Short Title.

COCHLEAR GANGLION CELL ACTIVITY IN THE GUINEA PIG.

Studies of Single Neurone Activity in the Cochlear Ganglion of the Guinea Pig.

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Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of PhD.

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ABSTRACT

Single cell recordings were obtained from the spiral ganglion of the basal turn of the guinea pig cochlea. The dependence of sharpness of tuning curves on cell sensitivity and the effects of respiratory impairment, perilymph removal and structural damage are discussed. It is tentatively concluded that the weight of evidence suggests the existence of a second frequency-selective mechanism in addition to the basilar membrane. However, the results also add a cautionary note, questioning the reliability of some basilar membrane data. Details of spontaneous activity agree with findings in cat. The occurence of a two-component positive spike suggests that the ganglion cell bodies are inexcitable. Electronmicroscopic and other studies support the conclusion that the action potential successively activates nodes on either side of the soma. This arrangement may reduce the risk of conduction block. None of the criteria used could distinguish between neurones related to inner and outer hair cells.

ABSTRACT

Des réponses unitaires dans le ganglion spirale furent enregistrées dans la partie basale de la cochlée chez le cobbaye. La sélectivité des courbes de réponses dépend de la sensibilité des cellules; les effets de l'insuffisance respiratoire, des dommages structurels et de l'enlèvement du liquide périlymphatique sont des points discutés. Les résultats suggèrent l'existance d'un second mécanisme de sélectivité de fréquence en plus de la membrane basilaire. D'autre part, les résultats indiquent que la validité de certaines données sur la membrane basilaire est discutable.

Les détails de l'activité spontanée concordent avec celles rencontrées chez le chat. L'existance d'un potentiel d'action positif en deux composantes indique que les soma des cellules du ganglion ne sont pas excitables. La microscopie électronique et d'autres études ont demontré que le potentiel d'action excite successivement les nodules sur chaque côté du soma: cette particularité peut prévenir le bloquage de conduction. Avec les critères utilisés, il fut impossible de faire la distinction entre les neurones reliés aux cellules ciliées internes et externes.

Addendum

My attention has been drawn to a publication by L.U.E. Kohlloffel concerning recordings from spiral ganglion cells in the cat cochlea (Facts and Models in Hearing (Springer - Verlag , Berlin , 1974, pp 193-203)). Dr. Kohlloffel communicated to me in 1973 that he was also trying such recordings , but I only became aware of his 1974 publication after this thesis was submitted.

CONTENTS

To my family and friends.

Acknowledgments

My special thanks go to my supervisor Dr. Geoffrey Manley, for his interest in this work and continually helpful critical appraisal of the results. Discussions with B.M. Johnstone, G. Yates and J.R. Johnstone of the Dept. of Physiology, University of Western Australia, were also extremely helpful. Without the unflagging energy and good humour of Lorraine Pawson, the care and breeding of experimental animals would have been far more burdensome than it was. Lastly, my deepest appreciation to all those friends, colleagues and professors whose advice and companionship were very real factors in the final completion of this thesis.

Preface

The work presented in the results section of this thesis contains the data from original research performed at McGill University, between September 1972 and November 1974.

The research involved single neurone recordings from the spiral ganglion in the basal coil of the guinea pig cochlea. This represents an original contribution to research on the peripheral auditory system, being to my knowledge, the first published records from single neural elements inside the mammalian cochlea. All previous single unit recordings in the primary auditory pathway have been obtained at more central locations, distant from the cell bodies of the ganglion. The advantages of this preparation for studying the mechanism of frequency selectivity and other properties of the auditory pathway, are seen in the five papers comprising the results section of this thesis. A direct spatial mapping of the neural output along the

basilar membrane is possible in the ganglion in contrast to the cochlear nerve trunk. The particular nature of the approach allows delicate manipulations such as perilymph removal and the placement of scala media microelectrodes to be easily performed, while maintaining contact with a single ganglion cell and measuring its responses. The first three papers are concerned with the frequency selective properties of the spiral ganglion cells. It should be mentioned that a brief report describing effects similar to those in paper I, appeared in the literature while this paper was in press (Evans, 1974). Paper IV contains the first reasonably complete description of spontaneous activity in the guinea pig primary auditory pathway and also contains data on the effects of anoxia. In the final paper, the extracellular spike shapes in the spiral ganglion are discussed and a hypothesis is proposed of the mode of impulse propogation in the bipolar ganglion cells.

If the papers in this thesis do not seem to conclusively solve the problem of the mechanism of frequency selectivity in the primary auditory pathway, this impression is not a false one. No complete explanation of the derivation of neural response properties within the cochlea is as yet forthcoming, and awaits the advent of better physical techniques and an investigative onslaught by researchers with a wide variety of skills.

INTRODUCTION

Format of the thesis

Five manuscripts, submitted to Science, J. Comparative Physiology and Brain Research constitute the results section of this thesis. Two of the manuscripts are in print in Science and J. Comparative Physiology. Each paper is presented in the format required by the appropriate journal. Thus, there is an introduction, methods, results, discussion and summary for each paper. A bibliography also accompanies each manuscript. For this reason, the Introduction which follows here is not intended to provide a comprehensive coverage and historical review of all those aspects of cochlear physiology dealt with in the manuscripts. Instead it attempts to provide a brief framework and an outline of the contentious issues in the field, so that the significance of the results can be better understood. A bibliography of material in this introduction and in the final discussion are also included.

Anatomy of the Auditory Periphery

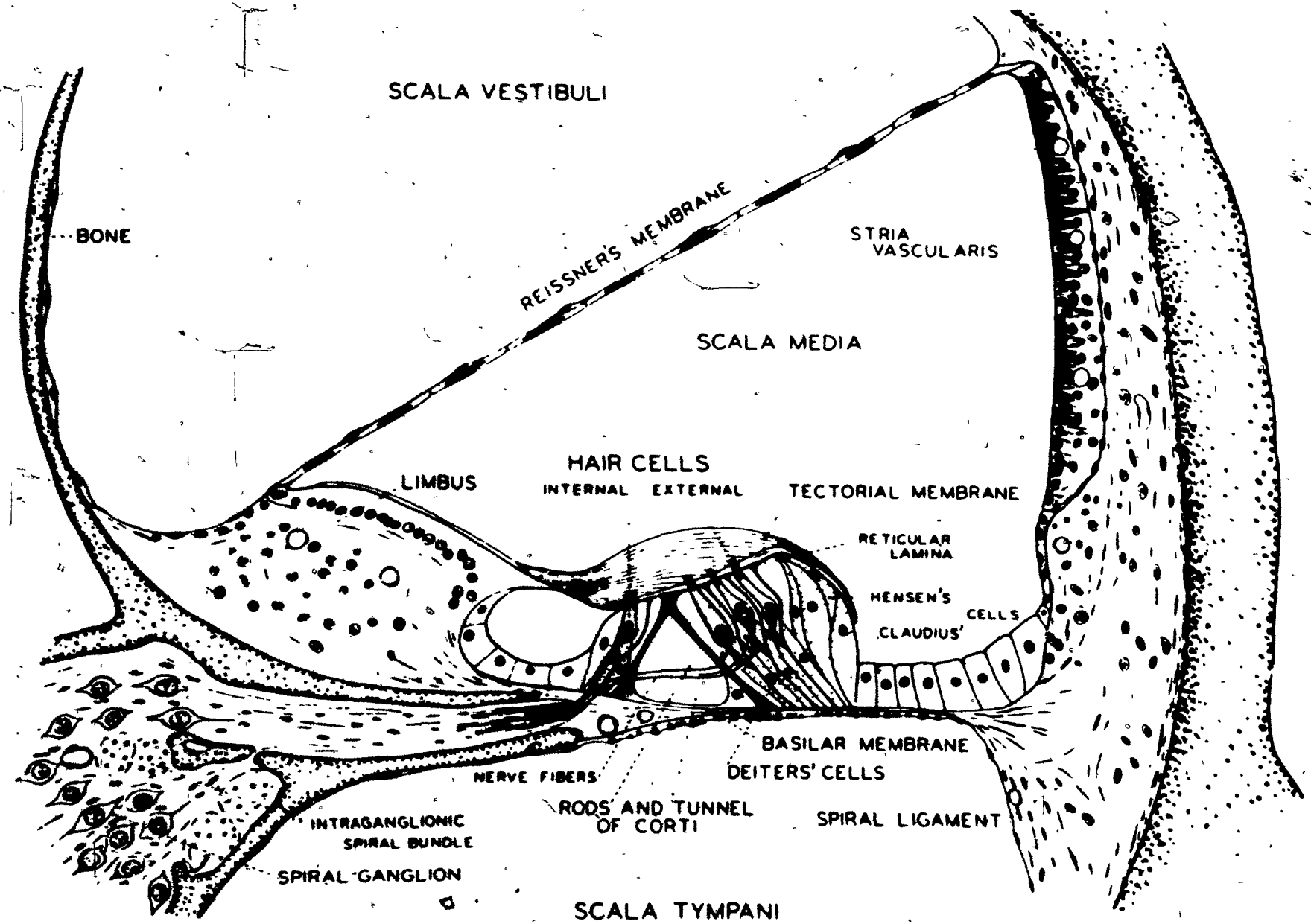
i) General

For the efficient delivery of airborne vibrations to the fluid filled cochlea, mammals have developed a complex middle ear structure. The tympanic membrane is connected by 3 ossicles, the malleus, incus and stapes to the scala vestibuli of the inner ear. The details of this ossicular chain differ from one species to another. As this is the most accessible of the peripheral auditory structures the mechanics of the middle ear have been intensively studied. Reliable data describing the frequency response of the middle ear have been obtained by various techniques, in the guinea pig and cat (Guinan and Peake, 1967; Johnstone and Taylor, 1969; Manley and Johnstone, 1974; Wilson and Johnstone, 1972). The middle ear in guinea pig appears to act as a band pass filter, having an essentially flat velocity response from 1 kHz to about 25 kHz (Manley and Johnstone, 1974). The rapid fall off in

amplitude beyond 25 kHz is now fairly well established (Manley & Johnstone, 1974; Wilson and Johnstone, 1972), and appears to be caused by factors other than simple mass-limiting.

There is some lack of accord on what correction should be applied to the response of inner ear structures to take account of the frequency response of the middle ear. Many authors correct amplitudes of vibration of the basilar membrane, or the threshold sound pressure level (SPL) of auditory nerve fibres by using the stapes amplitude response. Such a procedure is perhaps not too meaningful, since the amplitude of vibration of inner ear structures is apparently proportional to stapes velocity (Dallos and Durrant, 1972). It would be more reasonable to apply a middle ear velocity correction. This receives support from the closely corresponding shapes of the middle ear velocity response and the behavioural audiogram (Dallos, 1971; Manley and Johnstone, 1974). In the frequency range of interest in this thesis,

Fig. 1. Schematic diagram through one coil of the guinea pig cochlea. Taken from Davis (1954).


SCALA VESTIBULI
BONE
REISSNER'S MEMBRANE
STRIA VASCULARIS
SCALA MEDIA
HAIR CELLS
LIMBUS
INTERNAL
EXTERNAL
TECTORIAL MEMBRANE
RETICULAR LAMINA
HENSEN'S CELLS
CLAUDIUS'
BASILAR MEMBRANE
DEITERS' CELLS
NERVE FIBERS
RODS AND TUNNEL OF CORTI
SPIRAL LIGAMENT
INTRAGANGLIONIC SPIRAL BUNDLE
SPIRAL GANGLION
SCALA TYMPANI

the velocity response of the guinea pig middle ear is essentially flat, so that no correction would be necessary. This is assumed in the manuscripts and all neural thresholds are simply expressed as SPL at the eardrum. (See Appendix I).

The stapes communicates its vibrations to the fluid-filled vestibule which is connected to the scala vestibuli of the coiled cochlea. A schematic cross section through one coil of a typical mammal cochlea is shown in Fig. 1. Both the scala vestibuli and scala tympani are filled with perilymph, whose composition is similar to cerebrospinal fluid. The scala media or endolymphatic duct, contains endolymph which is high in potassium and chloride and very low in sodium (Bosher and Warren, 1971; Johnstone, 1967; Johnstone and Sellick, 1972).

The basilar membrane in mammals is narrow and stiff at the basal end and becomes wider and more flexible towards the apex (Bekesy, 1960). In guinea pig, the

entire membrane is 18.5 mm in length (Fernandez, 1952) and tapers from 600 microns wide at the apex to 200 microns wide at the basal end. It appears to be the tapering dimensions and gradient of stiffness which gives the basilar membrane its mechanical properties.

The Organ of Corti rests on the basilar membrane and consists of supporting cells, sensory hair cells, and nerve fibres. A schematic diagram of the Organ of Corti together with a light micrograph are shown in Fig. 2, 3. The detailed ultrastructure of the components of the Organ have been described by many authors (Engstrom and Wersall, 1958; Engstrom et al., 1966; Iurato, 1967; Spoendlin, 1966). Most noteworthy is that in mammals, the hair cells, whose apical ends are embedded in the reticular lamina, with hairs projecting into the scala media, are clearly differentiated into one row of inner hair cells and three to four rows of outer hair cells. The morphological differences between these two groups of receptors are well documented and

Fig. 2. A diagram of the mammalian Organ of Corti adapted from the work of Spoendlin (1973). Efferent fibres are shown as white and afferent fibres are black. The tectorial membrane is shown arbitrarily in contact with all hair cell stereocilia.

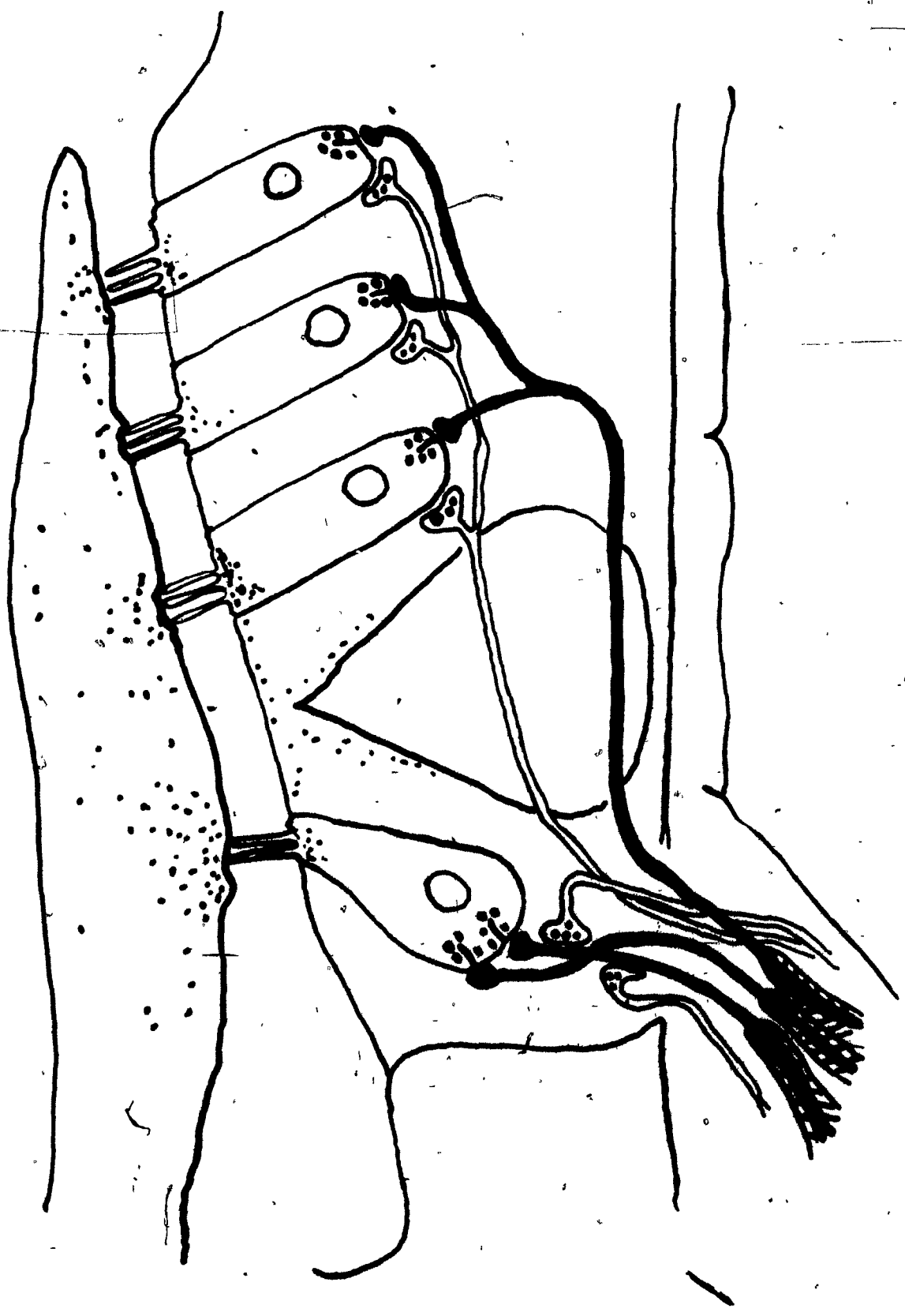
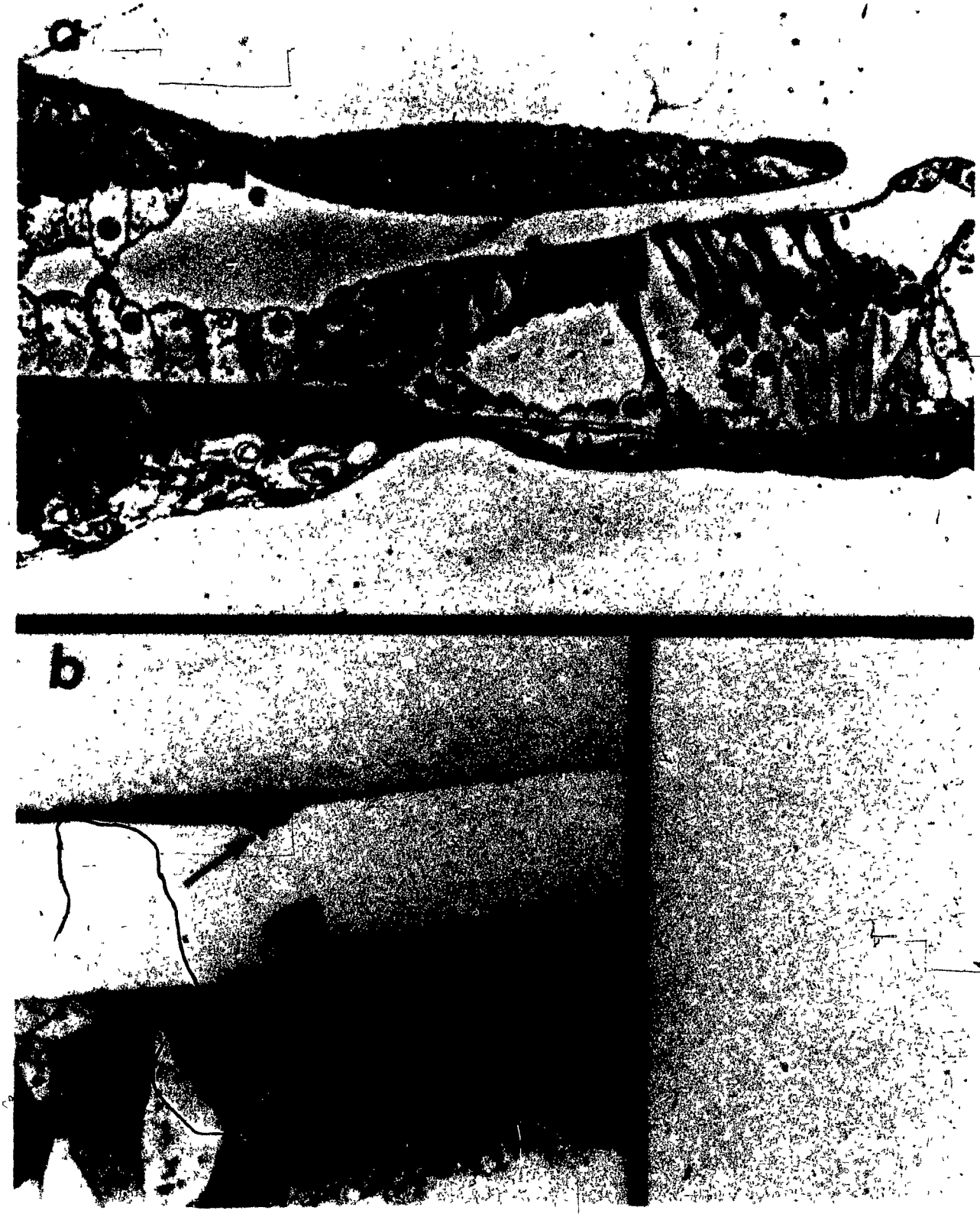


Fig. 3. a); Light micrograph of the guinea pig Organ of Corti. The section is tangential and was stained with toluidine blue and photographed with phase contrast. The darker area of tectorial membrane above the inner hair cells is indicated by an arrow.

b); A higher magnification of this region without phase contrast.


a
b

will not be discussed here except for the question of differences in the contact between the sterocilia and the tectorial membrane. This point is a controversial one.

