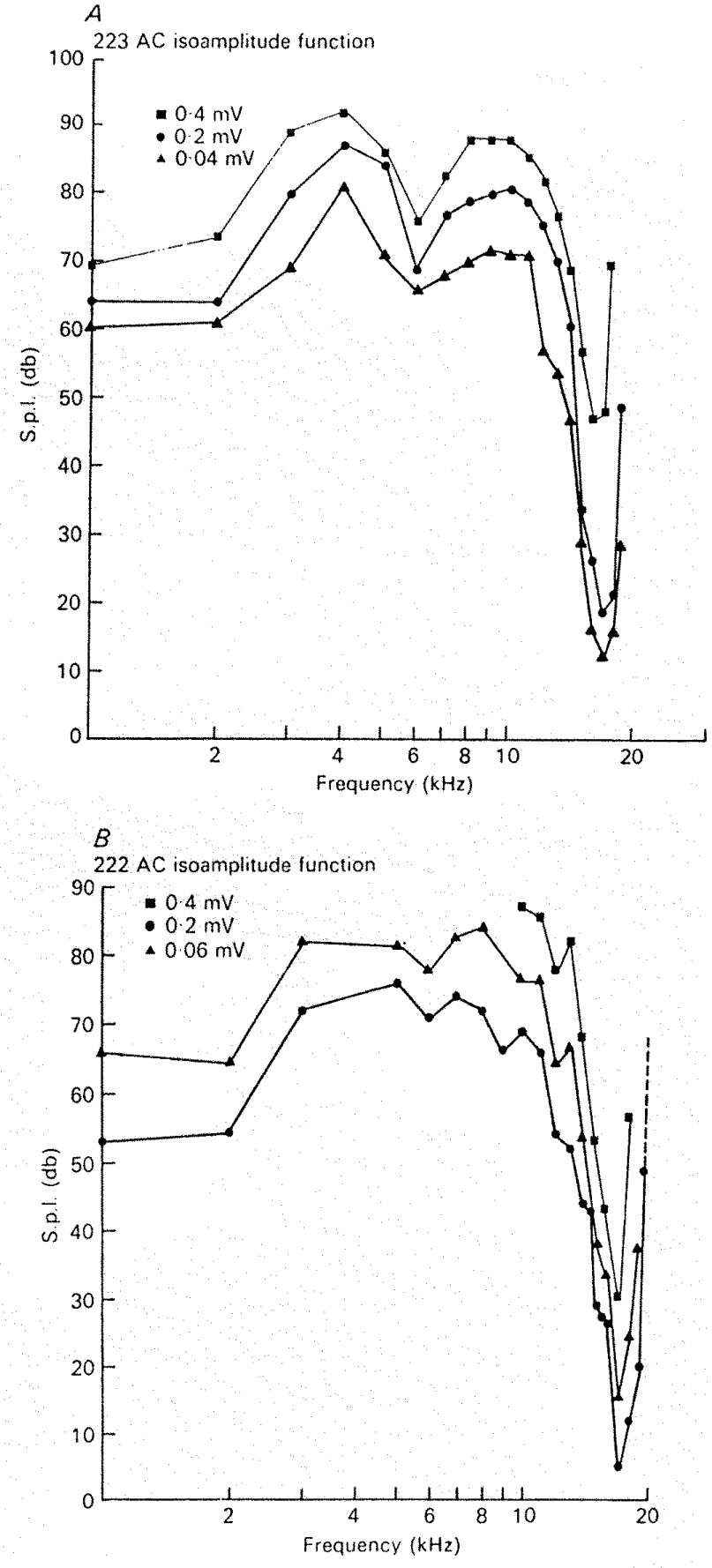

Fig. 6*A* and *B*. For legend see facing page.