Von Bekesy has described some of the mechanical properties of the amorphous, acellular tectorial membrane which overlies the stereocilia of both sets of hair cells (Bekesy, 1960). There is no doubt that the tops of the tallest stereocilia of the outer hair cells are in contact with the tectorial membrane (Angelborg and Engstrom, 1973; Engstrom et al., 1962; Lim, 1972). The situation for the inner hair cells is not so clear. Some authors believe that the inner hair cell stereocilia are freestanding (Billone and Raynor, 1973; Dallos et al., 1972), and that this must make their sensitivity and mode of stimulation different from the outer hair cells. However, the great distortion of the tectorial membrane by standard fixation procedures probably obscures the true picture. It has been shown that a

variety of apparent connections between both sets of hair cells and the tectorial membrane can be produced by different fixation procedures (Ross, 1974). Certainly, some published photographs do show the tectorial membrane in quite intimate contact with inner hair cell stereocilia (Angelborg and Engstrom, 1973; Flock, 1973). In osmium-fixed material the tectorial membrane is usually lifted well above both inner and outer hair cells. On the underside of the membrane above the inner hair cells is consistently seen a darker, apparently thicker region with an irregular edge (Fig. 3). It is possible that this is all that is left, after fixation, of an inner hair cell-tectorial membrane connection which is in some way different from the outer hair cell contact. This question is by no means settled, and the categorical statement by some authors that there is no tectorial membrane-inner hair cell connection, would seem to be premature to say the least. On anatomical grounds alone then, it cannot be stated that the mode

of stimulation of inner and outer hair cells is different. In addition, the fact that the basal body in both inner and outer hair cells is found at the distal, cuticle free edge of the cell, suggests that both sets of hair cells might have the same directional sensitivity to stereocilia displacement (Duvall et al., 1966; Flock et al., 1962; Wersall et al., 1965). In this respect it should be noted that Von Bekesy (1960) reported that maximal receptor responses in the region of the inner hair cells were obtained not by radial (as was the case for the outer hair cells), but by longitudinal displacements of the tectorial membrane. As pointed out by Duvall et al. (1966) this finding is at variance with the conclusion drawn from morphological studies of the location of the basal bodies, which imply that both inner and outer hair cells are stimulated by radial movement of the stereocilia. The explanation may be that the movement of stereocilia produced by displacement of the tectorial membrane from above (as in Von

Bekesy's experiments) is different from that produced by the normal method of displacement (indirectly by basilar membrane displacement).

ii) Innervation patterns.

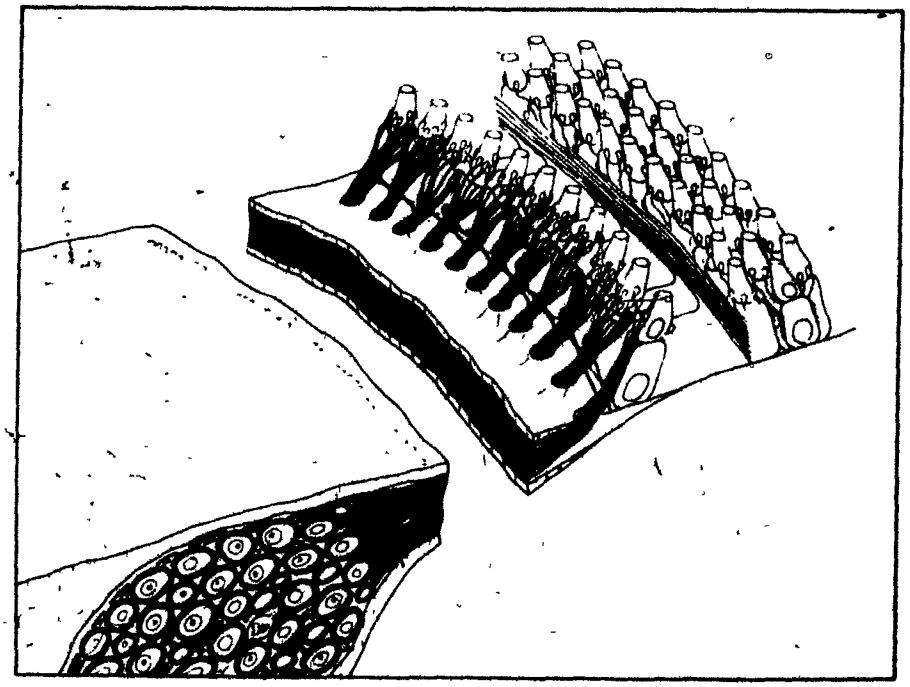
All nerve fibres to and from the Organ of Corti appear to enter the Organ in discrete bundles through the habenula perforata. It is at this point that all myelinated fibres abruptly lose their myelin sheaths so that all fibres within the Organ are bare. Schwann cells are also lacking and fibres often travel completely bare across fluid spaces within the Organ. However many fibres also travel in invaginations in supporting cells.

The afferent innervation in the mammalian cochlea appears to consist of two distinct groups; radial fibres which travel the short distance from the habenula to the inner hair cells, and the outer spiral fibres, which cross the tunnel of Corti to innervate the outer

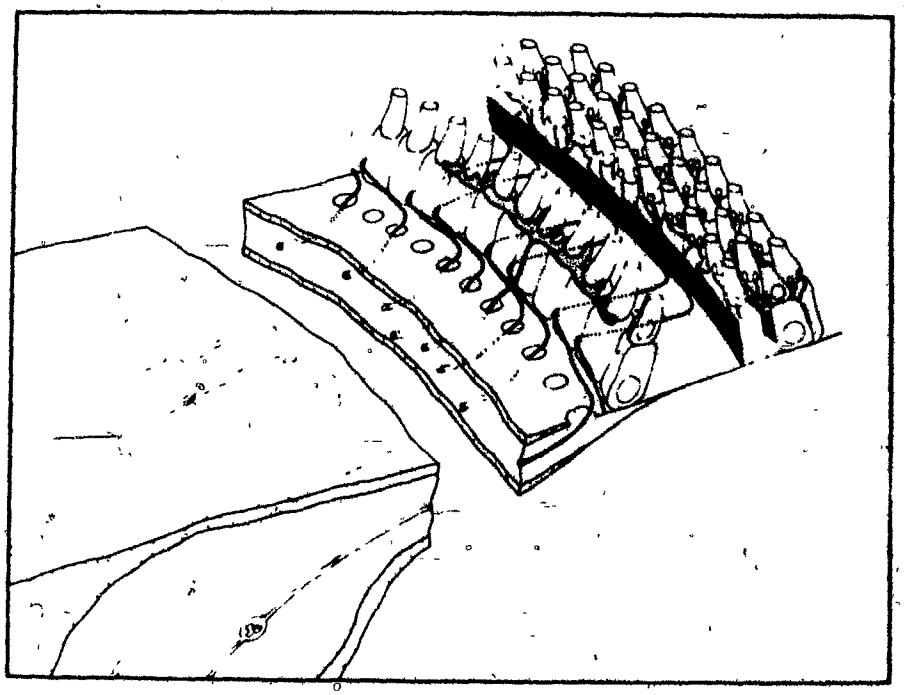
hair cells. The details of these two populations have been obtained by golgi techniques and by electronmicroscopy (Engstrom et al., 1966; Fernandez, 1951; Held, 1926; Lorente de No, 1937; Smith, 1967; Spoendlin, 1969, 1972), mainly in the cat, guinea pig and chinchilla. All authors agree that the density of the innervation of the inner hair cells far outweighs that of the outer hair cells. Spoendlin (1972) offers very specific data in the cat, claiming that 95% of all afferent fibres are radial fibres from the inner hair cells. These radial fibres innervate one hair cell each, and each inner hair cell receives about 10 fibres. The presence of synaptic bars, vesicles and synapic clefts indicates that the dendrites are excited by release of chemical transmitter from the hair cells. The much less numerous outer spiral fibres cross the tunnel of Corti to the outer hair cells, then travel in infoldings of the supporting Deiters cells in a basalward direction for up to 1 mm before beginning to form synapses. In con-

trast to the radial fibres, each outer spiral fibre forms synapses with many outer hair cells, and each outer hair cell is in turn innervated by several fibres. There is evidence from golgi studies in the guinea pig that the details of outer spiral fibre innervation are different in the basal and apical turns of the cochlea (Smith and Haglan, 1973). In the basal region one outer spiral fibre only innervates cells in one row of the three rows, whereas in the upper coils, these fibres can supply 2 or all 3 rows of outer hair cells. The terminal ramifications cover 70 - 280 microns. Spoendlin (1972) has also observed a small population (0.5%) of giant fibres which innervate approximately 10 inner hair cells at fairly regular intervals. A diagrammatic summary of this basic innervation pattern is shown in Fig. 4. It is not certain that this detailed pattern of innervation described by Spoendlin for cat and guinea pig is the same in all mammals, but it seems safe to say that the mode and density of innervation of the two populations

Fig. 4. Summary of the afferent innervation pattern obtained for the cat and guinea pig by Spoendlin (1972). The upper frame shows the total innervation, dominated by radial fibres to the inner hair cells. In the lower frame, the innervation provided by the small population of neurones is shown (see text).



This schematic representation summarizes the afferent innervation pattern of the organ of Corti with the two types of neurons. 95% of all neurons are type I and are connected in a direct radial direction with the inner hair cells. Only 5% of all ganglion cells are of type II and constitute the afferent innervation system of the outer hair cells.



. Schematic representation of the afferent innervation system of type II ganglion cells, after elimination of the type I ganglion cells by section of the cochlear nerve in the inner meatus. The type II neurons provide exclusively the afferent nerve supply to the outer hair cells by means of the outer spiral fibres and their numerous collaterals to the base of the outer hair cells. In addition there are a few giant nerve fibres which connect several inner hair cells and which also belong to the type II neurons.

of sensory cells is markedly different. This general picture was noted by some of the earliest histologists working on the cochlea. Spoendlin has reconstructed by serial sections the path taken by each bundle of fibres as it emerges into the organ of Corti. However, it is possible that some fibres were missed, and the task of locating very small areas of synaptic membrane in the electron microscope is not easy. The possibility should not be totally discounted that a few fibres innervate both inner and outer hair cells.

All authors are unanimous in concluding that there are no observable chemical synapses between afferent fibres within the Organ of Corti. Neither are membrane specializations associated with electrical synapses seen (Pappas et al., 1965; Pappas and Bennett, 1966).

Efferent fibres within the cochlea consist of the terminations of the crossed and uncrossed olivocochlear bundles, which arise from cell bodies in the vicinity

of the olivary nuclei in the medulla. An adrenergic innervation is also present. The number of efferent olivo-cochlear fibres in the medulla is small (500 crossed and 250 uncrossed in the cat)(Rasmussen, 1960), and they comprise an insignificant fraction of the total number of nerve fibres passing through the habenula (Spoendlin, 1972). Within the Organ of Corti, however, they ramify greatly to form several bundles. The inner spiral bundle runs underneath the inner hair cells and makes extensive synaptic contacts with the nerve endings of the radial dendrites. The spiral tunnel bundle travels along the basilar membrane and appears to send small bundles across the tunnel of Corti to the outer hair cells. The efferent contact in the outer hair cell region, in contrast to the inner hair cell area, is predominantly on the hair cells themselves. Both in the axo-dendritic synapses under the inner hair cells, and the endings on the outer hair cells, many vesicles are seen on the efferent (pre-synaptic) side. Once

again it is Spoendlin who claims that in the cat, the difference between efferent terminations at inner and outer hair cell regions is clear cut. According to him, the only efferent synapses in the region of the outer hair cells are on the hair cell base, and near the inner hair cells, only terminations on the radial fibres are seen (Spoendlin, 1973). In other species, this does not appear to be true. Smith and Rasmussen (1963) have reported axo-dendritic synapses underneath the outer hair cells in the chinchilla and guinea pig, and Angelborg and Engstrom (1973) claim to have identified efferent synapses on the inner hair cells as well as on the radial fibres themselves.

Little is known of the relative distribution of the crossed and uncrossed innervation between inner and outer hair cell regions. One brief report (Iurato, 1962) in the rat, claims that the axo-dendritic synapses under inner hair cells come exclusively from the uncrossed bundle, whilst endings on the outer hair cells

are from the crossed. This has not been verified, and there are grounds for caution in this matter (Desmedt and Robertson, in press; Klinke, 1974). It is very possible that the crossed bundle makes en passant synapses in the region of the inner hair cells before crossing the tunnel to the outer hair cells (Spoendlin, 1973).

The adrenergic innervation is little known or understood. Only recently has electronmicroscopic evidence on the location of adrenergic endings appeared (Densert, 1974). These appear to be restricted to the habenula region, as well as associated with blood vessels in the limbic lip and spiral ganglion.

iii) Spiral ganglion.

The spiral ganglion, variously called the cochlear ganglion or acoustic ganglion, contains primarily the bipolar cell bodies of the primary afferent neurones, whose final connections within the Organ of Corti, were discussed above. The single central process of each

ganglion cell exits from the cochlea via the modiolus to form the cochlear branch of the VIIIth nerve. Whereas the ganglion, following as it does the spiral course of the basilar membrane within the cochlea, presents a linear representation of the Organ of Corti innervation, the central processes of cells from all regions of the cochlea become fused in a complex fashion to form the cochlear nerve trunk (Sando, 1965). All the single unit recordings in this thesis are from the ganglion itself, and assuming a radial innervation a direct mapping of the Organ of Corti to nerve elements in the ganglion is thus possible. The ganglion is surrounded by thin bone, one wall of which abuts on the scala tympani, and this bone must be removed for microelectrode insertion. Also dispersed throughout the ganglion are blood vessels, unmyelinated adrenergic nerve fibres, and the olivocochlear fibres in the intraganglionic spiral bundle.

It is now firmly established that there are two populations of bipolar ganglion cells. 90-95% of the

cell bodies in cat and guinea pig are covered with a myelin sheath (Kellerhals et al., 1967; Spoendlin, 1972, 1973; Thomsen, 1967), and are ovoid in shape (20-25 by 15 microns). The peripheral and central processes of these cells are also myelinated and are 1-2 microns in diameter. A second population of cells, comprising only 5-10% of the total ganglion cell population, are smaller in size and are unmyelinated. They also send one process to the Organ of Corti and one to the cochlear nerve.

By the use of degeneration studies, Spoendlin has been able to show that these two populations probably correspond to the radial and outer spiral fibres innervating the inner and outer hair cells respectively (Spoendlin, 1972, 1971). This has been most clearly shown in cat, but appears to hold also for the guinea pig. While Spoendlin maintains that the unmyelinated cells are bipolar like the more predominant myelinated group, some authors differ. Ross (1973) claims that in

rat, these unmyelinated cells are in fact multipolar, and cites previous golgi studies to support this. All in all, Spoendlin's evidence in the cat and guinea pig seems fairly good. According to him there is evidence that the very small number of giant fibres which innervate the inner hair cells in multiple fashion, also come from the unmyelinated cells in the ganglion.

The detailed ultrastructure of the afferent ganglion cells has been reported by many workers Iurato, 1967; Kellerhals et al., 1967; Reinecke, 1967; Rosenblith and Palay, 1961; Spoendlin, 1972, 1973; Thomsen, 1967). The most pertinent feature is the presence of myelin on axons and cell bodies of the major ganglion cell population. There is also a suggestion that the processes of the unmyelinated cells are myelinated (Spoendlin, 1973). On the somas of the myelinated cells myelin is less compact than on the processes. Little attention has been given to the important question of the location of nodes of Ranvier on these cells (Rosenbluth and Palay,

1961), and some data on this and the implications of myelin distribution, are the subject of Paper V of this thesis.

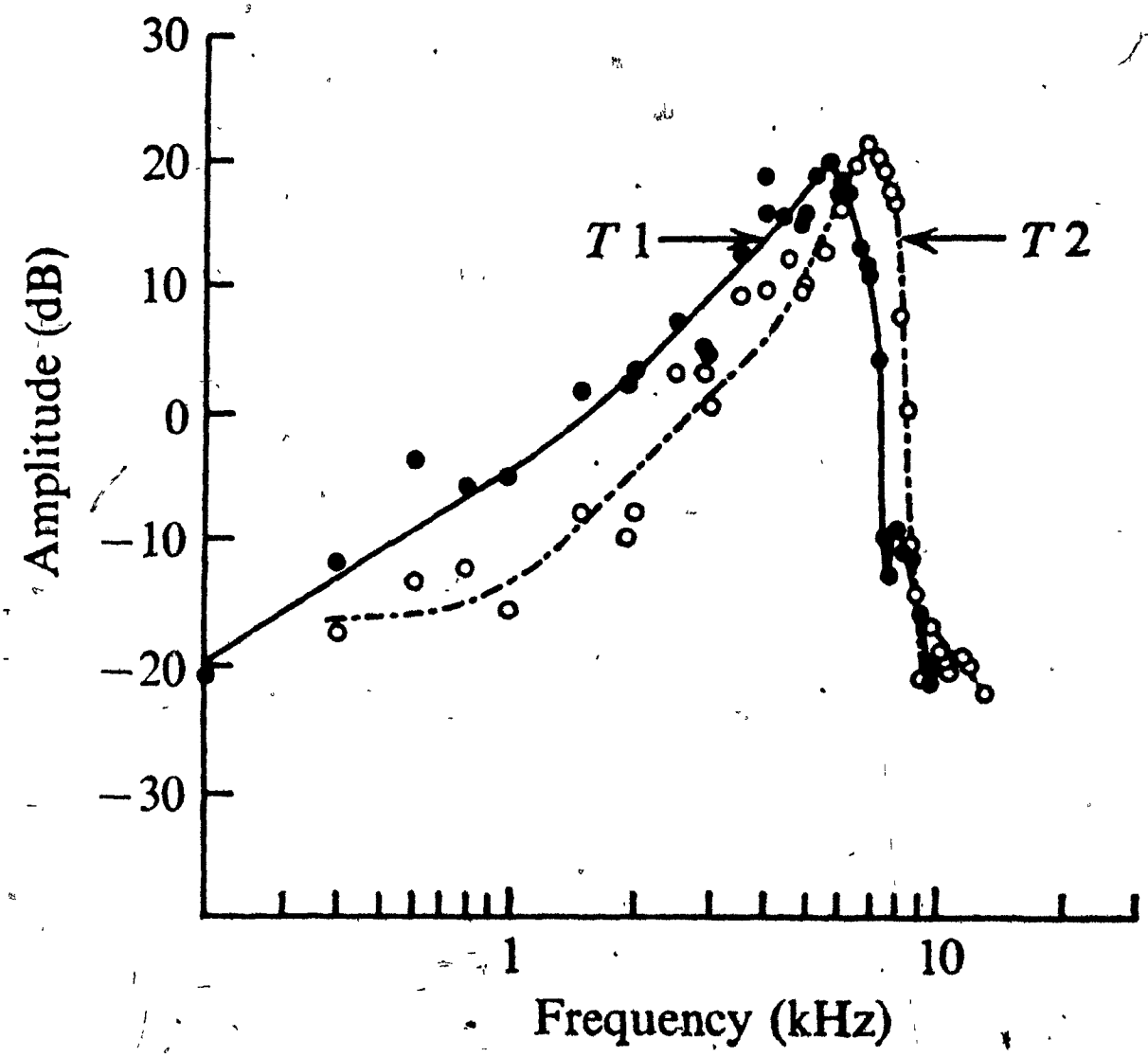
It seems that the peculiar pattern of innervation of the mammalian Organ of Corti is well established, at least for some species. The most extraordinary feature is that the predominant hair cell population, the outer hair cells, only make synapic contact with 5-10% of the primary afferent neurone pool. The implications of this arrangement for signal processing are obscure, but one important consequence is that, in single cell studies, either in the cochlear nerve or in the spiral ganglion, the vast majority of units recorded from probably emanate from inner hair cells. The smaller size of the ganglion cells innervating outer hair cells also increases the probability that they will be missed by microelectrode sampling techniques (Stone, 1973).

COCHLEAR PHYSIOLOGY

i) Basilar membrane mechanics

The vibration pattern set up on the basilar membrane by movements of the stapes was first observed optically by Von Bekesy and has since been studied by several techniques (Johnstone et al., 1967, 1970; Kohlloffel, 1973; Rhode, 1971; Wilson and Johnstone, 1972). The response to a continuous sinusoidal stimulus is the production of a travelling wave whose phase and amplitude varies along the length of the membrane. The amplitude of maximum vibration shifts in location depending on the frequency of the tonal stimulation. Low frequencies cause maximum vibrations near the apex and higher frequencies towards the narrower, stiffer basal end of the membrane. Modern practice is to measure the tuning curve, or frequency response of a particular point on the basilar membrane. A typical result is shown in Fig. 5. The high frequency fall-off beyond the peak has been reported to be up to 300dB/octave (Johnstone

Fig. 5. A typical basilar membrane mechanical tuning curve measured by the Mössbauer technique (from Rhode, 1971). Two tuning curves each at a different point on the basilar membrane are shown. The species is the squirrel monkey.


30
20
10
0
−10
−20
−30
Amplitude (dB)
T 1
T 2
1
10
Frequency (kHz)

and Yates, 1973), but more normally reported values are 100dB/octave. The actual value found seems to depend on a variety of factors; location on the membrane, state of the preparation (Rhode, 1973; Kohlloffel, 1973), perhaps animal species, and the laboratory in which the results are obtained. All authors are now agreed that the high frequency fall off does not continue indefinitely, but reaches a plateau some 30 - 50 dB below the amplitude peak. The low frequency slope of the amplitude function is very much less; a maximum reported value of 24 dB/octave when plotted against constant stapes displacement (Rhode, 1971), and is usally reported as 5-10 dB/octave. Table I summarizes the findings by different workers.

As discussed in the first three manuscripts of this thesis, all the present basilar membrane techniques suffer from disadvantages which are not considered important by many of the workers involved. The cochlea is an extremely sensitive system and it should be borne in

TABLE I

SPECIES	LOCATION mm from basal end	HIGH FREQ. SLOPE dB/octave	LOW FREQ. SLOPE dB/octave	AUTHORS
Guinea Pig	12	30	5	Bekesy, 1960
	2-3	100	5	Johnstone et al., 1967,1970
	1-4	130	2	Wilson & Johnstone, 1972
	4	340	5-15	Johnstone & Yates, 1973
	3	130	7	Kohlloffels, 1973
Squirrel Monkey	7	100-150	10-24	Rhode, 1971

mind that even the initial approach procedures (e.g. wide opening of the scala tympani, perilymph drainage, placement of radioactive sources on the membrane itself), as well as the inherent limitations of each technique, could obscure particular aspects of basilar membrane vibration which are perhaps present in the uninvaded preparation. This is dealt with more fully in papers I-III, and in the discussion following those papers. However, at this point mention will be made of one controversial and important topic; the existence of non-linear vibrations on the basilar membrane.

Von Bekesy (1960) and Johnstone et al. (1970), each reported that the basilar membrane vibrated linearly over all the frequencies examined, within the sound pressure range which could be investigated (120-140 dB for Von Bekesy, and 100-120 dB for Johnstone et al.). They thus inferred that the basilar membrane tuning curve they measured at these high sound pressure levels was similar to that at much lower intensities. This is

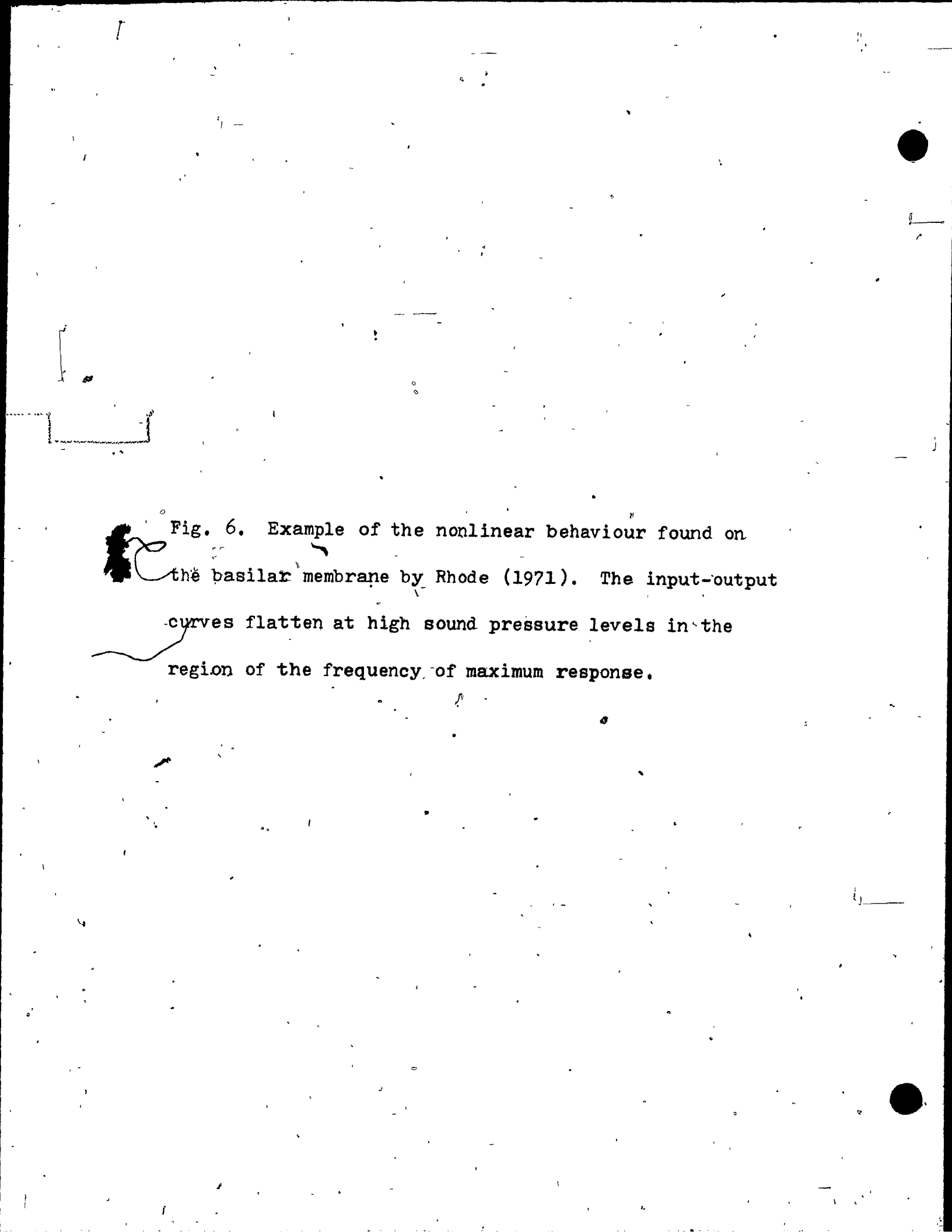
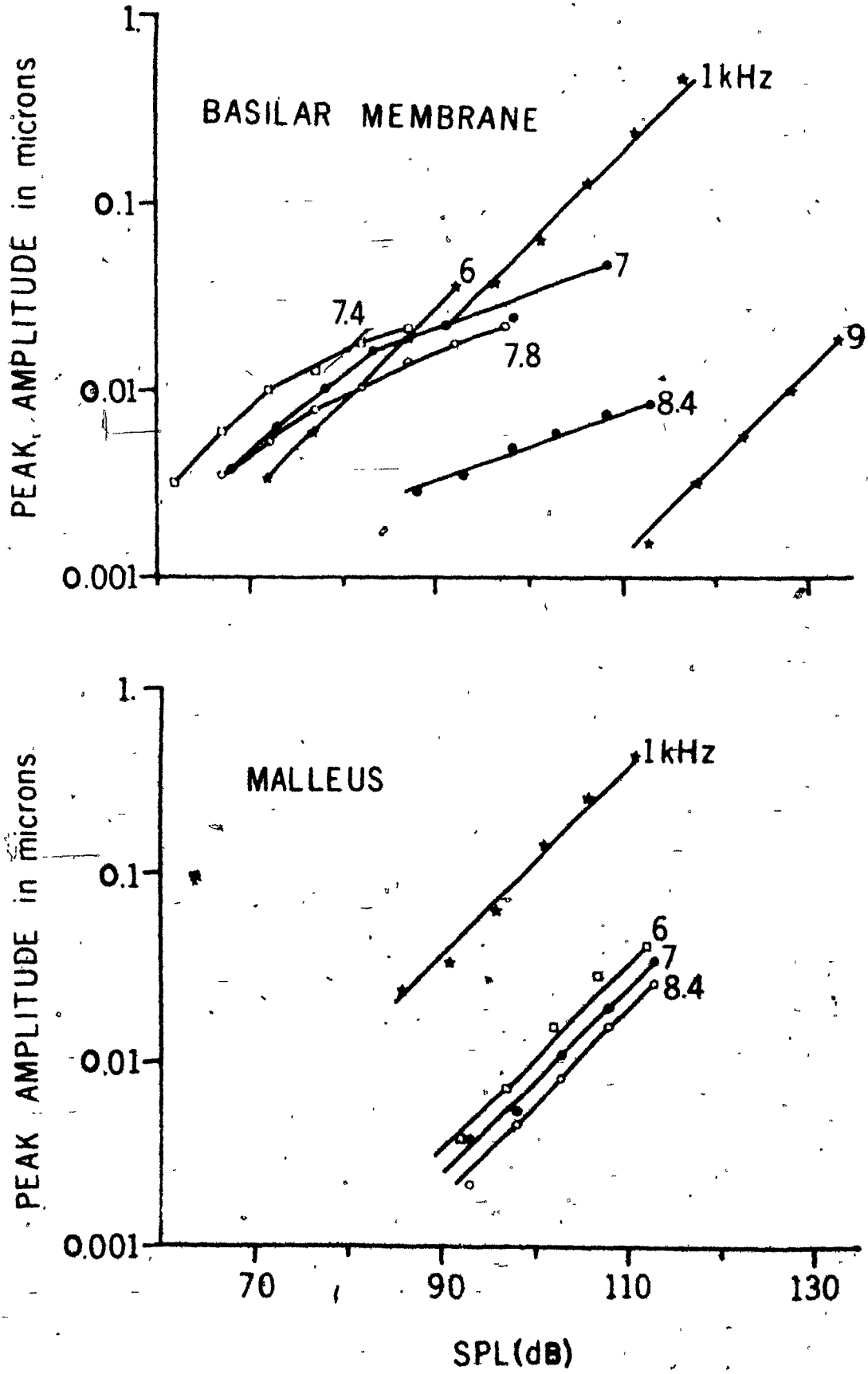
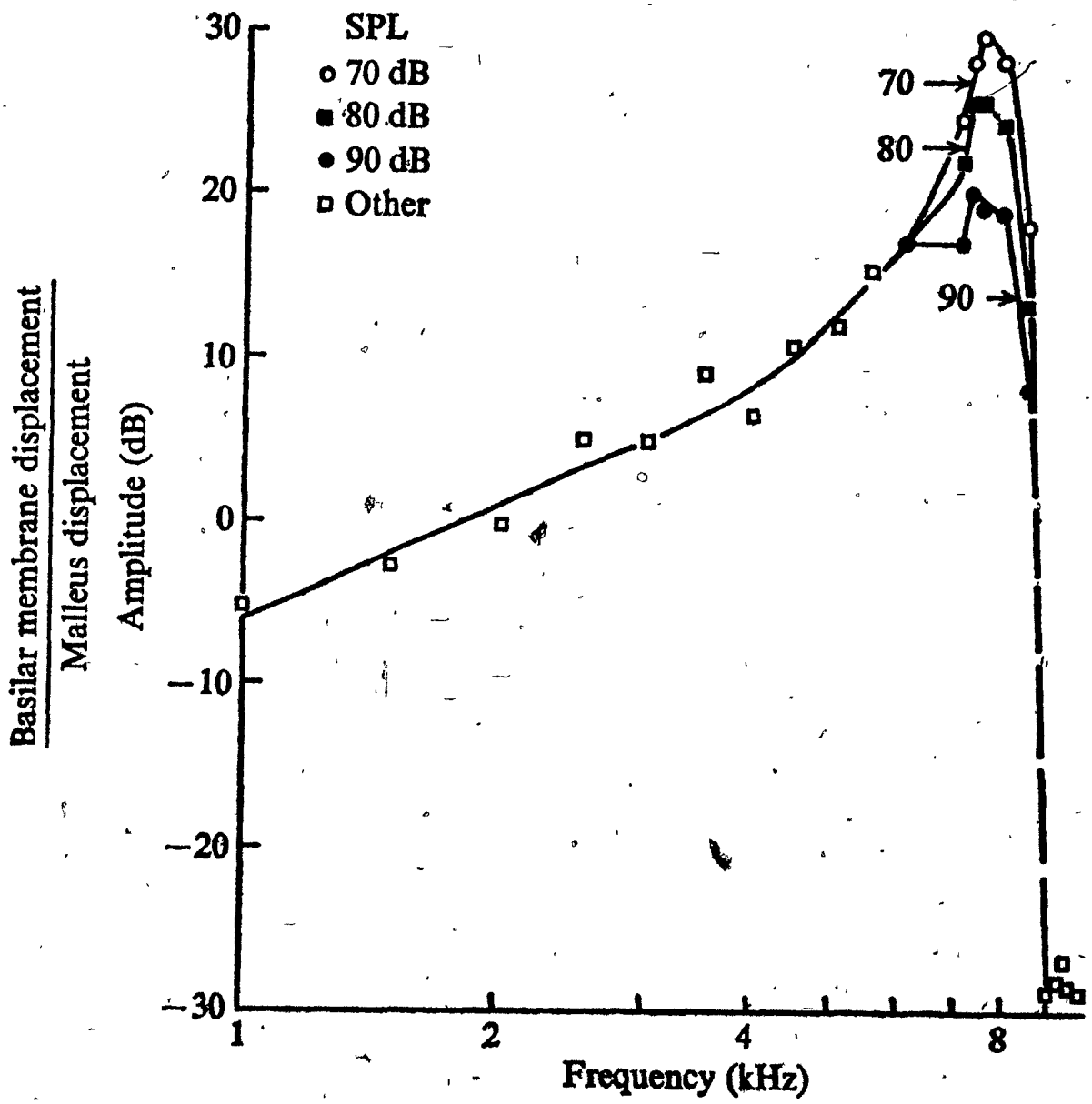


Fig. 6. Example of the nonlinear behaviour found on the basilar membrane by Rhode (1971). The input-output curves flatten at high sound pressure levels in the region of the frequency of maximum response.


BASILAR MEMBRANE
1kHz
6
7
7.4
7.8
8.4
9
PEAK AMPLITUDE in microns
1.
0.1
0.01
0.001
MALLEUS
1kHz
6
7
8.4
PEAK AMPLITUDE in microns
1.
0.1
0.01
0.001
70
90
110
130
SPL(dB)

important when considering the derivation of the neural tuning curves, considered in a later section. However, in 1971, Rhode reported a form of non-linearity on the squirrel monkey basilar membrane, which implied that tuning was not the same at different sound pressures. His initial results are reproduced in Figs. 6, 7. The input-output curves exhibit a flattening in the region of maximum vibration, whereas at other frequencies the behaviour is linear. The effect of this form of non-linearity is to produce a peaking of the basilar membrane tuning curve at lower sound pressures (Johnstone and Yates, 1973; Kim et al., 1973; Rhode, 1971). Again the limitations of the techniques do not allow sound pressures lower than 70-80 dB to be used. Subsequently, Wilson and Johnstone (1972) and Johnstone and Yates (1973) failed to confirm the existence of such a non-linearity in the guinea pig. A possible limitation of the technique of Wilson and Johnstone is the principle subject of paper II. A non-linearity of the type found

Fig. 7. Taken from Rhode (1971), showing the effect of the amplitude nonlinearity of Fig. 6 on the basilar membrane tuning at different sound pressure.


SPL
70 dB
80 dB
90 dB
Other
70
80
90
30
20
10
0
−10
−20
−30
1
2
4
8
Frequency (kHz)
Basilar membrane displacement / Malleus displacement
Amplitude (dB)

by Rhode is important when considering the shape of neurone response areas at low-sound pressure levels, so that the lack of agreement among authors is particularly frustrating.

As far as first hand information on the finer motion patterns of the Organ of Corti in response to basilar membrane movement are concerned, we still rely largely on the observations of Von Bekesy (1953, 1960), on fixed and living guinea pig cochleas. Most recent efforts have concentrated mainly on modelling (Billone and Raynor, 1973; Johnstone and Johnstone, 1966; Rhode and Geisler, 1967; Steele, 1973).

Bekesy reported that the pattern of motion of the hair cells embedded in the reticular lamina varied along the extent of the travelling wave. For regions basal to the point of maximum vibration, motion of the hair cells was radial, presumably producing a radial shear on the stereocilia. Apical to the peak, motion became progressively up and down, then longitudinal

till eventually it became undetectably small. Some limitations of these observations are the fact that they were obtained in the extreme apical region of the basilar membrane (below a best frequency of vibration of 1 kHz) and that they were observed at very high sound pressures. Just how the Organ of Corti responds at more basal locations and at reasonable sound intensities has never been directly observed. There is much scope for mechanical transformations of the basilar membrane motion before the actual hair cell excitation. It would be fair to say that though 20 years have passed since Von Bekesy's original observations, our ideas on these transformations are still largely conjecture.

ii) Resting potentials

In mammals, the scala media is maintained at a high positive potential (80-100 mV) with respect to blood and perilymph. The electrophysiology and electro-

chemistry of this potential have been intensively studied in guinea pig (Bosher and Warren, 1971; Johnstone and Sellick, 1972; Johnstone, 1967; Johnstone et al., 1973) and its generation does not appear to rely on the ionic gradients between endolymph and perilymph. This large endocochlear potential (+EP) is very sensitive to anoxia and after the death of the animal it falls within a few minutes to negative values (about -50 mV), produced largely by the potassium diffusion gradient from endolymph to perilymph. The +EP can be measured by insertion of KCl-filled microelectrodes into the scala media, either through the basilar membrane, Reissner's membrane or stria vascularis. The Organ of Corti extracellular spaces can be considered as approximately equipotential to perilymph. A large negative potential can be recorded inside the Organ of Corti (Bekesy, 1952; Tasaki et al., 1954). Dallos (1968) has discussed the various arguments for the source of this potential, and it is now generally agreed

that it is a normal intracellular potential recorded either from hair cells or supporting cells.

The implications of the +EP and the negative intracellular potential for transduction in the cochlea have been discussed by numerous authors (Davis, 1965; Honrubia et al., 1971; Honrubia and Ward, 1970; Johnstone et al., 1966). Most important is that there must be a large leakage current from scala media to scala tympani (Johnstone et al., 1966). The role of this current in the generation of receptor potentials in response to sound stimulation is discussed below.

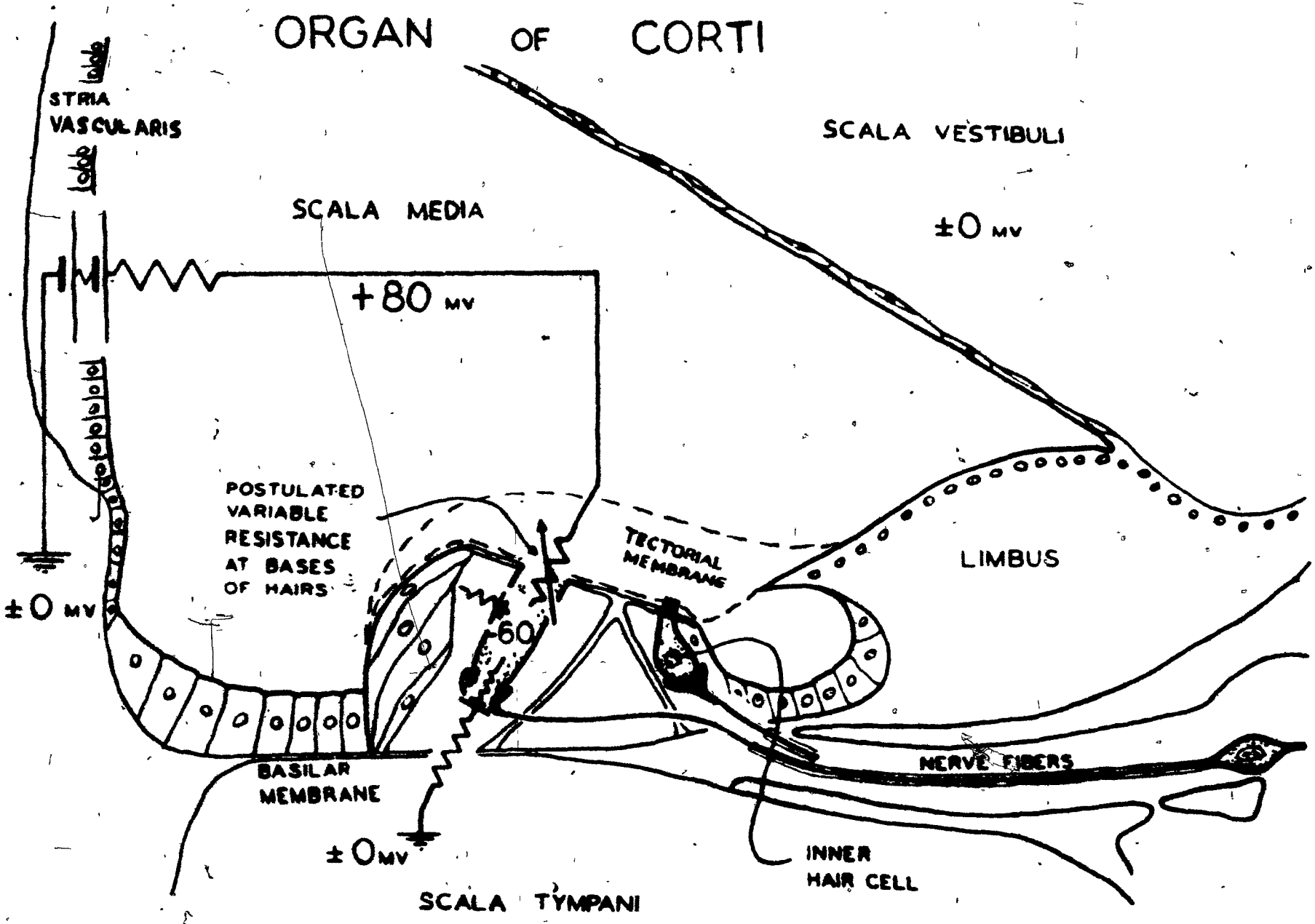
iii) Receptor potentials

Receptor potentials can be generally defined as the first potentials produced in sensory structures by the presentation of an appropriate stimulus. They may, or may not directly give rise to the generation of spike activity. In the case of the Organ of Corti, the receptor potentials are distinct from the generator and neural potentials which are initiated in the afferent neurones.