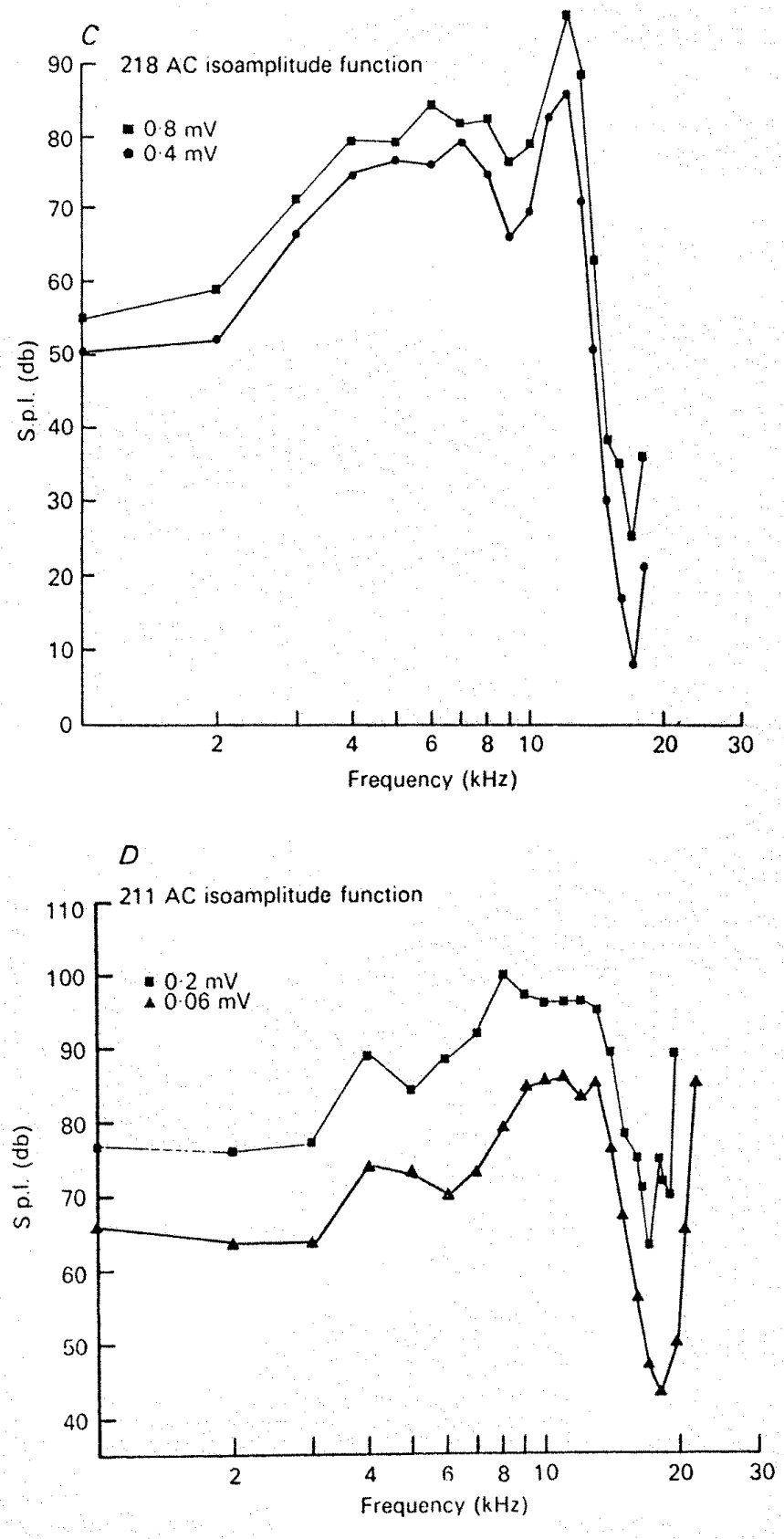

Fig. 6. Isoamplitude curves for the AC component of the receptor potential for three sensitive (*A*, *B* and *C*) and one (*D*) insensitive cell.