The current hypothesis for the production of receptor potentials by the hair cells is the mechano-electrical theory of Davis (1954, 1958, 1965)(Fig. 8). The hairs are assumed to be in contact with the tectorial membrane. Upward and downward movements of the basilar membrane cause shearing forces between opposing points on the tectorial membrane and the reticular lamina and thence bending of the hairs. This is assumed to cause a resistance change in some region of the hair cell and thus a change in the standing current discussed above. If an electrode is placed in the scala media, scala tympani or scala vestibuli, this modulation of the standing leakage current will be registered as a change in potential difference between the electrode and a remote reference, usually placed in the neck muscles.

Two types of receptor potential are in fact found in response to a tone stimulus. The cochlear microphonic (CM) is an AC potential which follows closely

Fig. 8. A summary of the potentials within the cochlea which are the basis of the mechano-electrical theory of Davis. Taken from Davis (1965).


ORGAN OF CORTI
STRIA VASCULARIS
SCALA VESTIBULI
SCALA MEDIA
±0 MV
+80 MV
POSTULATED VARIABLE RESISTANCE AT BASES OF HAIRS
TECTORIAL MEMBRANE
LIMBUS
±0 MV
60
BASILAR MEMBRANE
NERVE FIBERS
±0MV
INNER HAIR CELL
SCALA TYMPANI

the waveform of the stimulus. Also found in response to the same stimulus is a DC potential, the summating potential (SP) whose origin is not as easily explained as the CM. The summating potential appears positive in the scala media on the basal slope of the travelling wave envelope and negative at the peak. Positive summating potentials also become negative as the sound pressure is raised (Honrubia and Ward, 1969). There is evidence from intracellular recordings in the lizard basilar papilla, that the summating potential is reflected in changes in the hair cell membrane potential. (Mulroy et al., 1974).

This variable resistance theory of CM and SP generation now has considerable experimental support (Honrubia et al., 1971; Honrubia and Ward, 1970; Johnstone et al., 1966; Johnstone and Johnstone, 1966; Kurokawa, 1965). Upward displacements of the basilar membrane (away from the scala tympani) produce decreases in the scala media access resistance and thus a fall in

the EP. Downward basilar membrane displacements result in a rise in EP and resistance. The consequence of this theory, verified by experiment, is that the magnitude of both the CM and the SP will be directly related to the magnitude of the potential differences across the cochlear partition (i.e. the magnitude of the hair cell leakage current).

The CM has been more intensively studied than the SP. Dallos' group maintain that the CM in the normal cochlea is proportional to basilar membrane displacement (in agreement with Von Bekesy, (1960))(Dallos et al., 1972) and that this CM is dominated by the output of the outer hair cells. This is not unreasonable in view of their larger number. However these authors also maintain that the inner hair cells are responsive to basilar membrane velocity and are some 30 dB less sensitive than the outer hair cells. Their evidence is obtained from cochleas in which the outer hair cells have been selectively eliminated by the

ototoxic drug kanamycin. Inner hair cells were scored as present and "normal" by light microscopy. There is considerable reason to believe that these remaining inner hair cells are not cytologically normal (Wersall, 1973), and the originators of such experiments should provide evidence on this point. Wersall (1973) states "It is remarkable how well preserved the cuticle and the hairs might be in a cell with advanced protoplasmic disintegration.". In addition, the study of Kiang et al., (1970) on cats poisoned with doses of kanamycin similar to those used by Dallos' group, failed to elicit responses to the most intense auditory stimulation from cochlear nerve fibres emanating from regions with apparently intact inner hair cells and no outer hair cells. This is a strong indication that inner hair cells which appear to be structurally normal in the light microscope are highly abnormal from a functional point of view. Even if the inner hair cells are structurally normal in such preparations it might be

supposed that the cellular rearrangement and loss of many stereocilia-tectorial membrane contacts accompanying vast outer hair cell losses would alter the mechanical properties of the organ of Corti. As it would thus seem hasty to infer the normal properties of inner hair cells from such kanamycin poisoned cochleas, judgement will be reserved here on the conclusions of Dallos and his co-workers. Of interest is the fact that the postulate of inner hair cells responsive to basilar membrane velocity is based on the assumption that the inner hair cell stereocilia are not attached to the tectorial membrane and are stimulated principally by viscous forces (Dallos et al., 1972). As we have seen, this belief, which is also the basis of several models (Billone and Raynor, 1973), is not founded on firm evidence and may in fact be incorrect.

Though the cochlear microphonic is not studied in this thesis, the findings of other workers on this receptor potential are relevant to the central problem

of the first three papers of this thesis. For this reason, some aspects of the cochlear microphonic will be discussed at length here.

The CM is usually measured by placing gross differential electrodes, one each in the scala vestibuli and scala tympani. Though this differential technique undoubtedly has advantages over single-ended recording (Dallos, 1969), there is evidence that, in the basal turn of the cochlea at least, a spatial filtering effect occurs which limits the usefulness of such measurements. This effect is apparently caused by the nature of the hair cells as out-of-phase generators in fluid-filled compartments (Kohlloffel, 1970, 1971; Lazlo et al., 1972; Whitfield and Ross, 1965), and only limited insight can be gained into the nature of individual hair cell output from such records. An interesting effect has been found which is consistent with the operation of this spatial filter (Yates, personal communication; Yates et al., in press), where partial drainage of perilymph from the

scala tympani allows high frequency slopes of up to 100dB/octave to be measured in CM tuning curves by a gross single-ended electrode placed on the spiral lamina. This contrasts with high frequency slopes of only 30-40dB/octave measured in the same region by the differential electrode technique (Dallos, 1973a). Another effect of such drainage is to shift the SP peak from the high-frequency CM slope (Dallos, 1973a) to correspond exactly with the CM peak.

An amplitude-limiting nonlinearity is present in the cochlear microphonic and Dallos (1973a) has reported that one of the effects of this CM saturation at high intensities, is to produce a peaking of the CM tuning curve at lower sound intensities (Fig. 4 in Dallos, 1973a). The parallel between this behaviour and Rhode's basilar membrane non-linearity is obvious, but the effect is by no means dramatic. It is not clear how the use of gross electrodes which pick up the responses from large numbers of CM generators may minimize this and other nonlinear phenomena.

The question of where distortion products found in the cochlear microphonic are generated, is an important one in view of the controversy regarding nonlinearities in the basilar membrane vibration. All the available psychophysical evidence (Goldstein, 1967; Goldstein and Kiang, 1968; Smoorenburg, 1972) suggests that the cubic distortion product $2f_1 - f_2$ is transduced at its appropriate place on the basilar membrane. However, in this respect and in numerous others the behaviour of this distortion product in the CM is very different. Dallos and Sweetman (1969) and Sweetman and Dallos (1969) report that $2f_1 - f_2$ cannot be cancelled by a third tone at two locations simultaneously. The explanation is that this and other distortion products in the cochlear microphonic are not present as true travelling waves and peak at the location of the primaries or fundamental tones. Also, Dallos et al., (1969) have shown that distortion products in the cochlear microphonic are affected differently from pure tones by polarization of the coch-

lear partition. Other discrepancies are that the magnitude of $2f_1 - f_2$ in the CM does not vary with f_2/f_1 or intensity as would be expected from the pschoacoustical data. Neither are the relative levels of distortion products in the cochlear microphonics in agreement with the neural correlates of such nonlinearities. Because of these data, Dallos (1973b) suggested that in fact the CM might not be important in the transduction process and that the distortion products in the neural output of the cochlea must be generated at some as yet unspecified stage.

Basilar membrane models incorporating a nonlinearity of the type observed by Rhode can generate distortion products whose behaviour is in reasonable agreement with psychophysical data (Kim et al., 1973). It has also recently been shown that the distortion products generated by such models do peak at the location of the fundamentals in agreement with the CM data of Dallos and Sweetman (Hall, 1974).

Wilson and Johnstone (1972, 1973), on the basis of Dallos' data and their own failure to find significant levels of $2f_1 - f_2$ in the basilar membrane vibration postulate that the nonlinear elements responsible for the generation of neural distortion products are central to both the basilar membrane and the CM generation step. They postulate that the locus is within the hair cell itself. The fact that CM and basilar membrane data are recorded in the guinea pig, and psychoacoustical data are only available for man, cat and squirrel monkey may mean that the discrepancies mentioned above are not important. However, if species differences are not important and if the CM measurements with gross differential electrodes are meaningful, then the postulate of Wilson and Johnstone must be seriously considered.

The relative roles of the CM and SP in the excitation of afferent dendrites is obscure. Upward movement of the basilar membrane, corresponding to a drop in hair cell resistance and a decrease in +EP, is excitatory to

the auditory nerve fibres. Thus if the CM provides excitatory current, only one phase can be used. At high frequencies (above about 5 kHz and up to 100 kHz in the bat) it is difficult to see how such a rapidly alternating potential could effectively release transmitter from the hair cells. Perhaps the negative SP becomes important at these frequencies.

iv) Generator potentials

Actual recording of generator potentials from within the afferent dendrites has not been achieved in mammals. However, data from lateral line organs (Flock et al., 1973) and the saccular macula of teleosts (Furukawa and Ishii, 1967) show that excitatory postsynaptic potentials (EPSPs) are produced in the afferent dendrites, both spontaneously, and in response to sound stimuli, or deflection of sensory hairs. All these studies utilize low frequencies and show EPSP's in approximate synchrony with the expected excitatory phase of the micro-

phonic response. Presumably, these EPSP's spread decrementally to a more distant spike initiation point, perhaps at the point where myelination begins (at the habenula in the organ of Corti). Mulroy et al., (1974) have recorded all-or-none spikes in the lizard basilar papilla at a point between the habenula and the first nodes of the ganglion cells. For the outer spiral fibres in the Organ of Corti, the situation may be a little different. The question has been raised as to whether these fibres are so small and so long that decrementally conducted EPSP's would be too small at the habenula to initiate action potentials. The possibility should therefore be considered that propagated action potentials exist in these long unmyelinated dendrites. There is some evidence for dendritic spikes in other systems (Llinas and Nicholson, 1969). However, in some invertebrate sensory systems, decremental conduction appears to take place effectively in fibres just as fine and long as the mammalian outer spiral fibres (Patton and Kater, 1972; Shaw, 1972).

v) Neural Recordings

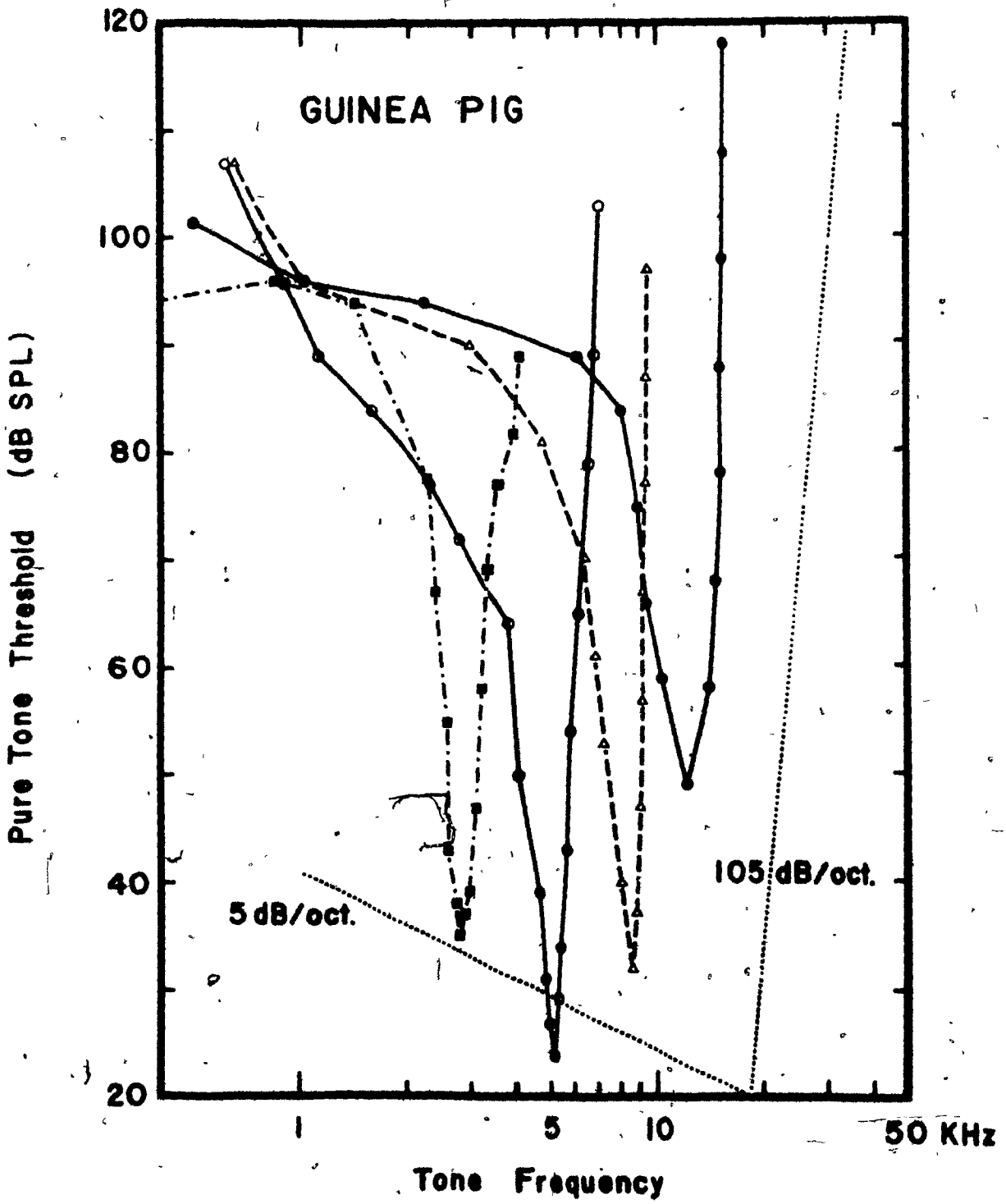
Since the work of other researchers relevant to each manuscript are discussed therein, this will not be comprehensively covered here. Many single fibre recordings have been obtained from the cochlear nerve in the internal auditory meatus of cat, guinea pig, and squirrel monkey (Evans, 1972; Kiang, 1965; Rose et al., 1974, 1971, 1967). Single unit thresholds have been shown to agree reasonably well with the behavioural audiograms (Evans, 1972), and most authors are now agreed that the variation in single unit thresholds at a given best frequency in a single animal is quite small (not greater than about 20dB) (Evans, 1972; Kiang, 1965). There is a large amount of data available on the spontaneous activities (cat) and response properties of these primary neurones and only one aspect relevant to the following papers will be emphasized here. These are the tuning curves or threshold-frequency response areas which define the threshold sound pressure level for a given fibre at each

frequency of sound stimulation. These tuning curves are consistently sharper than the basilar membrane tuning curves which have been reported for similar regions in the same species (Fig. 9) (Evans, 1970; Evans and Wilson, 1973). One exception to this is the tuning curves of fibres with best frequencies less than about 1kHz, which are in substantial agreement with the original basilar membrane curves of von Bekesy in this region (Evans and Wilson, 1973).

The problem of whether there must be an additional mechanism subsequent to the basilar membrane tuning to explain the single unit tuning curves, or whether the present basilar membrane measurements are inadequate, constitutes the central problem of the first three papers of this thesis.

The high frequency slopes measured within 25dB of the best frequency thresholds are not too different from the maximum reported basilar membrane values but there is a very large discrepancy between the low frequency slopes

Fig. 9. A comparison of the neural tuning curves of cochlear nerve fibres of the guinea pig and the basilar membrane data of Johnstone et al. (1970). The fine dotted line shows the mechanical data, and it is obvious that the slopes of this curve are very much less than in the neural tuning curves. Taken from Evans (1970).


GUINEA PIG
120
100
80
60
40
20
Pure Tone Threshold (dB SPL)
5 dB/oct.
105 dB/oct.
1
5
10
50 KHz
Tone Frequency

near the best frequency for the neural and mechanical tuning. In addition, it has been reported (Evans, 1972) that the high frequency neural slopes can reach maximum values of 1000dB/octave which is very greatly different from the maximum mechanical values. There is no sign in the neural curves of the high frequency plateau observed in the basilar membrane vibration by Wilson and Johnstone (1972), Johnstone and Yates (1973) and Rhode (1971).

This question of an additional mechanism is directly related to the above discussion on the site of generation of distortion products in the cochlea. The particular nature of the nonlinearity found by Rhode raises the possibility that both the sharp neural tuning and the generation of some distortion products can be explained by nonlinear basilar membrane mechanics. As outlined above, there are arguments and data both for and against this hypothesis and several authors believe that both sharp neural tuning and the generation of nonlinear distortion products in the neural output are properties of

an additional unspecified mechanism. This matter is examined in the results section of this thesis and in the discussion following the papers.

A second problem in neural recordings so far is the failure to detect single units in the cochlear nerve which can be assigned to the inner and outer hair cells with any degree of certainty. As discussed above, the particular innervation of the organ of Corti is bound to result in a low probability of sampling fibres emanating from outer hair cells. Intensive studies of a large number of cochlear nerve fibres in the cat have failed to reveal two populations which can reasonably be attributed to inner and outer hair cells (Kiang, 1965; and personal communication). Evans (1972) does report the presence of a small number of nerve fibres with high spontaneous rates which failed to show a significant increase in discharge rate but did show synchronization to low frequency stimuli. From a reading of Evans (1972) it appears that he did not consider that these were es-

pecially related to the outer hair cells. Nomoto et al., (1964) classified fibres into two groups on the basis of their rate versus intensity functions, but the particular behaviour they observed has not been confirmed. In addition, their smaller population comprised nearly 30% of their sample of 66 fibres which is not in very good agreement with Spoendlin's quantitative estimates of outer spiral fibre density.

The above introduction has shown that there are many important and unresolved problems in the primary pathway. The papers which now follow do not attempt to provide a complete explanation for any of these problems. The general properties of the spiral ganglion cells in the basal turn of the guinea pig cochlea are presented together with some interesting findings which throw some light on the neural tuning question.

PAPER I

Manipulation of Frequency Analysis in the Cochlear Ganglion of the Guinea Pig*

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Introduction

It is known that primary auditory neurones exhibit great frequency selectivity in their response to sound stimuli (e.g. Kiang, 1965; Evans, 1972). This has been shown to be true both for pure tone and impulse stimuli (Moller, 1970; de Boer, 1969). Studies on the VIIIth nerve axons of mammals show that a single primary neurone responds with greatest sensitivity to a particular frequency (the characteristic frequency, cf.). On either side of the cf. the sensitivity of the fibre decreases very rapidly. This frequency selectivity has been traditionally expressed as the tuning curve, a diagrammatic representation of the threshold sound pressure level (SPL) at various frequencies. A convenient indication of the frequency selectivity derived from the tuning curve is the "sharpness quotient" or Q_{10dB}; the cf. divided by the bandwidth of the tuning curve 10 dB above threshold at the cf.

The mechanism underlying this frequency selectivity is currently one of the major research problems in peripheral auditory physiology. Studies of basilar membrane vibration in the guinea pig suggest that in regions with a maximal vibration at frequencies greater than about 1kHz, the mechanical tuning of points on the basilar membrane is much less sharp than the corresponding neural tuning curves (Johnstone et al., 1970; Johnstone and Yates, 1973; Wilson and Johnstone, 1972). One study in the squirrel monkey (Rhode, 1971) has revealed a mechanical non-linearity in the basilar membrane vibration which results in a sharpening of the basilar membrane tuning as lower sound intensities are used. This non-linearity, which could be used to reconcile the neural tuning curves derived at low (threshold) sound levels with the basilar membrane tuning normally measured between 70 and 100 dB SPL, has not been found in the guinea pig. In fact the guinea pig basilar membrane has been reported to vibrate linearly down to sound

levels as low as 40 dB (Wilson and Johnstone, 1972). Whether this necessitates the presence of some additional physiological filter to account for the sharpness of neural tuning curves or whether there may still be some inadequacy in present measurement techniques of basilar membrane vibration is not certain.

In this study we report extracellular recording from 197 single cochlear ganglion cells in the acoustic (spiral) ganglion of guinea pigs. The ganglion cells are the somata of the bipolar afferent cells whose axons form the cochlear branch of the VIIIth nerve. It is shown that under some conditions the sharpness of tuning curves of these cells is variable and can approach the pattern of mechanical tuning which has been reported on the basilar membrane.

Materials and Methods

Guinea pigs (150-400 g) were anaesthetised with 36 mg/kg of Nembutal, following an injection of 20 μg of Atropine sulphate. A tracheotomy was performed, the animals were relaxed with 0.05 ml of Quelecin (Roche) and artificially ventilated. Rectal temperature was maintained at 38.5°C. The cochlea was approached ventro-laterally through the acoustic bulla. The scala tympani of the basal cochlear turn was opened with a sharp scalpel taking care not to damage the spiral ligament close to the basilar membrane. The round window was not removed and the scala tympani hole was made a small as was feasible (usually about 700 X 500 μ). Perilymph was allowed to remain in the scala tympani, excess seepage into the bulla being taken up with fine cotton wicks. Through the hole in the wall of the scala tympani, the modiolus and osseous spiral lamina could be visualised. A small hole (50 μ diameter) was

made with a fine steel pick in the thin bone of the spiral lamina overlying the spiral ganglion.

Extracellular recordings were obtained from the bipolar ganglion cells by introducing metal-filled glass microelectrodes (Frank and Becker, 1964) of tip diameter 5-8μ through the hole in the spiral lamina. The movement of the electrode was controlled with a remote hydraulic microdrive located outside the sound-proof room in which all experiments were conducted. Tone burst stimuli were delivered in a closed sound delivery system calibrated from 1 kHz to 25 kHz. A probe tube incorporated in the delivery tube was used to measure sound pressure levels at the tympanic membrane. Accurate and reproducible placement of the probe tube in each experiment was made by resecting the external auditory meatus and fully exposing the tympanic membrane up to within 1 mm of the edge of the tympanic ring. The sound delivery tube was sealed over the tympanic ring with "Vaseline" and a transparent tip

to the end of the delivery tube allowed the threaded probe tube to be accurately positioned in relation to the tympanic membrane. Broad-band white noise was used as a search stimulus although the single cells could generally be detected by their spontaneous activity. Single unit data were tape-recorded and analysed off-line with a PDP8 computer. Tuning curves were obtained during the experiments by using standard criteria to estimate the single cell thresholds to pure-tone stimuli (Kiang, 1965).

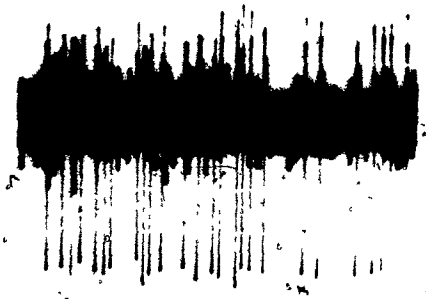
After the experiment the cochlea was fixed by perfusion with 1% osmium tetroxide and further dissected to expose the basilar membrane. The preparation was then photographed to obtain measurement of distances on the basilar membrane relative to ganglion recording locations. The recording site in the ganglion was verified histologically.

Fig. 1 A--D. Patterns of activity of spiral ganglion cells. A Oscilloscope tracings of activity of a single ganglion cell a) spontaneous activity, b) response to 15 kHz 10 dB above threshold, c) response at 15 dB above threshold. B Interspike interval histogram of the spontaneous activity of a sensitive ganglion cell. Bin width 0.1 msec. C Interspike interval histogram of injury discharge in an insensitive ganglion cell. Bin width 1.5 msec. D Typical responses to tone burst stimuli. Peristimulus-time histograms of the response to cf. tone-burst stimuli in two cells. Bin width 2.0 msec. Position of tone burst indicated by bar. Rise-fall time of tone burst was 10 msec. The cell in (a) had a spontaneous activity of 90/sec and is responding to a tone burst 15 dB above threshold. In (b) the cell has a spontaneous activity of 14/sec and is responding at 10 dB above threshold.

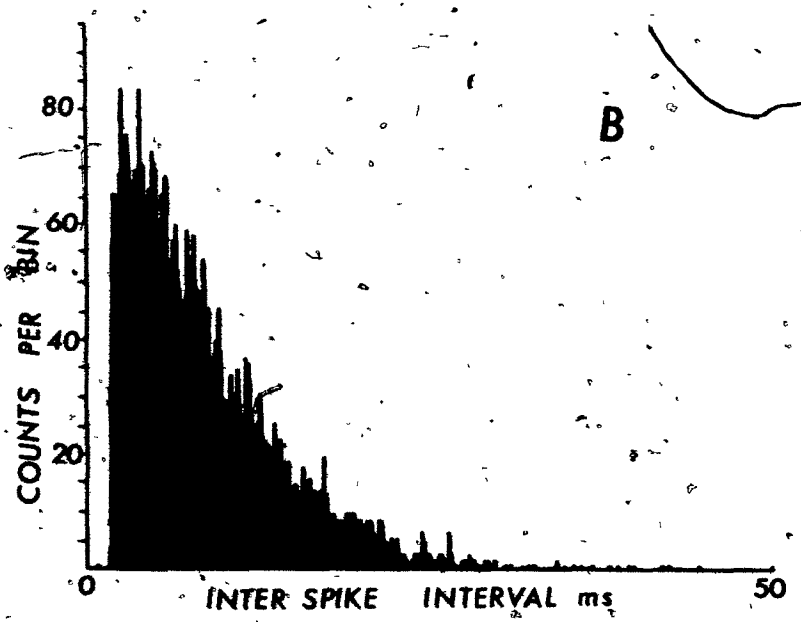
A

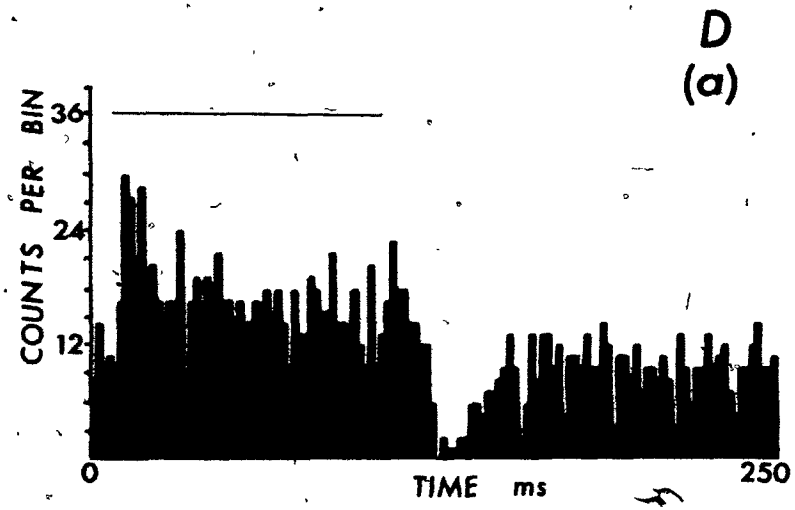
a

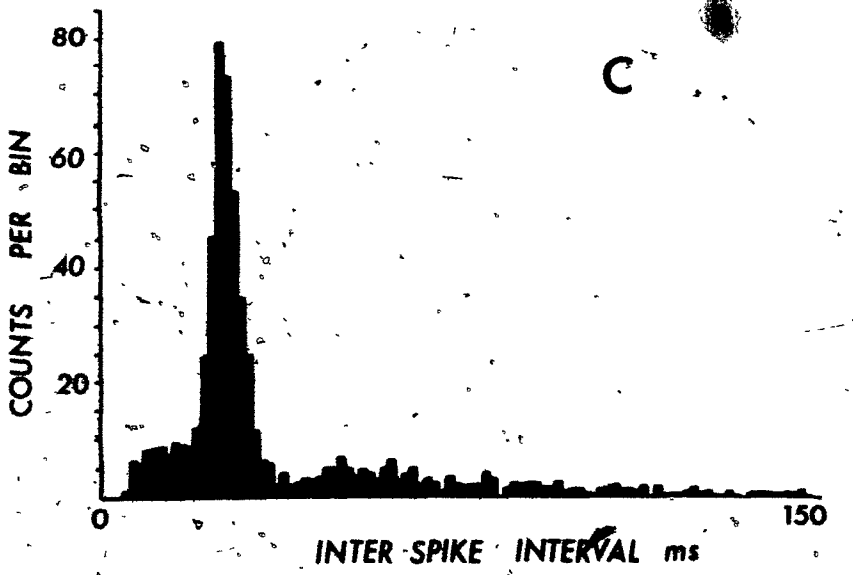
b

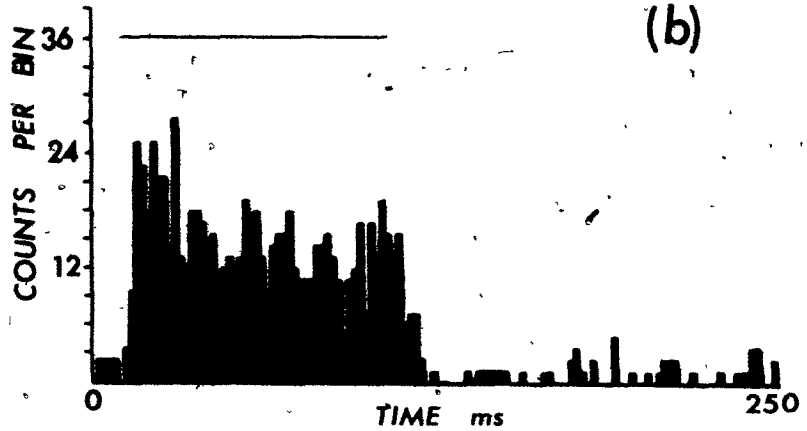


c


B
80
60
40
20
COUNTS PER BIN
0
INTER SPIKE INTERVAL ms
50


D
(a)
36
24
12
COUNTS PER BIN
0
TIME ms
250


C
80
60
40
20
COUNTS PER BIN
0
150
INTER SPIKE INTERVAL ms


(b)
36
24
12
COUNTS PER BIN
0
TIME ms
250

Results

a) Spontaneous Activity and Tone Burst Responses

In any one penetration at a single recording location in the ganglion, a maximum of 5 successive cells was encountered. Histological study showed that the ganglion in the basal turn was between 100 and 200 μ deep with 8-15 cells in the path of a single electrode track in the most favorable locations. The vast majority of cells recorded from showed spontaneous activity, ranging from a few spikes/sec. to as high as 130/sec. Considerable variation in rate of spontaneous activity was found in different cells of a single animal, but in all those animals in good condition, the interspike interval histogram of the spontaneous activity showed a shape such as in Fig. 1B. This pattern of spontaneous activity is similar to that reported for the VIIIth nerve fibres in cat (Kiang, 1965). The spontaneous activity did not arise from uncontrolled room noise, since this pattern of spontaneous activity was still seen

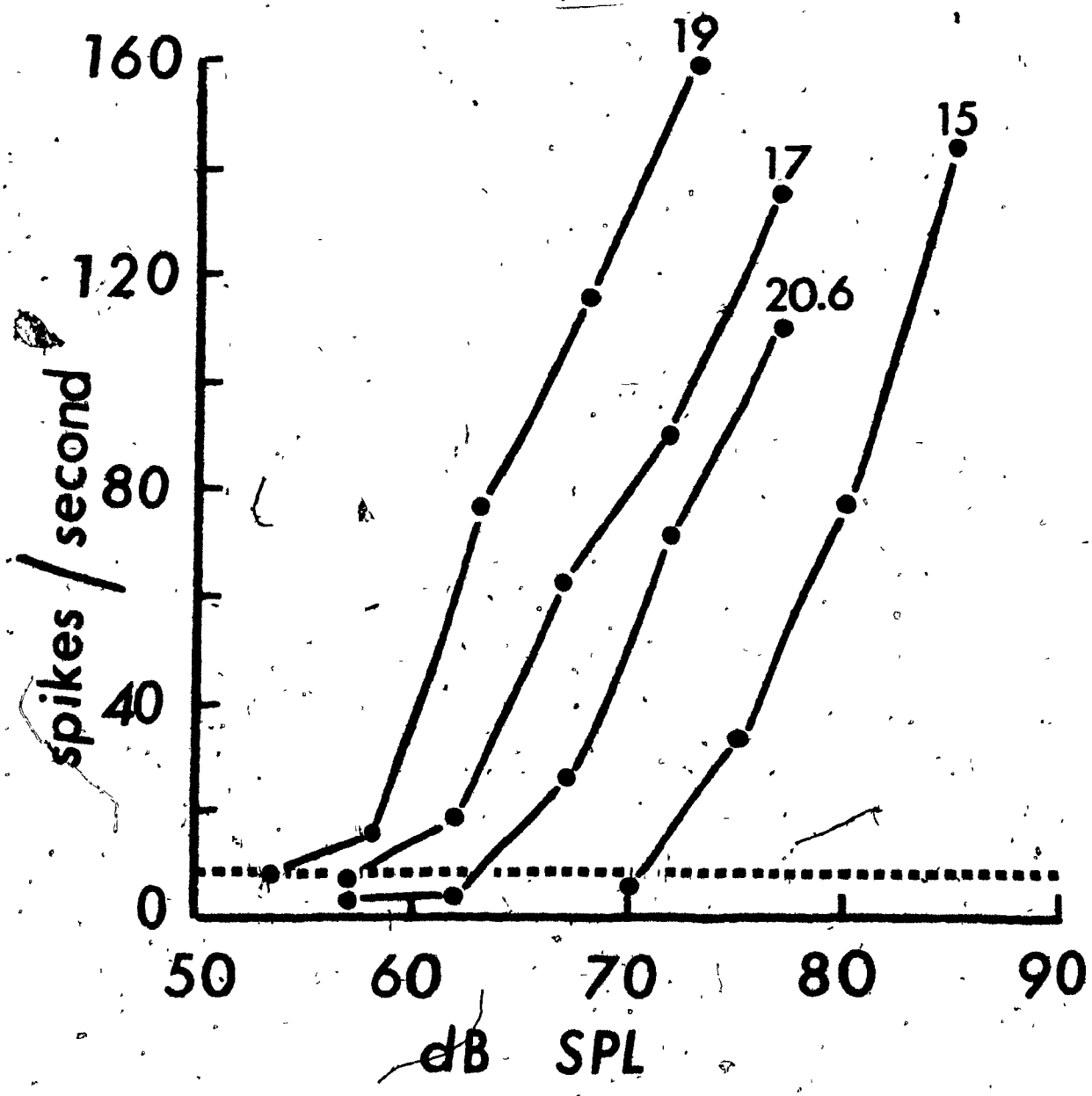
after interruption of the ossicular chain (attenuating the conduction of airborne sounds by about 30 dB). Also, the accumulation of fluid in the bulla during recording from a single cell frequently caused up to 20 dB rise in pure tone thresholds without causing a noticeable reduction in spontaneous firing rate. The maintenance of spontaneous activity depended on the integrity of the Organ of Corti, for destruction of the basilar membrane resulted immediately in an almost complete cessation of spontaneous activity.

Animals were often encountered with unusual patterns of spontaneous activity. The rate of spontaneous firing in these animals was very low, often zero, and the cells were sometimes first detected by rapid injury discharge caused by advance of the electrode. The interval histograms of such injury discharge showed a large symmetric peak (Fig. 1C). In such animals the cells frequently did not respond to the normal level of search stimulus and their thresholds to tone stimuli

were invariably elevated, indicating a pathological condition either of the cochlea or the whole animal. There was no reason to believe in these animals that the dissection had caused any gross damage to the Organ of Corti.

Responses to tone burst stimuli (Fig. 1D) showed a pattern which was uniformly similar to those reported in other species in studies on the cochlear nerve axons (Nomoto et al., 1964; Kiang, 1965). Rate-intensity functions at different frequencies were also computed for many cells. The slopes of these functions were very similar for frequencies both on and off the cf. in the majority of cells studies (Fig. 2). The dynamic range that could be investigated was limited to about 20 dB above threshold due to the presence of a large cochlear microphonic response at higher intensities that made discrimination of individual spikes difficult.

Fig. 2. Rate-intensity functions at different frequencies for a cell with a cf. at 19kHz. The rate was measured as the number of spikes falling within ten 100 msec tone bursts. Functions are shown at 19, 17, 15 and 20.6 kHz. Sound pressure is relative to 2×10^{-4} dynes cm^{-2}.


160
120
80
40
0
spikes / second
19
17
20.6
15
50
60
70
80
90
dB SPL

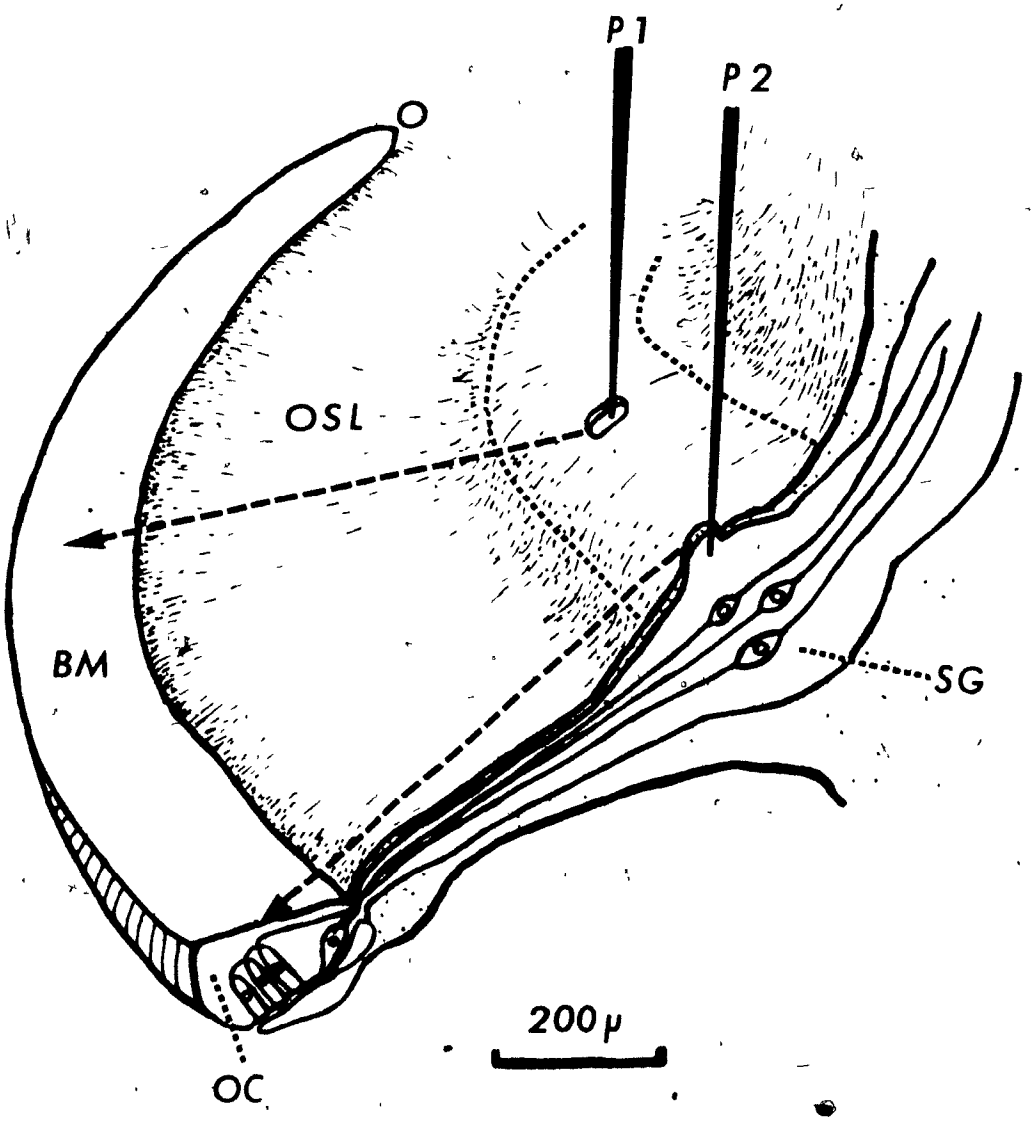
b) Distribution of Characteristic Frequencies in the Ganglion

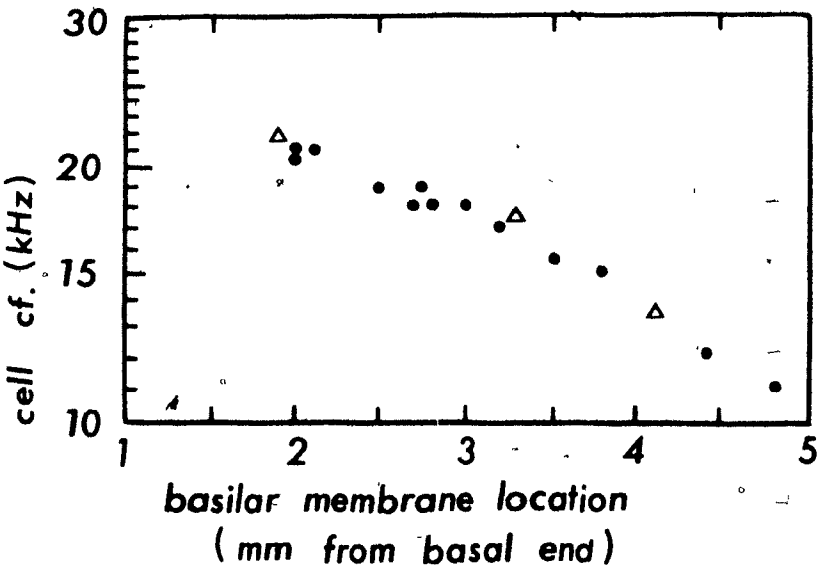
At any given recording location all the cells encountered had the same cf. This cf. varied systematically with the recording location in the ganglion and, in the accessible area in the basal turn of the cochlea, ranged from 11kHz to 21 kHz. In photographs of the osmium-stained cochleas the myelinated peripheral processes of the bipolar cells could be seen to follow an orderly path from the recording site in the ganglion to the basilar membrane (Fig. 3). Distances from the basal end of the basilar membrane to points on the membrane could be measured from such photographs and related to a particular recording site in the ganglion (visible as a puncture in the osseous spiral lamina). In Fig. 4 is shown data from 8 animals relating the cf. of cells in the ganglion to the corresponding distance along the basilar membrane. The distribution of frequency along the basilar membrane

Fig. 3. Photograph of the guinea pig cochlea with bony capsule and stria vascularis removed. The basilar membrane has been stripped from the basal turn.
G Point of entry of electrode into spiral ganglion.
N Myelinated peripheral processes of ganglion cells visible through the osseous spiral lamina. B Inner edge of the basilar membrane. Stained with Osmium tetroxide.