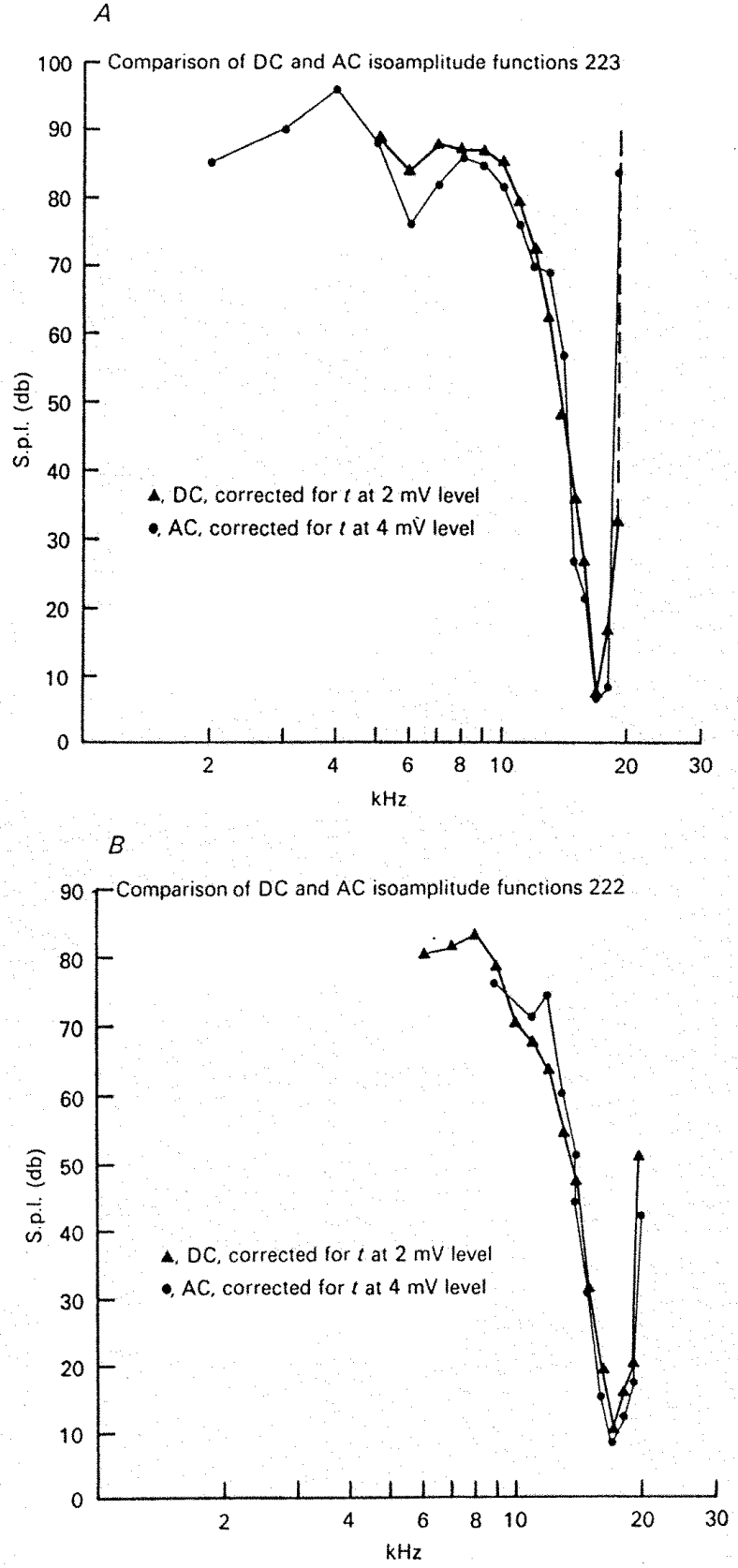

Fig. 7. Comparison between the DC isoamplitude curves at the 2 mV level and the AC isoamplitude curves at the 4 mV level, corrected for the time constant of the cell, for two different cells (*A* and *B*).

e.g. the mechanoelectric time constant of the transducer process, because extracellular recordings of the AC component are not subject to this attenuation (see section 4 below). We have measured the time constant for seven cells (section 6) including cells 222 and 223 for which we have also measured the AC component. In order to see if the AC and DC components are tuned in an identical fashion we have

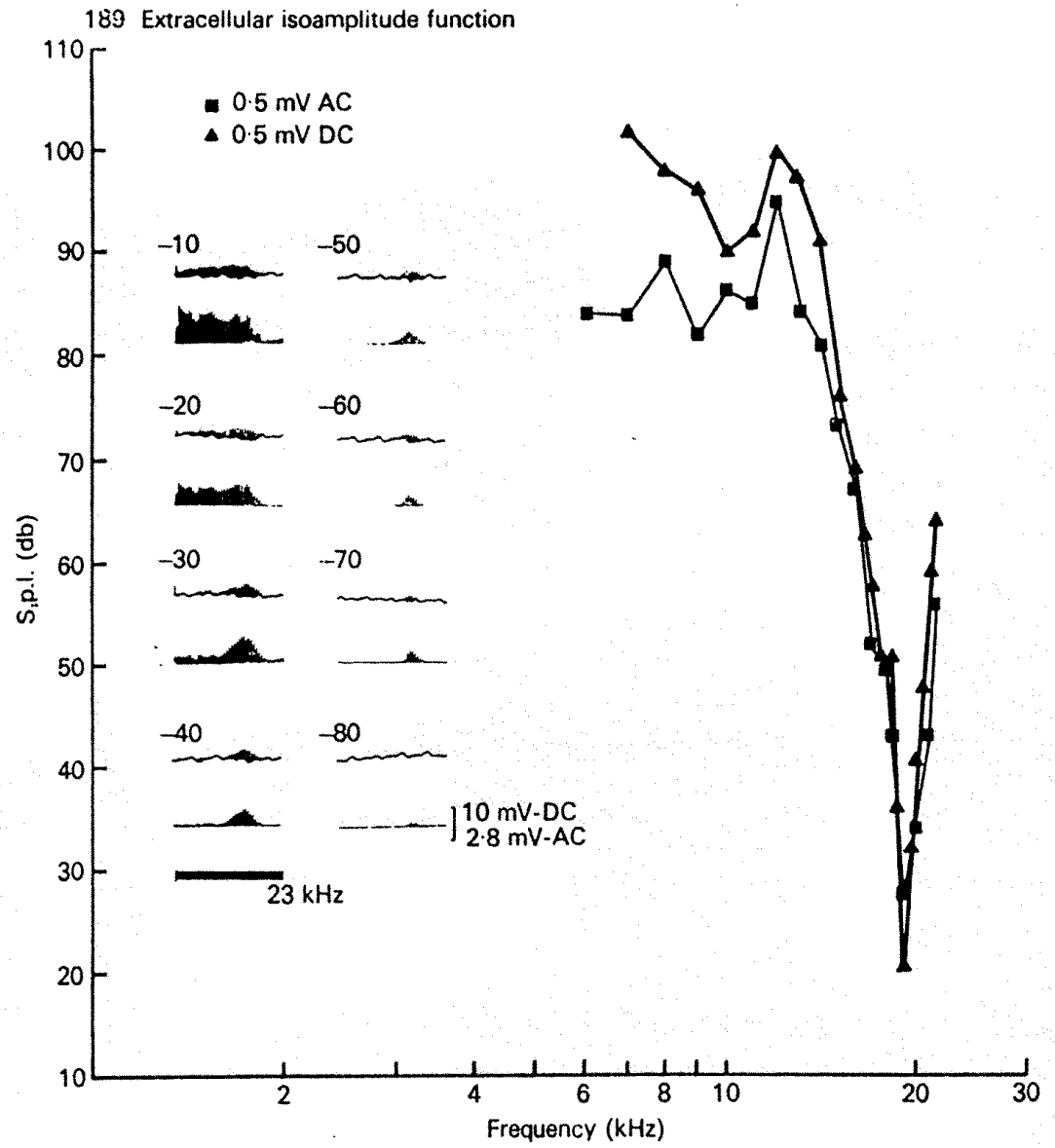

Fig. 8. Isoamplitude curves at the 0·5 mV level for the extracellularly recorded DC and AC components. Inset: DC (upper trace) and AC (lower trace) response to 80 msec tone burst recorded extracellularly from the organ of Corti. Legend as for Fig. 2.