G
N
B

Fig. 4. Relation between characteristic frequency in the ganglion and distance along the basilar membrane. The drawing shows the method of measurement. BM basilar membrane; OC organ of Corti; SG spiral ganglion; OSL osseous spiral lamina; P2 and P1 two different penetration points in the ganglion. Closed circles are experimental measurements from 8 animals, triangles are the location of displacement maxima on the basilar membrane taken from Wilson and Johnstone (1972).


P1
P2
OSL
BM
SG
200 μ
OC

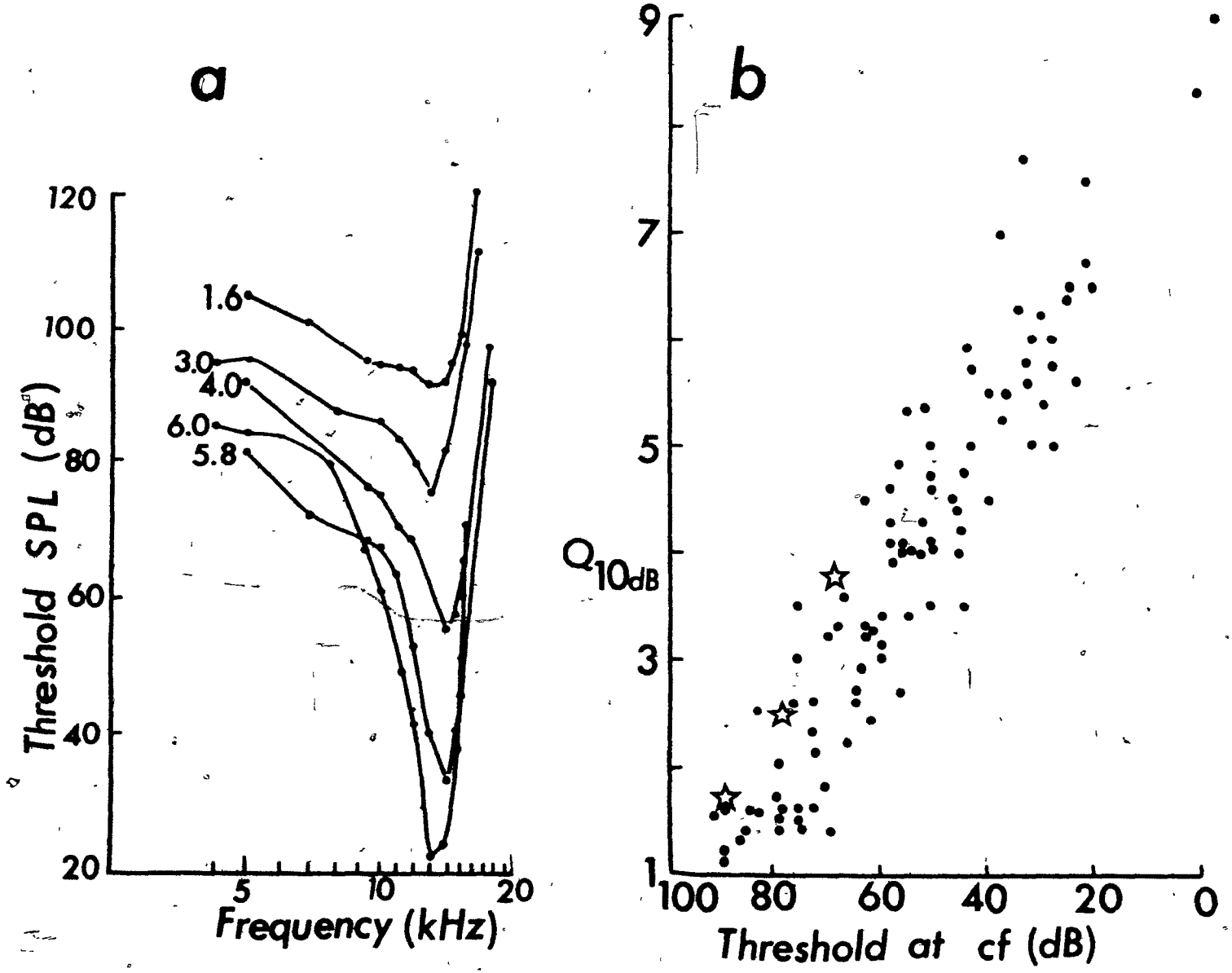

30
20
15
10
cell cf. (kHz)
1
2
3
4
5
basilar membrane location
(mm from basal end)

determined by this method agrees well with available data on the location of displacement maxima on the guinea pig basilar membrane (triangles in Fig. 4 from Wilson and Johnstone, 1972).

c) Tuning Curves

It was found that both the sensitivity and the sharpness of the tuning curves of spiral ganglion cells varied considerably between animals. In a single animal however, there was little variation in these parameters. The mean range of threshold in a single animal at each cf. was only 6.7 dB (S.D. = 3.9) and Q_{10dB} was 0.9 (S.D. = 0.6). Some typical tuning curves from different animals are shown in Fig. 5a. It can be seen that those units with high thresholds have broad tuning curves while the tuning curves are progressively sharper at lower threshold sound intensities. Fig. 5b shows the pooled data from 94 cells from different animals with cfs. ranging from 12-19 kHz. There is

Fig.5 a and b. Variations in Q_{10dB} and thresholds. a) Representative tuning curves each from a different animal showing the gradation in sensitivity and sharpness of the tuning curves. b) Pooled data from 94 cells showing the close relationship between Q_{10dB} and the threshold at the cf. The range of cfs. included is 12-19 kHz; closed circles are the neural data from the present study. Stars are the Q_{10dB} values from Rhode (1971) of the basilar membrane vibration at the 8 kHz point in squirrel monkey.

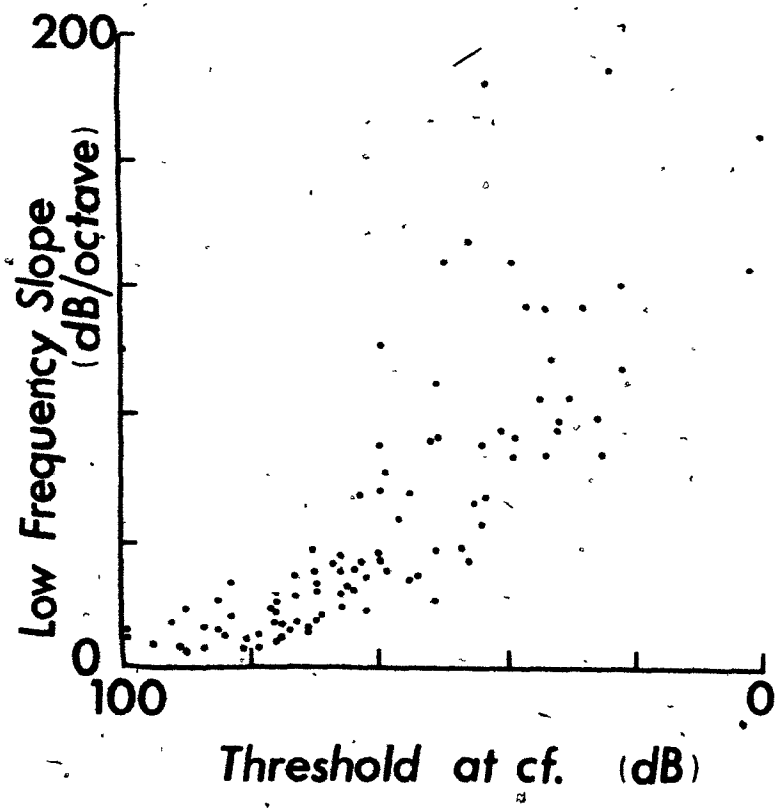

a
120
100
80
60
40
20
Threshold SPL (dB)
1.6
3.0
4.0
6.0
5.8
5
10
20
Frequency (kHz)
b
9
7
5
3
1
Q_{10dB}
100
80
60
40
20
0
Threshold at cf (dB)

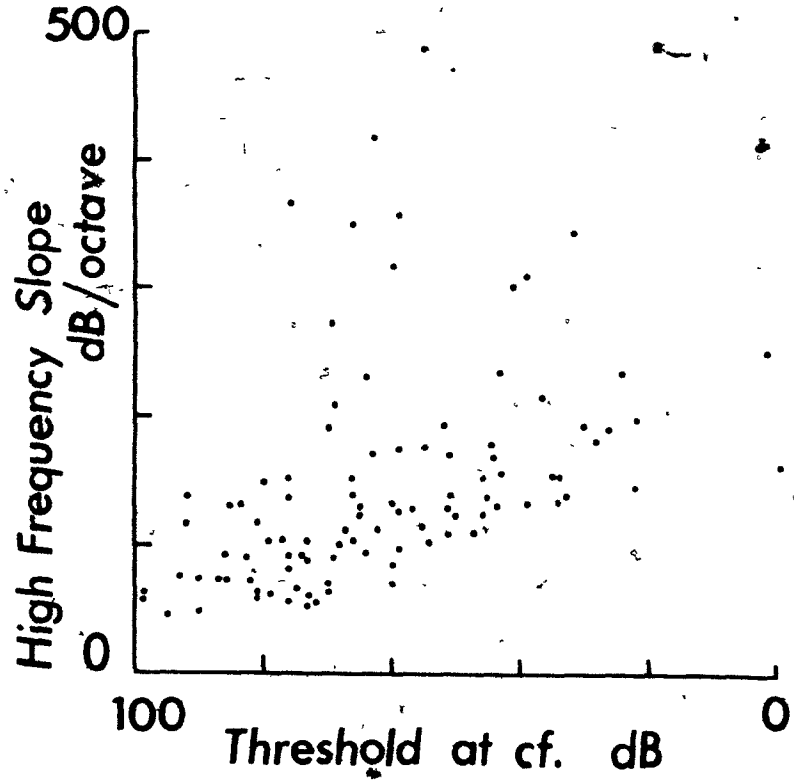
a consistent linear relationship between the Q_{10dB} and the sensitivity at the cf. throughout the intensity range investigated. Those neurons with especially high thresholds at the cf. (greater than about 70 dB SPL) showed the unusual patterns of spontaneous activity referred to above.

The low and high frequency slopes of the tuning curves were measured for the same 94 cells. Wherever possible these slopes were computed between 3 and 23 dB above the cf. threshold (Evans, 1972). In many of the higher threshold cells this was not possible in the case of the low frequency slope, owing to the lack of a steeply rising portion to the curve near the cf. The low frequency slopes for these cells were therefore determined on the straightest portion of the curve available near to the cf. The low and high frequency slopes are plotted against threshold at the cf. in Fig. 6.

On investigating the basis of this variation in

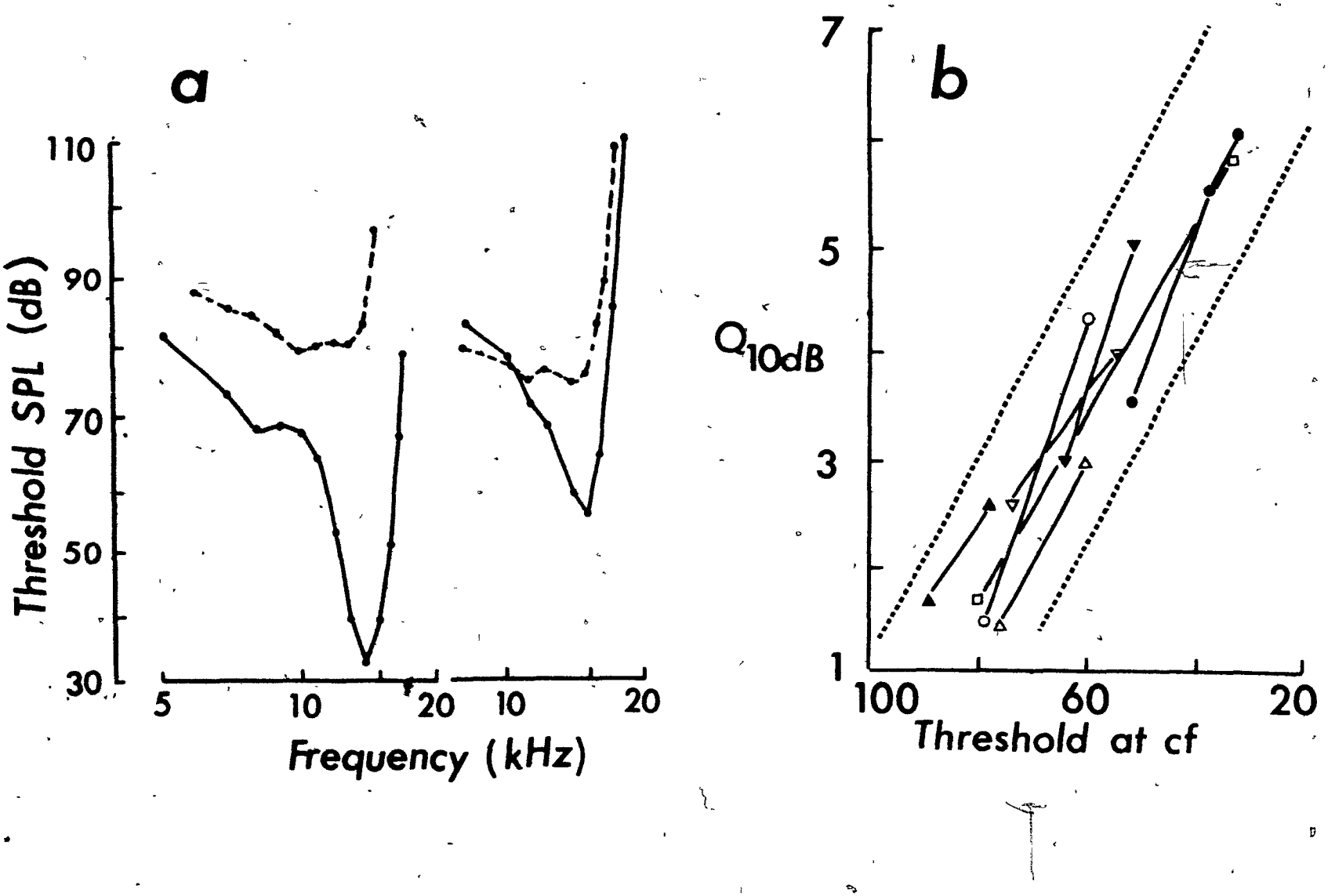
Fig. 6. Low and high frequency slopes of the tuning curves of the 94 units of Fig.5b . The method of measurement of slopes is discussed in the text. The slopes are presented plotted against threshold sound intensity at the cf.


200
0
Low Frequency Slope
(dB/octave)
100
0
Threshold at cf. (dB)


500
0
High Frequency Slope
dB/octave
100
0
Threshold at cf. dB

thresholds and tuning curves between animals, it was found that tuning curves could be deliberately altered by reducing the rate of artificial ventilation. A reduction in respiratory rate from 60-30 strokes/min caused a rise in the threshold at the cf. and a fall in the spontaneous rate (by up to 80%) within 2 min. The rate of ventilation could be adjusted to give an elevated but steady threshold at the cf. and a tuning curve for the cell could again be determined. The changes in the tuning curve produced are illustrated for 2 cells in Fig. 7a. In Fig. 7b changes in Q_{10dB} and threshold induced by the above procedure are shown for 7 cells. For all of the cells except one (squares in Fig. 7b) the changes in the tuning curve and the threshold were reversible within one minute of restoration of the normal rate of ventilation. In the one cell referred to, the animal died as a result of the respiratory impairment. In another cell (closed circles in Fig. 7b), the threshold recovered to a level more sensitive than the

Fig. 7a and b. The effect of deliberate slowing of the ventilation on tuning curves and sensitivity. a) Tuning curves for 2 cells in different animals. Solid lines: before slowing ventilation, broken lines: after slowing respiration and with a steady elevated threshold at the cf. b) The effect in 7 cells (including the 2 shown in (a) of slowing ventilation. Lines join the Q_{10dB} and cf. threshold points before and after respiratory impairment. All cells except one (open squares) showed a fully reversible change, in tuning curve and sensitivity. One cell (closed circles) showed an increase in both sharpness and sensitivity when the respiration rate was returned to normal. Dotted lines show the range of the pooled data of Fig. 5b.


a
110
90
70
50
30
Threshold SPL (dB)
5
10
20
10
20
Frequency (kHz)
b
7
5
3
1
Q_{10dB}
100
60
20
Threshold at cf

initial threshold when the respiratory rate was returned to normal. The tuning curve which accompanied this increase in sensitivity was sharper (a higher measured Q_{10dB}) than the initial curve.

The alterations in Q_{10dB} and threshold produced by this respiratory impairment are consistent with the relationship between Q_{10dB} and threshold shown for the pooled data of Fig. 5b in which the respiration was not deliberately interfered with. It therefore seems reasonable to suggest that varying degrees of physiological malfunction (possibly anoxia) can explain the wide variation in Q_{10dB} and threshold found in spiral ganglion cells between different animals.

Discussion

Data presented above show that the orderly distribution of characteristic frequencies in the spiral ganglion is consistent with the known location of displacement maxima on the basilar membrane (Fig. 4). This

finding indicates that it is at the peak of the travelling wave envelope that maximum stimulation of the hair cells occurs. A scheme of the type suggested by Tonndorf (1962) derived from cochlea models, in which radial shearing forces spatially separated from the travelling wave peak constitute the effective stimulus to the hair cells does not therefore seem to be the case in the intact guinea pig cochlea. If the excitation of the hair cells is brought about by radial shear, as seems likely in view of the directional polarization of stereocilia, this radial shear must, in the basal turn of the guinea pig cochlea, be maximal at or very close to the travelling wave envelope.

The findings on the variability of neural tuning are relevant to the problem of whether the neural tuning curves can be accounted for purely on the basis of the mechanical tuning of the basilar membrane, or whether a second filter of unknown nature has to be postulated between the basilar membrane tuning and the

generation of spikes in the afferent fibres. The occurence of high threshold, broadly tuning cochlear nerve fibres has been occasionally noted (Kiang et al., 1970; Evans, 1972) and Evans (1972) has observed a dependence of Q_{10dB} on sensitivity. However, it has not been previously demonstrated that there is a close relationship between Q_{10dB} and threshold at the cf., throughout the entire measured intensity range (Fig. 5b). The use of the spiral ganglion preparation, which allows a detailed study of a restricted range of characteristic frequencies is probably the explanation for the above relationship being clearly observed in the data reported here. The data of Figs. 5, 7 shows a steady degradation of the ~~frequency~~ selectivity of cochlear ganglion cells over a large range of sound intensity. Even cells which have initially quite broad tuning curves can be further altered by deliberately impairing the ventilation. The experiments on the manipulation of the respiratory rate show that the

frequency selectivity of single cells is metabolically sensitive in a reversible fashion, and that tuning curve changes are closely related to changes in sensitivity.

The slopes of tuning curves at intensities above about 70dB are comparable with those reported for mechanical tuning in this region of the guinea pig cochlea. Johnstone et al. (1970) report low and high frequency slopes on the basilar membrane of 12 and 100dB /octave respectively. In a later report (Johnstone and Yates, 1973) the slopes were reported as 15 and 300db/ octave. When corrected to constant sound pressure level at the eardrum, these low and high frequency slopes do not differ significantly from the slopes of neural tuning curves with thresholds at the cf. greater than about 70 dB. The data on the basilar membrane tuning cited above were obtained at sound intensities between 70 and 100 dB.