corrected the AC component of cells 222 and 223 for a 6 db/octave roll-off using their own time constants of 0·48 and 0·49 msec respectively. This resulted in an increase in the apparent amplitude of the AC component to approximately twice that of the DC component throughout the frequency range. Moreover there was close correspondence between the isoamplitude curves for the DC component at 2 mV and the corrected AC component at 4 mV (Fig. 7*A* and *B*).

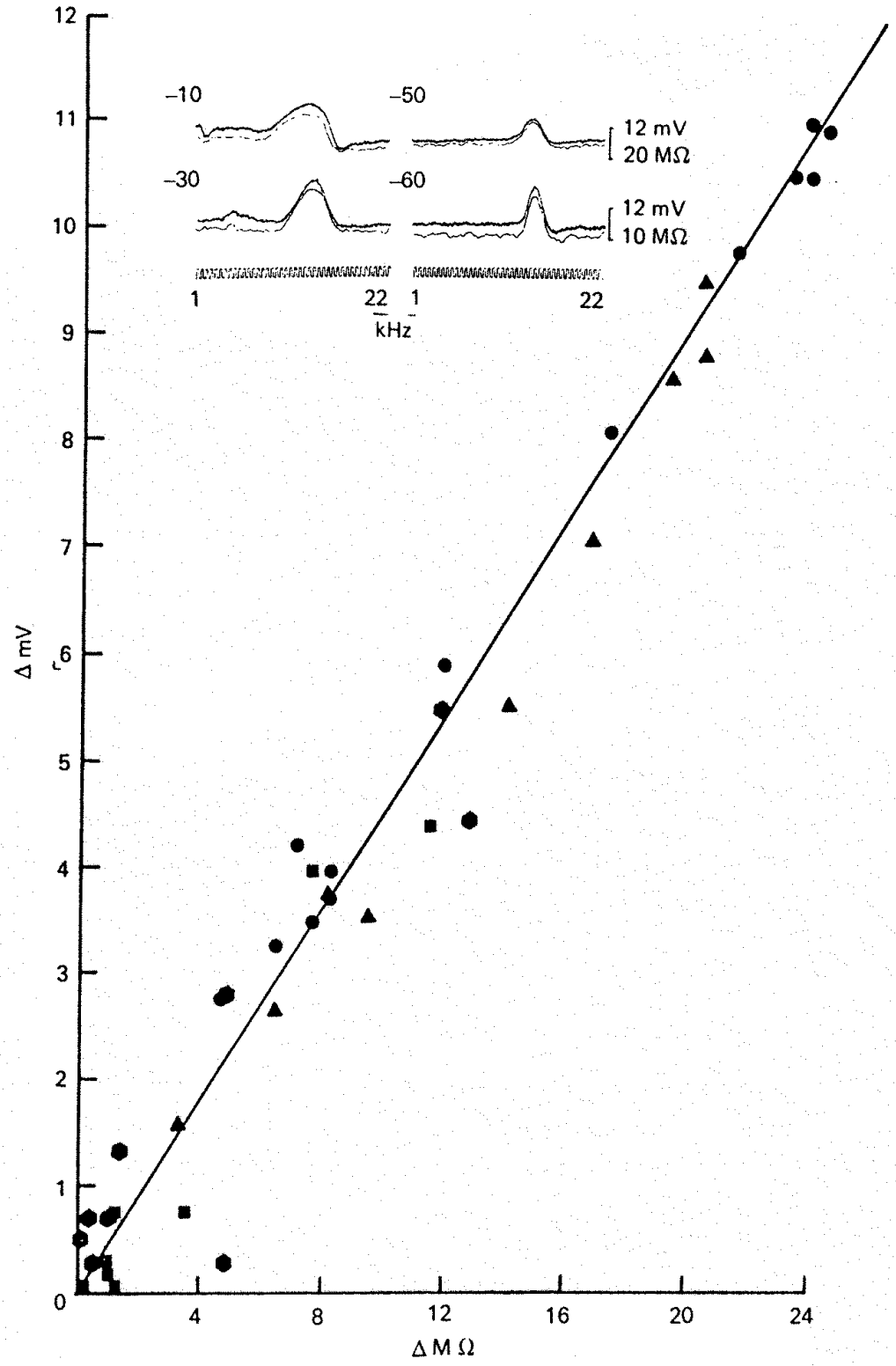

Fig. 9. Relationship between receptor potential and resistance change from a presumed inner hair cell in response to a swept pure tone. Inset: DC potential change (upper trace) and resistance change (lower trace) recorded simultaneously from a presumed inner hair cell in response to a linearly swept pure tone. The numbers by the side of each pair of traces indicate the attenuation of the sound system, the frequency scale indicates 500 kHz intervals.

4. *Isoamplitude tuning curves for extracellularly recorded receptor potentials*

DC and AC receptor potentials, maximally about 3 mV in amplitude (Fig. 8) may be recorded in the organ of Corti adjacent to the inner hair cells. In certain electrode positions these potentials are tuned similarly to intracellular potentials except that the AC component is not subject to attenuation by the time constant of the hair cell (Fig. 8). This is in contrast to the broad tuning obtained from electrodes placed superficially on the basilar membrane or in the scala media. The exact position of the electrode within the organ of Corti was not determined and therefore the contribution of inner and outer hair cells to the extracellularly recorded receptor potentials is unknown.

5. *Resistance changes in hair cells during sound stimulation*

Sound stimulation causes a decrease in resistance of the hair cell which is closely associated with the potential change. This relationship is linear and is illustrated in Fig. 9. Similar plots were made for eight other hair cells and their slopes were expressed in nA, the mean being $0{\cdot}62 \pm 0{\cdot}18$ nA (Table 2). At high s.p.l.s (*ca.* 100 db) the resistance of the hair cell decreases by about 50 % of the total cell resistance (Table 2) in those cells in which the total membrane resistance was measured. Resistance changes were never observed to accompany receptor potentials when they were recorded either extracellularly or intracellularly in supporting cells. The resistance change was independent of the frequency of the measuring current, indicating that the impedances we observed were resistive and not due to changes in capacitance of the hair cell membrane.

6. *Time constant, specific resistance and specific capacitance of hair cells*

The time constant, derived from the ratio of the real and imaginary components of the impedance changes (Pinto & Pak, 1974) measured for seven hair cells, varied from 0·31 msec in cells with small resting potentials to 0·76 msec in cells with large resting potentials (Table 2). The higher values are believed to be more representative of the time constant of hair cells. The specific resistance and capacitance were calculated for three hair cells in which the total membrane resistance was measured when the recording electrode was withdrawn from the cell. The specific resistance and capacitance of the hair cell membrane were based on the assumption that the inner hair cells correspond to a cylinder of radius 5 μm and length 40 μm based on measurements by Duval, Flock & Wersall (1964) for the guinea pig cochlea and was calculated to be $733 \pm 120 \Omega/cm^2$ and $0{\cdot}87 \pm 0{\cdot}29$ F/cm^2 respectively. These are comparable to the values of $1 k\Omega/cm^2$ and 1 μF/cm^2 for cell membranes (Cole, 1968).

7. *Tonotopic distribution of hair cells along the basilar membrane*

DC receptor potentials have been recorded intracellularly and extracellularly from inner hair cells with c.f.s ranging between 14·5 and 32 kHz and their tonotopic distribution has been mapped on the basilar membrane with respect to the hook (the basal extreme of the basilar membrane). Fig. 10 shows the frequency distribution of the hair cells along the basilar membrane and the cut-off frequencies of the mechanical

TABLE 2. Details of the measurement of potential and real and imaginary impedance changes in response to a swept pure tone. The real resistance and capacitance of the cells was calculated according to the method of Pinto & Pak (1974)

Expt. No.	Characteristic frequency (kHz)	Resting potential (mV)	Time constant (msec)	Slope current mV/MΩ (nA)	Total resistance (MΩ)	Total capacitance (10^{-12}F)	Specific resistance (Ω/cm²)	Specific capacitance (μF/cm²)
186	15	25	0·47	0·76	—	—	—	—
188(1)	15·5	20	0·45	0·81	—	—	—	—
188(2)	16	45	0·76	0·45	48	15·8	680	1·1
222	17·5	30	0·48	0·55	61·5	7·8	870	0·55
223	17	20	0·49	0·56	—	—	—	—
225	17	30	0·63	0·37	46	13·7	650	0·97
231	17·5	20	0·31	0·81	—	—	—	—

tuning curves determined by Wilson & Johnstone (1975) and for the characteristic frequencies for the cell bodies of the auditory nerve (spiral ganglion cells) plotted by Robertson & Manley (1974).