The dependence of sharpness of neural tuning

curves on sensitivity is not inconsistent with the mechanical non-linearity reported by Rhode (1971) in the 7-10 kHz region of the squirrel monkey basilar membrane. This non-linearity is present near the region of maximum displacement and results in a sharpening of the basilar membrane tuning as lower sound intensities are used. In Fig. 5b the mechanical sharpness quotients obtained by Rhode at three sound intensities are included for comparison with the neural data. If this non-linearity persists to even lower intensities, the mechanical tuning could fully account for the neural tuning curves. The dependence of Q_{10dB} on threshold demonstrated here may thus be explained by a direct or indirect effect of metabolic impairment on the sensitivity of the afferent neurones. For neurones with higher thresholds the broader tuning curves may simply reflect the altered mechanical tuning at these higher intensities. It has been reported that a non-linearity of the type found by

Rhode could account for many other peripheral phenomena, such as two-tone inhibition and the generation of combination tones (Johnstone and Yates, 1973).

Though the above explanation is an attractive one, it has not been possible to demonstrate this type of non-linearity in the basal turn of the guinea pig cochlea. Wilson and Johnstone (1972) report linearity of basilar membrane vibration down to 40 dB SPL. Johnstone and Yates (1973) reported that the only substantial non-linearity they could detect was on the high frequency slope of the mechanical tuning curve. It may be that the non-linearity is indeed present here but is not as apparent as in the region in which Rhode's measurements were made. This receives some support from the observation that in the guinea pig and cat, neural tuning curves of cochlear nerve fibres are sharpest between 8-10 kHz and show a definite decrease in Q_{10dB} above this frequency (Evans, 1972; Kiang, 1965). This 8-10 kHz region is precisely where Rhode obtained his measurements

in the squirrel monkey. Rhode also reported that the non-linearity he observed was fragile and could be absent when structures close to the basilar membrane were damaged. The exposure of the scala tympani required for studies on basilar membrane vibration in the guinea pig is very extensive and could possibly disrupt some delicate structural component responsible for a mechanical non-linearity. However, extensive opening of the scala tympani and drainage of perilymph has been reported not to alter the tuning curves of cochlear nerve fibres (Evans, 1970).

If indeed a mechanical non-linearity of the type found by Rhode is peculiar to the region of the squirrel monkey cochlea which he studied, then another mechanism is needed to explain the sharpness of neural tuning curves, as well as their variability reported here. Some authors (Wilson and Johnstone, 1972; Smoorenburg, 1972) have postulated a second filter in addition to the basilar membrane mechanical tuning. Such a filter

would serve to sharpen the frequency selectivity of primary cochlear units. The results presented here show that any such filter, if it exists, must be metabolically sensitive and must be intimately related to the sensitivity of the cochlear neurones. The nature of the hypothetical second filter is still a matter for discussion.

Summary. 1. Recordings of extracellular activity were obtained from single cells in the spiral ganglion of the basal turn of the guinea pig cochlea.

2. The spatial distribution of characteristic frequencies of cells in the ganglion was consistent with published data on the location of displacement maxima on the basilar membrane.

3. Large variations in the sharpness of single cell tuning curves were seen between animals. These variations were closely linked to sensitivity differences.

4. The tuning curves of single cells could be made less sharp by slowing the rate of artificial ventilation. These tuning curve changes were reversible and intimately associated with alterations in sensitivity and spontaneous activity.

5. The data point either to the presence of a mechanical non-linearity, or a physiologically vulnerable second filter, as the explanation for the sharpness of neural tuning curves in cochlear nerve fibres.

References

DeBoer, E.: Initiation of nerve impulses in the inner ear. Proc. kon. ned. Akad. Wet 72, 129-151 (1969)

Evans, E.F.: Narrow tuning of the responses of the cochlear nerve fibres emanating from the exposed basilar membrane. J. Physiol. (Lond.) 208, 75-76P (1970)

Evans, E.F.: The frequency response and other properties of single fibres in the guinea pig cochlear nerve. J. Physiol. (Lond.) 226, 263-287 (1972)

Frank, K., Becker, M.C.: Microelectrodes for recording and stimulation. In: Physical techniques in biological research, vol. 5 (Nastuk, W.L., ed.), New York: Acad. Press 1964

Johnstone, B.M., Taylor, K.J., Boyle, A.J.: Mechanics of the guinea pig cochlea. J. acoust. Soc. Amer. 47, 504-509 (1970)

Johnstone, B.M., Yates, G.: Basilar membrane tuning. Proc. 85th Meeting Acoust. Soc. Amer. (1973)

Kiang, N.Y.S.: Discharge patterns of single fibres in the cat's auditory nerve. Cambridge (Mass.): M.I.T. Press 1965

Kiang, N.Y.S., Moxon, E.C., Levine, R.A.: Auditory nerve activity in cats with normal and abnormal cochlea. In: Sensorineural Hearing Loss, p. 241-273 (Wolstenholme, G., Knight, K., Eds.) London: Churchill 1970

Moller, A.R.: Studies of the damped oscillatory response of the auditory frequency analyzer. Acta physiol. scand. 78, 299-314 (1970)

Nomoto, M., Suga, N., Katsuki, Y.: Discharge patterns and inhibition of primary auditory nerve fibres in the monkey. J. Neurophysiol. 27, 768-787 (1964)

Rhode, W.S.: Observations of the vibration of the basilar membrane of squirrel monkeys using the Mössbauer technique. J. acoust. Soc. Amer. 49, 1218-1231 (1971)

Smoorenburg, G.F.: Combination tones and their origin. J. acoust. Soc. Amer. 52, 615-632 (1972)

Tonndorf, J.: Time/frequency analysis along the partition of cochlear models. J. acoust. Soc. Amer. 34, 1337-1350 (1962)

Wilson, J.P., Johnstone, J.R.: Capacitive probe measures of basilar membrane vibration. Symp. on Hearing Theory, LPO Eindhoven (1972)

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The previous paper was concerned primarily with the lability of frequency selectivity in the spiral ganglion cells and with a comparison of the neural and basilar membrane tuning curves. In the discussion, the question was raised as to the validity of the basilar membrane data of some researchers. It was suggested that wide opening of the scala tympani and drainage of perilymph might affect the basilar membrane tuning. Though the experiment of Evans (1970) implies that this is not true, it was thought advisable to repeat this experiment with the spiral ganglion preparation, since this allows more careful monitoring of the fluid level in the scala tympani. The following paper describes the results of this investigation.

PAPER II

Cochlear Neurons: Frequency Selectivity Altered by Perilymph Removal

Donald Robertson

It has long been held that there is a serious discrepancy between the filtering properties of the basilar membrane and the frequency selectivity of single primary auditory neurons (1-3). Mechanical measurements, usually obtained at high sound pressures, indicate a broad tuning of the basilar membrane, whereas the tuning curves of primary neurons (obtained at threshold sound pressures), are much sharper. Rhode (4) and Rhode and Robles (5) have reported a nonlinearity in basilar membrane vibration which consists of a flattening of the input-output curves in the region of the peak of the membrane tuning curve. This nonlinearity results in a sharpening of the mechanical tuning curve at low sound pressures. Such a nonlinear behavior could be used to bring the neural and mechanical data into close agreement and thus eliminate discrepancies between basilar membrane and primary neural tuning (6, 7). There is, however, some conflict between these authors' findings and those of other workers. In par-

ticular, the failure to find a nonlinearity in the guinea pig cochlea (3, 8, 9) has lead some authors to state that the neural tuning curves cannot be explained solely on the basis of basilar membrane mechanics. Additional filtering processes have therefore been postulated (2, 3, 10). How much of this disagreement in mechanical measurements could be caused by species differences or by variations in technique is not clear. The results that are reported here show that removal of perilymph from the scala tympani could constitute a source of error in several of the mechanical measurements.

The technique of recording from the bipolar cell bodies of the spiral ganglion has been described elsewhere (7). This procedure permits stable recording from single cells in a chosen and restricted frequency range in the basal cochlear spiral, while continuously monitoring the cochlea during experimental manipulations. The frequency selectivity of the ganglion cells

was estimated by measuring the tuning curves (threshold versus frequency response curves) (7, 11).

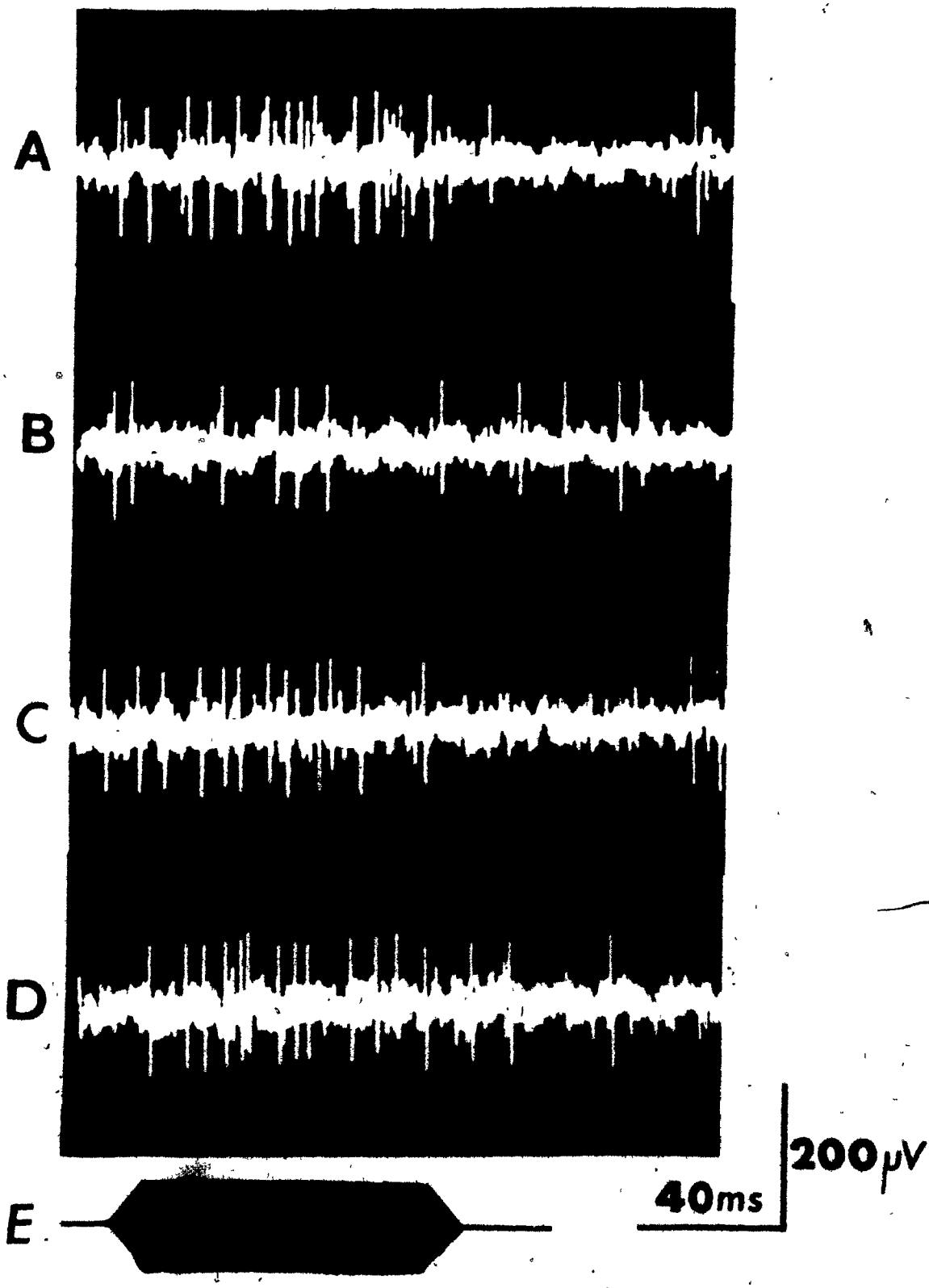
As previously reported, sharp and sensitive tuning curves could be obtained from the ganglion cells after limited opening of the scala tympani (7). These curves are similar to those obtained from eighth nerve axons (1, 2, 11). From this, it is inferred that opening of the scala tympani without fluid removal does not substantially alter the neural tuning curves. On this point the results are in agreement with the findings of Evans (12), who recorded from the eighth nerve axons (the central processes of the spiral ganglion cells). However, in contrast to Evans' results, the removal of perilymph bathing the basilar membrane caused drastic alterations in the sensitivity and frequency selectivity of single ganglion cells.

Preliminary experiments were performed by measuring tuning curves at a chosen location in the ganglion in the basal coil. After the recording microelectrode was withdrawn, a small cotton wick was used to gently

suck out perilymph from the scala tympani, so that no large fluid bridges existed between the walls of the scala tympani and the basilar membrane. Care was taken not to touch the basilar membrane. The microelectrode was then reinserted at the same location and tuning curves of single cells were again measured. In all experiments (four animals and 15 cells), tuning curves obtained after fluid removal were about 20 dB less sensitive and very much broader than those obtained when the scala tympani was full of perilymph. When the wick was removed and the perilymph allowed to refill the scala tympani, further cells encountered showed a partial recovery of both the sensitivity and frequency selectivity.

More conclusive results were obtained by continuously recording the response of the same cell before, during, and after perilymph drainage. When a single ganglion cell had been isolated and its tuning curve obtained, perilymph was sucked out of the scala tympani

Fig. 1. Oscilloscope tracings of the response of a single ganglion cell in the guinea pig cochlea to a tone burst at the best frequency. The intensity of the tone burst was 10 db above threshold for the cell with the scala tympani full of perilymph. Responses were recorded (A) before draining perilymph, (B) with perilymph removed, (C) with perilymph only partially replaced, but covering basilar membrane, and (D) with scala tympani refilled. (E) Approximate position of tone burst.

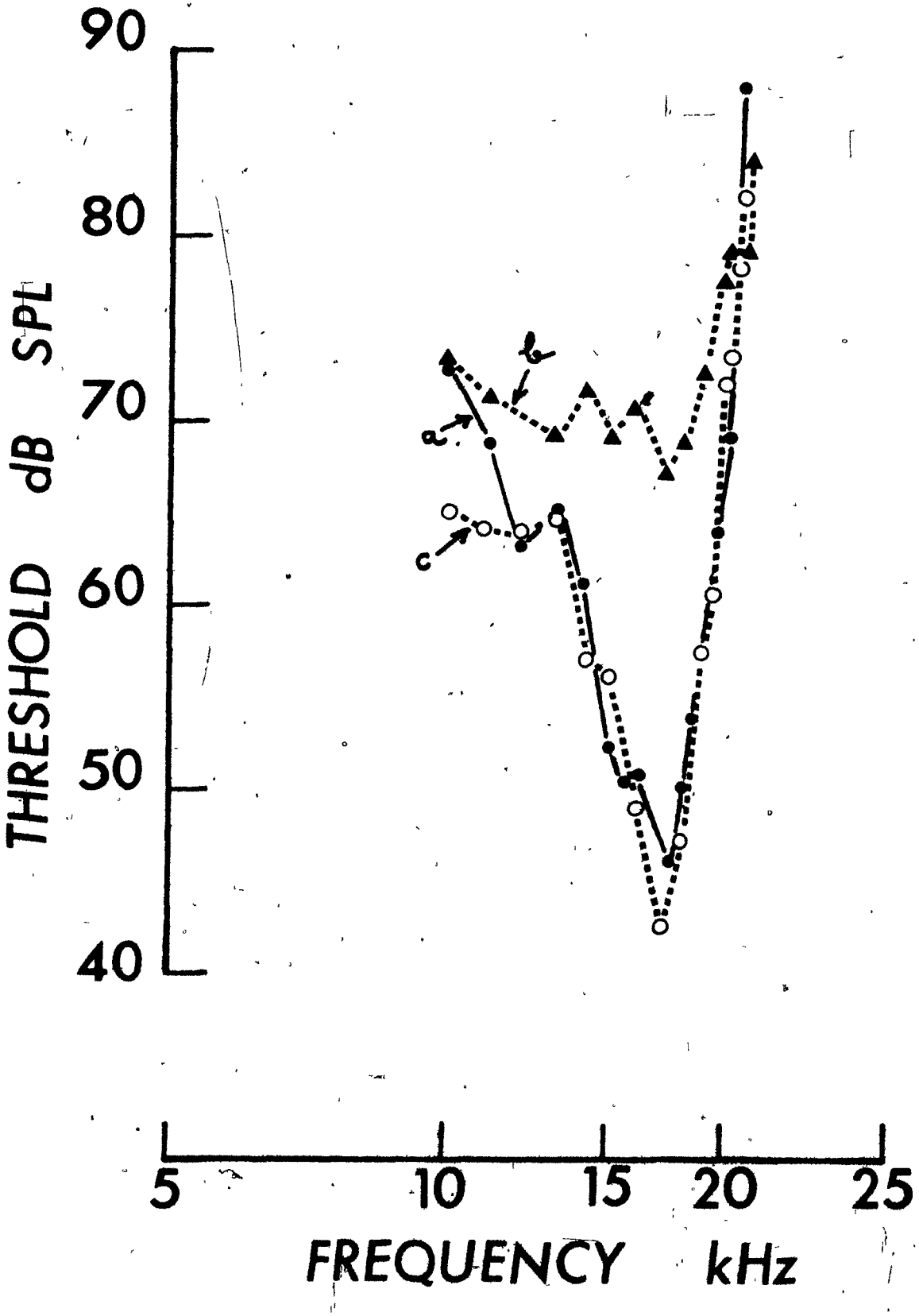

A
B
C
D
E
200μV
40ms

while the response of the cell was continuously monitored. As soon as the fluid bridge between the wall of the scala tympani and the basilar membrane was removed, the cell's sensitivity dropped by about 20 dB. In five cells in three different animals it was possible to maintain contact with the cell throughout this procedure. The sensitivities at the best frequency fell by 18, 12, 21, 16, and 17 dB. When the wick was removed and perilymph allowed to flood back into the scala, the response of the cells recovered (Fig. 1). The tuning curves of these five cells were drastically altered by perilymph removal. This change, which occurred together with the sensitivity change, consisted of a reduction in high-frequency slope and a dramatic fall in low-frequency slope so that the sharpness of the tuning curve was reduced (Fig. 2). The replacement of perilymph, as well as causing a return of sensitivity, resulted in a substantial recovery of the frequency selectivity. In one case the tuning curve recovered completely (Fig. 2), but

in the other cells the recovery was not perfect. In all cases the recovered tuning curves were much sharper than those when the perilymph was removed and the sensitivity returned to within 5 dB of the original. The long low-frequency tail of the curves was not plotted below 10 kHz so as to minimize the time required for the estimations.

The effects described above are probably not due to a disruption of neural processes, since histograms of both the mean rate and the interspike interval of the spontaneous activity were not altered by perilymph drainage. The rapid reversibility also argues against a deterioration of neural mechanisms. The effects appeared to be immediate when the final meniscus between the basilar membrane and the walls of the scala tympani was removed or replaced. Masking effects caused by wick placement or fluid movements are certainly not important, since the spontaneous activity, thresholds, and tuning curves were not affected by simply placing the cotton

Fig. 2. Tuning curves of a single cell in the basal coil of the cochlea: (a) initial, (b) immediately after perilymph removal, (c) after refilling the scala tympani. The cell is not the same as the one in Fig. 1. The time between obtaining each of the curves was approximately 3 minutes.


90
80
70
60
50
40
THRESHOLD dB SPL
a
b
c
5
10
15
20
25
FREQUENCY kHz

wick in the scala tympani. In Fig. 1C, the sensitivity had returned even though perilymph, now covering the basilar membrane, was still rising in the scala tympani.

These findings on the effect of fluid removal conflict with the results of Evans (12) who recorded from the eighth nerve axons in the internal auditory meatus. Evans' experiments did not allow recording from the same axon during the perilymph removal, nor did they permit such strict control of the cochlear location investigated. Direct visual observation of the cochlea was also probably difficult during neural recording in Evans' experiments. It is therefore possible that Evans, while removing the bulk of the perilymph from the scala tympani, did not remove that fluid lying directly over the basilar membrane.