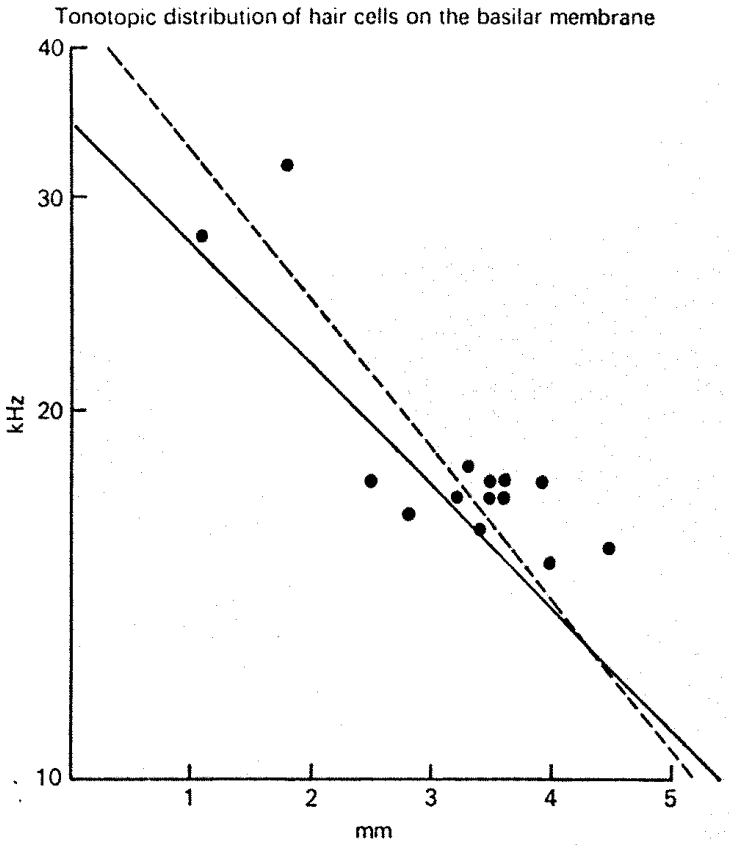

Fig. 10. Tonotopic distribution of hair cell characteristic frequencies on the basilar membrane. Uninterrupted line shows the distribution of ganglion cell characteristic frequencies from Robertson & Manley (1974). Dashed line represents the cut-off frequencies of the basilar membrane mechanics measured with a capacitance probe by Wilson & Johnstone (1975).

DISCUSSION

These experiments have attempted to resolve two questions of basic interest about the peripheral auditory system which have been subjects of speculation for many years; namely, what is the nature of the hair cell receptor potential, and how do the tuning properties of inner hair cells compare with those of primary afferent fibres, and the mechanics of the basilar membrane?

Hair cell receptor potential

Our results substantiate Davis' (1957) proposal that the c.m. and s.p. are both receptor potentials in the cochlea, and that they are associated with a change in the ohmic resistance of the hair cells. We have recorded receptor potentials from inner hair cells within AC and DC components which correspond to the c.m. and s.p. respectively in that the former follows the wave form of the stimulus, and the latter its envelope. Furthermore, we have recorded intracellular resistance changes which follow exactly the DC component (Fig. 9). However, our data departs from Davis'

model in that the current flow through the apex of the hair cell is not modulated by the resistance changes. In fact it remains remarkably constant over a wide range of frequency and intensity.

The transduction process in hair cells is believed to be a mechano-electric rectification of the input waveform (Flock, 1965; Flock, Jørgensen & Russell, 1973; Hudspeth & Corey, 1977). Such a mechanism provides the basis of the AC component of the receptor potential. It is tempting to propose that the DC component is provided by smoothing the AC component with the low pass electrical characteristics of the hair cell which attenuate the AC component at a rate of 6–9 db/octave with respect to the DC component (Fig. 4) at frequencies above the cut-off frequency of the hair cell (210–600 Hz). The attenuation and smoothing of the AC component are not observed extracellularly since the resistance and capacitance to ground are small. If the magnitude of the AC component is corrected for the attenuation due to the hair cell resistance and capacitance using the cells time constant, the AC component becomes approximately twice as large as the DC component over the frequency range and the 4 mV isoamplitude curve of the AC component is identical to the 2 mV isoamplitude curve of the DC component. These relationships are to be expected if the DC component is derived from the smoothed rectified waveform of the AC component.

The passive electrical properties of the hair cells determine the relative importance of the AC and DC components in neural excitation. Hair cells in the acousticolateralis system excite afferent fibres through the release of chemical transmitter (Furukawa & Ishii, 1967; Flock & Russell, 1976) which is controlled by the level of hair cell depolarization (Sand, Ozawa & Hagiwara, 1975). At low frequencies of auditory stimulation, below the cut-off frequency of the hair cell, transmitter release will be under the dominant control of the AC component. At 5 kHz, where phase-locking to the auditory stimulus by primary auditory fibres disappears (Rose, 1970), the AC component is only 4 % of the DC component. It is, therefore, apparent that at 14–20 kHz, which corresponds to the best frequencies of hair cells in the basal turn of the cochlea, from which most of our recordings have been made, the DC component is entirely responsible for neural excitation. We have estimated the amplitude of the DC component that corresponds to neural threshold, and 2 mV seems a reasonable approximation of this value as seen by comparing the a.p. threshold with the threshold for the 2 mV isoamplitude curve in Table 1.

Comparison between hair cell and neural frequency tuning curves

The isoamplitude tuning curves of the AC and DC components of the intracellular receptor potential and the threshold tuning curves of single fibres in the auditory nerve of guinea-pigs, both of which are termed frequency tuning curves (f.t.c.s), closely resemble each other in several respects. The high and low frequency slopes of the hair cell f.t.c.s measured within 5 and 25 db of the tip fall within the ranges of 300–600 db/octave, and 100–300 db/octave respectively, which are similar to the values measured by Evans (1972) for the corresponding slopes of neural tuning curves in the guinea-pig. The sharpness of neural and hair cell f.t.c.s expressed as $Q_{10\ db}$ fall within the same range of 6–11 and the difference between the low frequency tail and tip of the f.t.c. is comparable to those of neural f.t.c.s (Table 1) (Evans, 1972; Robertson & Manley, 1974). Furthermore the tuning properties of hair cells and nerve

fibres exhibit a similar independence of s.p.l. (Geisler, Rhode & Kennedy, 1974), i.e. the characteristics of the hair cell and neural f.t.c.s are largely independent of the amplitude of receptor potential and threshold criterion selected to derive f.t.c.s. It is well known that neural f.t.c.s are labile in that the thresholds for the tips vary according to the condition of the animal and after experimental manipulation (Evans & Klinke, 1974; Evans, 1975; Robertson, 1974; Robertson & Manley, 1974). Hair cell f.t.c.s display a similar variation in sensitivity as illustrated by Fig. 5*E* and *F* to surgical damage occurring during the experiment. The conclusion that the f.t.c.s of auditory fibres are derived solely from the frequency and amplitude response of the individual inner hair cells, which they innervate, seems inescapable.

The characteristics of the receptor potential intensity functions and their relationship to neural rate functions

The properties of hair cell f.t.c.s and consequently neural f.t.c.s are determined by the intensity functions of the receptor potentials. Neural rate functions have been plotted for cat auditory nerve fibres by Sachs & Abbas (1974). These can be compared to the intensity functions of hair cells provided it is remembered that several transformations occur between the production of a receptor potential in a hair cell and the generation of impulses in the afferent fibre innervating it. For example, the release of chemical transmitter from the hair cell, the depolarization of the post-synaptic membrane and the initiation of impulses in the nerve. These are all rate limited functions whose characteristics have not been measured in the peripheral auditory system and are likely to account for differences between hair cell intensity functions and neural rate functions. One obvious difference between neural rate functions and hair cell intensity functions is that the former is sigmoidal while the latter rises linearly from the noise level before saturating. It may be that the hair cell intensity function is also sigmoidal but the lower inflexion is masked by the noise level. However, the lower inflexion of the neural rate function is more likely to be caused by the limitations of the synaptic and spike generating mechanisms, as predicted by the model of Schroeder & Hall (1974) for mechanical to neural transduction. The important similarity between hair cell intensity functions and neural rate functions is that the level at which saturation occurs declines progressively with increasing frequency through and above c.f. The former characteristic determines the range over which the filter characteristics remain constant. If the level at which the hair cell f.t.c. is plotted is outside the linear portion of the intensity function at c.f., i.e. corresponding to plotting an f.t.c. in response to very loud sound then the tuning curve will become broader, the difference between the tip and low frequency shoulder will be decreased and the c.f. of the f.t.c. will shift to lower frequencies (Fig. 5*A*, *B* and *C*). This finding corresponds to the behaviour of neural f.t.c.s in response to loud sound (Evans, 1977).

The basis of frequency analysis in the cochlea

In the absence of experimental data concerning the transduction of mechanical movement to hair cell receptor potential and subsequent initiation of impulses in the auditory nerve, a large number of models have been put forward to explain the discrepancy between basilar membrane and neural frequency selectivity. These models start with the broad tuning of the basilar membrane and propose some

sharpening mechanism to produce the tuning properties of the auditory nerve. They may be divided into three classes. Firstly, there are mechanical models that propose that the vertical or measured motion of the basilar membrane is not directly responsible for stimulating the hair cells, but that this motion undergoes some transformation at the top of the hair cells to produce sharpening (Tonndorf, 1960; Duifhuis, 1976; Steele, 1973). Secondly, there are neural models which propose that neural interaction occurs within the organ of Corti to produce sharpening of the response of the auditory nerve or hair cell (Zwislocki, 1974). Thirdly, electrical interaction models which propose that receptor potentials from adjacent hair cells interact electrotonically (Manley, 1977). The data presented in this paper provide a basis for assessing these models.

Since the tuning properties of auditory nerve fibres can be fully accounted for by those of the individual inner hair cells, we can exclude a neural basis for frequency tuning of the auditory fibres. Neural sharpening through electrical interaction between hair cells can also be excluded because simultaneous measurements of resistance change and the DC receptor potential recorded from inner hair cells demonstrate that the potential change is exactly mimicked by the resistance change. It could be argued that the receptor potential and the resistance change are not causally related to each other but that some of the potential change is due to electrotonic spread from adjacent hair cells. However adjacent hair cells would have to be tuned identically otherwise the relationship between the receptor potential change and the resistance change would not be linear (Fig. 9). Electrical interaction of this kind could produce a change in gain of the hair cells but not a change in the shape of the f.t.c., i.e. to produce sharper tuning.

Thus we are left with the conclusion that the tuning is due to the mechanical properties of the cochlear partition. Measurement of basilar membrane vibration in the guinea-pig in the same basilar membrane region as the hair cell studies (Johnstone & Boyle, 1967; Wilson & Johnstone, 1975) indicate that the vibration is linear at s.p.l.s between 40 and 120 db (the largest range over which linearity was tested) and that it has approximately low pass characteristics. These measurements thus differ from the intracellular hair cell studies in that the low frequency slopes of the mechanical f.t.c. are close to zero compared with the hair cell low frequency slopes of 45–253 db/octave. The high frequency slopes of the hair cell f.t.c.s are steeper than those of the mechanical f.t.c.s, being 340–500 db/octave and 60–245 db/octave respectively.

The two sets of data share common c.f. or cut-off frequencies as is seen from Fig. 10. However, the intensity functions for the hair cell receptor potentials at c.f. are almost fully saturated throughout s.p.l.s of 40–120 db over which the mechanics show complete linearity. We have found that damage to the cochlea causing raised a.p. thresholds for tones above 10 kHz produced a reduction of the saturation effect around c.f. and a marked reduction in the low frequency slope (cell 211, Table 1, Fig. 3*F*). It is conceivable therefore that the lack of similarity between the guinea-pig mechanical measurements and the hair cell measurements could be due to damage to the cochlea and loss of the sharply tuned response during measurement of the basilar membrane tuning properties. However, Evans & Wilson (1975) measured mechanical tuning properties of the basilar membrane and f.t.c.s from the auditory

nerve concurrently in the cat and found normal neural f.t.c.s coexisting with basilar membrane mechanics that are essentially the same as those measured in the guinea-pig.

Thus in the first turn of the guinea pig cochlea, there is a clear discrepancy between the shape of the mechanical f.t.c.s of the basilar membrane and the shape of the f.t.c.s of the hair cell receptor potentials. According to Davis' (1957) model of cochlear transduction and from experiments on hair cells in other acousticolateralis receptors (Harris, Frischkopf & Flock, 1970; Flock, 1965, 1971), the excitatory stimulus is a shearing displacement of the sensory hairs on the apical surface of the hair cells and it has been thought that in the organ of Corti the shearing displacement would be proportional to the basilar membrane movement. Since it is generally accepted that the AC component of the receptor potential reflects the mechanical stimulus to the hair cell as it does in hair cells in other sense organs (Flock, 1971; Weiss, Mulroy & Altman, 1974; Hudspeth & Corey, 1977; Sand *et al.* 1975) we must conclude that the shear displacement to the hair cells does not reflect the frequency response of the basilar membrane. Several models have been proposed to explain why this should be the case (Tonndorf, 1960; Duifhuis, 1976; Steele, 1973; Allen, 1977).

A slightly different picture has emerged from measurements of basilar membrane motion at the 6–8 kHz region of the squirrel monkey with the Mössbauer technique by Rhode (1971). The response of the basilar membrane to constant s.p.l. has essentially low pass characteristics similar to those of the guinea pig. However he found a substantial non-linearity at the c.f. and down the high frequency slope. The lower frequencies were linear. These are qualitatively similar to the non-linearities observed in the hair cell receptor potential. However the measurements do not account for the neural and hair cell f.t.c.s quantitatively. The difference is most noticeable in the frequency region just below c.f. where the basilar membrane measurements lack a sharp valley of about 40 db that always occurs in the neural data (Geisler *et al.* 1974).

The similarities between Rhode's data and the receptor potential measurement is most striking when the predictions of a model devised by Kim, Molnar & Pfeifer (1973) based on Rhode's data are considered. Intensity functions predicted for the model at frequencies around c.f. are remarkably similar to those measured for the intracellular receptor potential. They predict a decrease in c.f. with increasing s.p.l. due to a cross-over of the intensity functions at high s.p.l. Hence the vibration of the basilar membrane in the 6–8 kHz region of the squirrel monkey may reflect, to a limited degree, the shear displacement which excites the hair cells.

The lability of cochlear tuning properties

Neural f.t.c.s have been shown to be metabolically labile in response to anoxia resulting in an increase in threshold and broadening of the f.t.c. A simple magnitude change in the DC component of the potential receptor of a rise in threshold of the afferent synapse could not fully account for these changes as we have previously suggested (Russell & Sellick, 1977*a*) since for very sensitive cells an orderly decrease in $Q_{10\ db}$ for increasing criterion isoamplitude curves does not occur. Indeed the $Q_{10\ db}$ may become larger initially with increased isoamplitude criterion as shown in the neural data by Evans (1975). Rather the systematic increase in $Q_{10\ db}$ and thres-

hold of spiral ganglion cells observed during anoxia by Robertson (1974) must be due to changes in the properties of the stimulus to the hair cells. For example, it is known that the stereocilia of hair cells are composed of actin (Flock, 1977) and that their stiffness properties are metabolically labile. It is conceivable that a change in coupling between the tectorial membrane and the reticular lamina during anoxia could account for the loss of tuning.

Experiments in which the outer hair cells have been presumed to be selectively destroyed by ototoxic drugs indicate that they have an important role in the maintenance of normal neural f.t.c.s (Dallos, Ryan, Harris, McGee & Ozdamar, 1977; Evans & Harrison, 1976). Our results would suggest that the outer hair cells have a mechanical function or that the ototoxic drugs disrupt the structure of the organ of Corti to such an extent that the mode of stimulation of the inner hair cells is altered.

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