One interpretation of the results reported here is that removal of fluid broadens the mechanical tuning of the basilar membrane and that this alteration is faithfully reflected in the neural tuning curves. This

notion could resolve some of the contradictions in previous mechanical measurements. Rhode (4) found considerably sharper basilar membrane tuning in the squirrel monkey than that reported by Wilson and Johnstone (3) for the guinea pig. Rhode used a Mössbauer technique and took pains to leave perilymph in the scala tympani. Wilson and Johnstone used a capacitance probe and were obliged to completely remove perilymph to obtain their measurements. A further disagreement between these authors concerns the question of nonlinear basilar membrane motion. Rhode reported a nonlinearity in the region of the peak of basilar membrane displacement, which results in a sharpening of the membrane tuning at lower intensities, while Wilson and Johnstone found linearity down to 40 dB. Although the possibility of a species difference cannot be ruled out, the present results suggest that Rhode's results reflect more reliably the true basilar membrane response. Results of laser measurements in the guinea pig show

linearity, but these have only been reported in post-mortem cochleas (13). Rhode (14) has shown that basilar membrane nonlinearity disappears after death. The results of Johnstone and colleagues are difficult to assess in this light, as these authors did not pay special attention to the level of perilymph. They did, however, state that drying the basilar membrane reversibly broadened its tuning (8) and they have recently reported the presence of a small nonlinearity in the guinea pig (15). It is thus possible, if these results are considered together, that the sharpness of neural tuning curves may be accounted for by the pattern of vibration of the basilar membrane in the living, fluid-filled cochlea.

A second interpretation is that the broadening of neural tuning curves reflects, not an alteration in basilar membrane vibration, but a disruption of the functioning of a second filter of unknown nature, whose existence is considered necessary by some authors (2,

3, 10). The disruption could involve a structural change, perhaps a reversible displacement of the tectorial membrane (16), or an alteration in the flow of current in some important extracellular pathway. The lack of effect of fluid removal on spontaneous activities, however, implies that standing current levels through the hair cells are not altered, since it is known that polarization of the cochlear partition strongly affects rates of spontaneous activity in primary fibers (17). The results reported here do not make it possible to choose between these various hypotheses. However, by revealing a major source of error in some previous mechanical measurements, they again pose the question whether a second filter is necessary in addition to the basilar membrane to account for the sharpness of primary auditory frequency selectivity.

Abstract. Removal of perilymph from the scala tympani of the guinea pig cochlea reversibly broadened the tuning curves of single spiral ganglion cells emanating from the basilar membrane. Thus, fluid continuity along the membrane is essential for normal cochlear function, in particular for sharp neural tuning curves. These data reveal a possible source of error in some estimates of basilar membrane motion and suggest a reappraisal of current concepts of the mechanism of sharp tuning in primary auditory nerve fibres.

National Research Council and a grant from the Banting Research Foundation.

References and Notes

1. E.F. Evans, J. Physiol. (Lond.) 208, 74P (1970).
2. _____, ibid. 226, 263 (1972).
3. J.P. Wilson and J.R. Johnstone, in Symposium on Hearing (Eindhoven, Netherlands 1972), p. 172.
4. W.S. Rhode, J. Acoust. Soc. Am., 49, 1218 (1972).
5. _____ and L. Robles, ibid. 54, 268 (1973).
6. A.R. Moller, Q. Rev. Biophys. 5, 59 (1972); D.O. Kim, C.E. Molnar, R.R. Pfeiffer, J. Acoust. Soc. Am. 54, 1517 (1973).
7. D. Robertson and G.A. Manley, J. Comp. Physiol 91, 363 (1974).
8. B.M. Johnstone and K. Taylor, in Frequency Analysis and Periodicity Detection in Hearing, R. Plomp and G.F. Smoorenburg, Eds. (Sijthoff, Leiden, 1970), p. 84.
9. B.M. Johnstone, K.J. Taylor, A.J. Boyle, J. Acoust. Soc. Am. 47, 504 (1970).

10. E.F. Evans and J.P. Wilson, in Basic Mechanisms in Hearing, A.R. Moller, Ed. (Academic Press; New York, 1973), p. 519.

11. N.Y.S. Kiang, Discharge Patterns of Single Fibers in the Cat's Auditory Nerve (MIT Press, Cambridge, Mass., 1965).

12. E.F. Evans, J. Physiol. (Lond.) 208, 75P (1970).

13. L.U.E. Kohllofel, Acustica 27, 66 (1972).

14. W.S. Rhode, in Basic Mechanisms in Hearing, A.R. Moller, Ed. (Academic Press, New York, 1973), p. 49.

15. B.M. Johnstone and G. Yates, J. Acoust. Soc. Am. 54, 268 (1973).

16. C.R. Steele, in Basic Mechanisms in Hearing, A.R. Moller, Ed. (Academic Press, New York, 1973), p. 69.

17. T. Konishi, D.C. Teas, J.S. Wernick, J. Acoust. Soc. Am. 47, 1519 (1970).

18. I thank G. Manley for support and advice during this work, and L. Pawson for technical assistance. Supported by grants A6368 and E2582 from the Canadian

It was shown in the preceding paper, that the report by Evans (1970) on the effects of perilymph drainage on neural tuning curves was inaccurate. Drainage of perilymph does in fact produce large changes in the tuning curves of single spiral ganglion cells, so that the drained curves resemble the mechanical data on basilar membrane vibration in the guinea pig. This finding has many implications, which have been mentioned in the paper. Not the least of these is the doubt cast on the validity of Wilson and Johnstone's (1972) basilar membrane measurements. Since these authors report linearity of basilar membrane vibration in the drained cochlea, it might be interesting to see if nonlinear behaviour, as well as tuning, is altered by fluid drainage. The most easily observed nonlinear phenomenon in the primary auditory neurones is two-tone inhibition. The effects of fluid removal and other variables on this aspect of the behaviour of spiral ganglion cells is now examined.

PAPER III

Changes in Inhibition and Response Curves of Primary Auditory Neurones in the Guinea Pig Spiral Ganglion.

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Montreal, Que. CANADA

Running Title: Inhibition in cochlear ganglion cells.

Introduction

The only inhibitory phenomenon that does not employ efferent activity which has so far been found in the mammalian primary auditory pathway, is the so-called two-tone inhibition (Nomoto et al., 1964; Arthur et al., 1971; Kiang, 1965; Sachs and Kiang, 1968; Sachs, 1969). In this phenomenon, presentation of a tone in the correct frequency and intensity range can reduce the firing of a single neurone to a different tone, usually fixed at the best, or characteristic frequency of the unit (the cf.). Little is known of either the mechanism or possible functional significance of this inhibition. Some of the arguments for and against mechanical, neural and electrotonic mechanisms have been discussed by Arthur et al. (1971). Various models are also published in the literature (Pfeiffer, 1970; Kim et al., 1973; Furman and Frishkopf, 1964) but do not offer a complete explanation of the effect. It is also not known whether this phenomenon may be analagous to lateral inhibition in other

sensory systems (Ratliff, 1961.) by perhaps sharpening the excitatory response area. Though neural interconnections between cochlear afferents seem to be ruled out (Evans and Wilson, 1973), an electrotonic mechanism might still be able to operate. In any case, it is not at all clear how an inhibitory phenomenon which involves the simultaneous presentation of two tones, relates to the single-tone threshold-frequency response curve (tuning curve).

It has recently been shown that the complete removal of perilymph from the scala tympani of the guinea pig cochlea reversibly alters the tuning curves of single spiral ganglion cells (Robertson, 1974). This alteration consists of a loss in sensitivity of about 20dB and a dramatic broadening of the tuning curve. In the present paper, the effect of this treatment, and of gross structural damage, on two-tone inhibition in these cells, is reported.

Methods

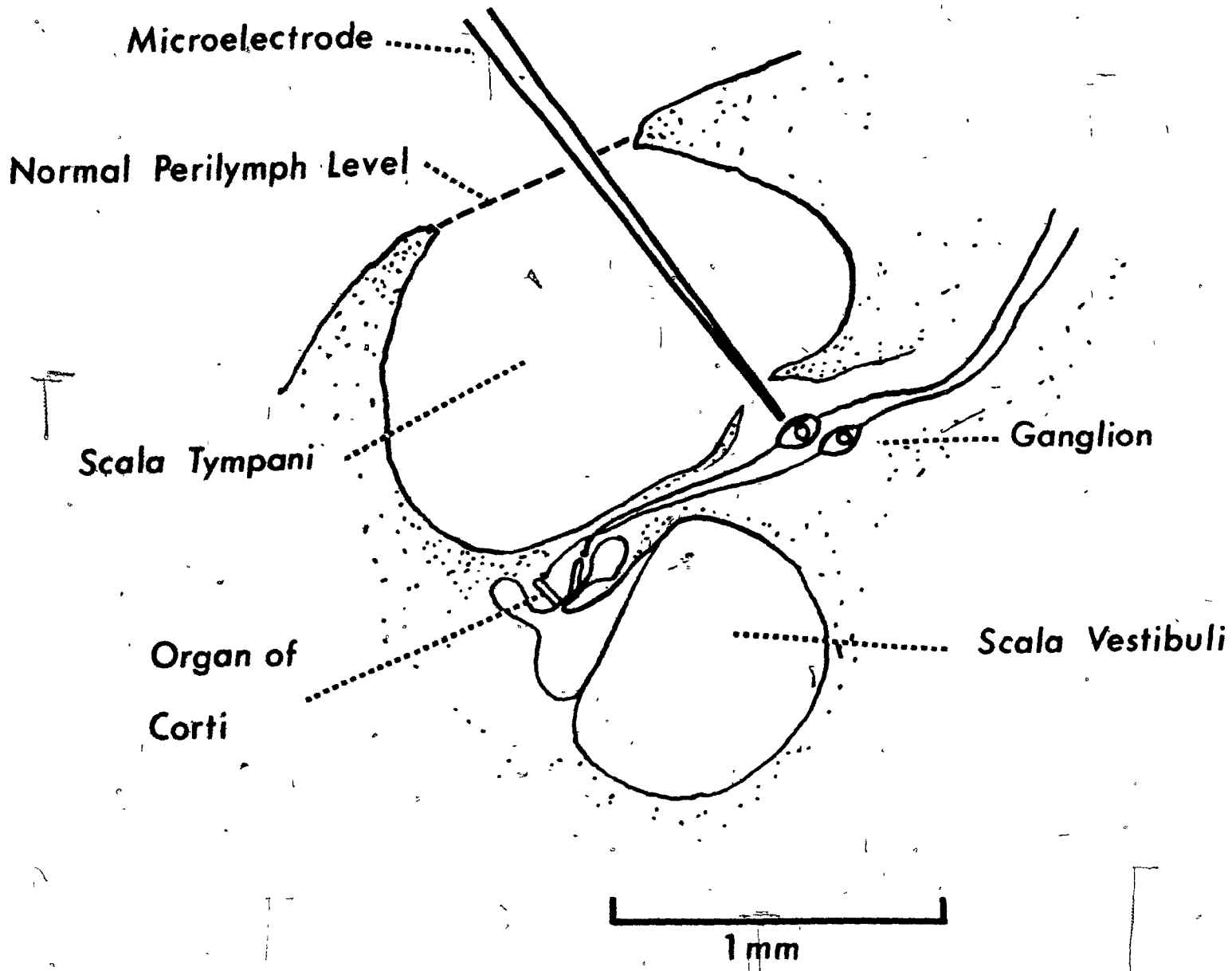
The technique, which has been described in detail (Robertson and Manley, 1974), involved extracellular recordings from single ganglion cells in the basal turn of the guinea pig cochlea. Guinea pigs (150-400g) were anaesthetized with 35mg/kg of pentobarbital sodium, relaxed with Quelecin (Roche) and artificially ventilated. Metal filled microelectrodes with a gold-platinum ball tip (diameter 5-8 microns) (Frank and Becker, 1964) were introduced into the ganglion with a remote hydraulic microdrive. A schematic representation of the recording situation is shown in Fig. 1.

Two-tone inhibition was studied in 57 units in 15 animals. For the investigation of this phenomenon, two tone bursts with 5 ms rise-fall time were presented as shown in Fig. 3. The longer (50 ms) tone was at the cf. of the neurone and an estimated 10dB above threshold at that frequency. The shorter tone

was 30 ms long and situated inside the cf. tone. The frequency and intensity of this tone were varied in order to investigate two-tone inhibition. Post-stimulus histograms (PSTH) of the responses to repeated stimulus sets were computed with a PDP8 computer, both on-line and off-line using tape-recorded data. In cases where two-tone inhibition was weak or absent (see results), the intensity of the cf. tone was varied to see if inhibition could be produced by some other stimulus combination.

The single-tone tuning curve of each cell was also measured (Robertson and Manley, 1974) as well as the mean rates of spontaneous activity.

Fig. 1. Schematic representation of the experimental recording situation. In the fluid drainage experiments, perilymph was removed from the scala tympani by placing small cotton wicks through the dissected hole in the wall of the scala tympani.


Microelectrode
Normal Perilymph Level
Scala Tympani
Ganglion
Organ of
Corti
Scala Vestibuli
1 mm

Results

Two-tone inhibition in the guinea pig cochlear ganglion cells was found to resemble that reported for cochlear nerve fibres of other species. That is, for a tone at the cf., inhibition of firing could be produced by tones of the correct frequency and intensity in restricted bands above and below the cf. (Fig. 2, 3). Inhibition of a cf. tone could often be produced by a second tone up to 5 dB below the threshold for excitation by the second tone alone (Sachs and Kiang, 1968).

In cat, Sachs and Kiang (1968) have reported that two-tone inhibition of this form was found in every primary cochlear nerve fibre which was investigated. In the guinea pig spiral ganglion preparation however, Robertson and Manley (1974) have reported that much variation can occur from one animal to another in the sensitivity and sharpness of the neural tuning curves. In the present study, strong two-tone inhibitory

Fig. 2. General shape of inhibitory and excitatory response areas in sharply tuned ganglion cells. Solid lines; threshold-frequency response area (tuning curve) for the presentation of a single tone. Dotted lines; frequency-intensity bands of a second tone (T_2) which causes a reduction of firing to T_1 whose frequency and intensity are indicated by the star.

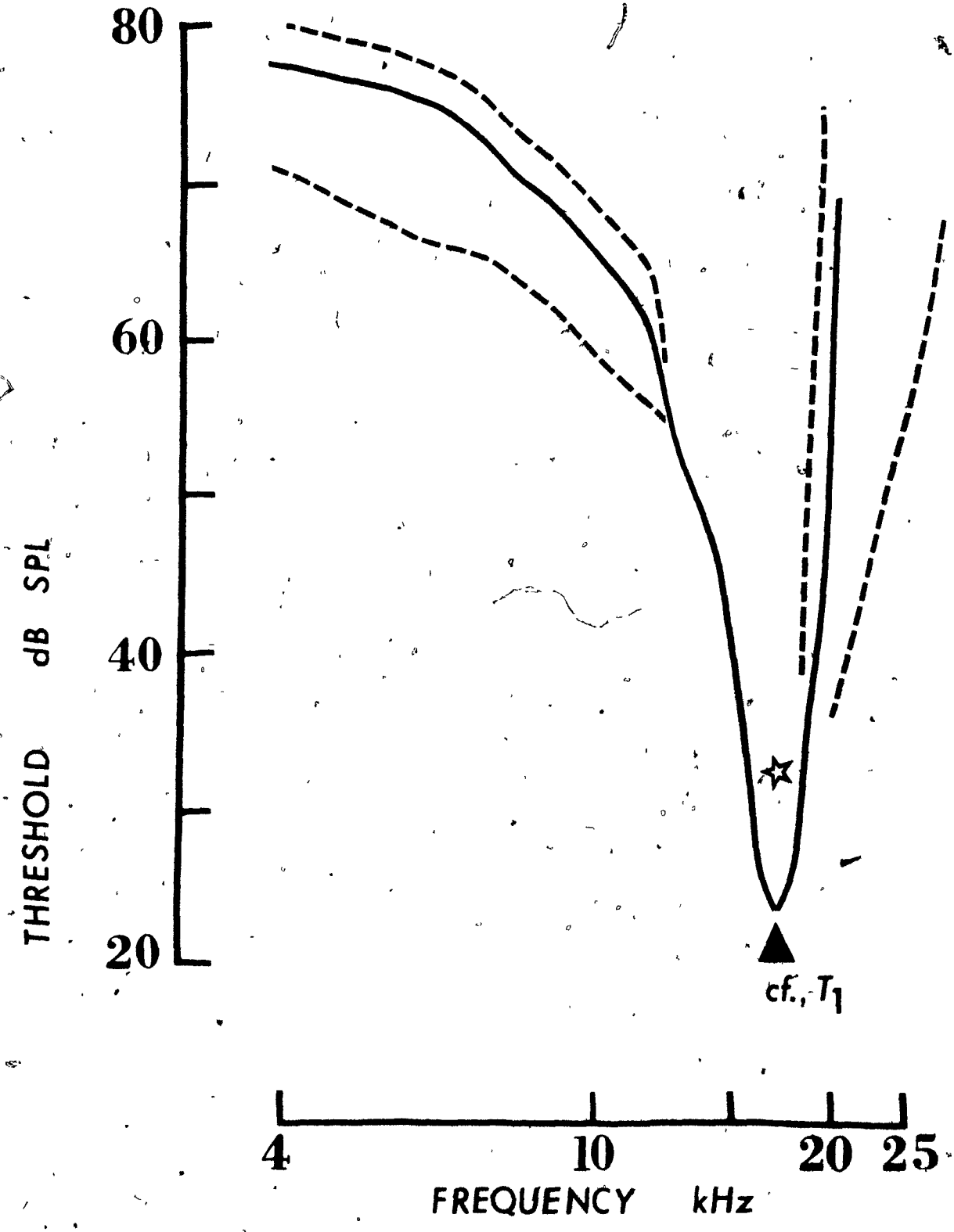
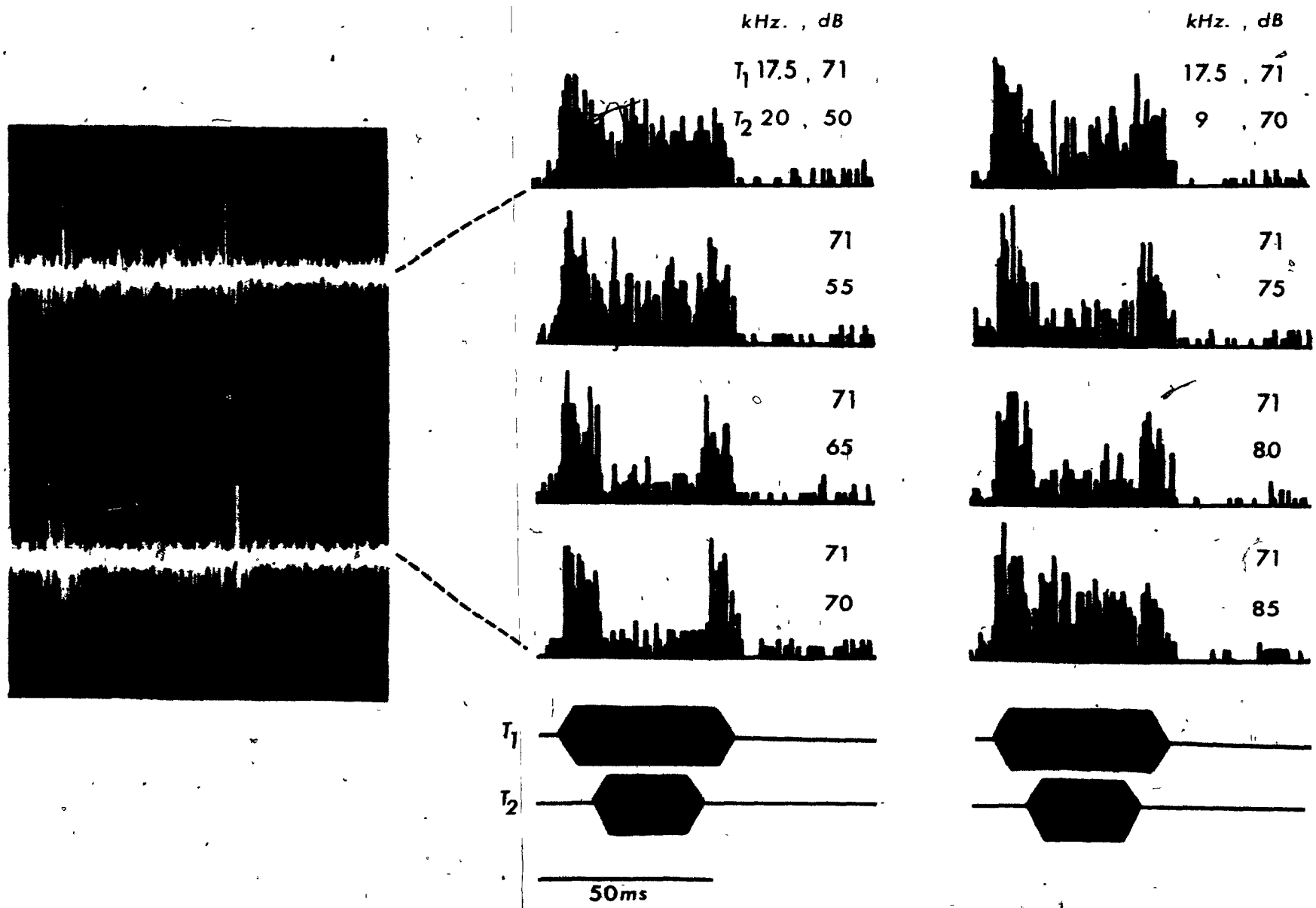

80
60
40
20
THRESHOLD dB SPL
cf., T_1
4
10
20
25
FREQUENCY kHz

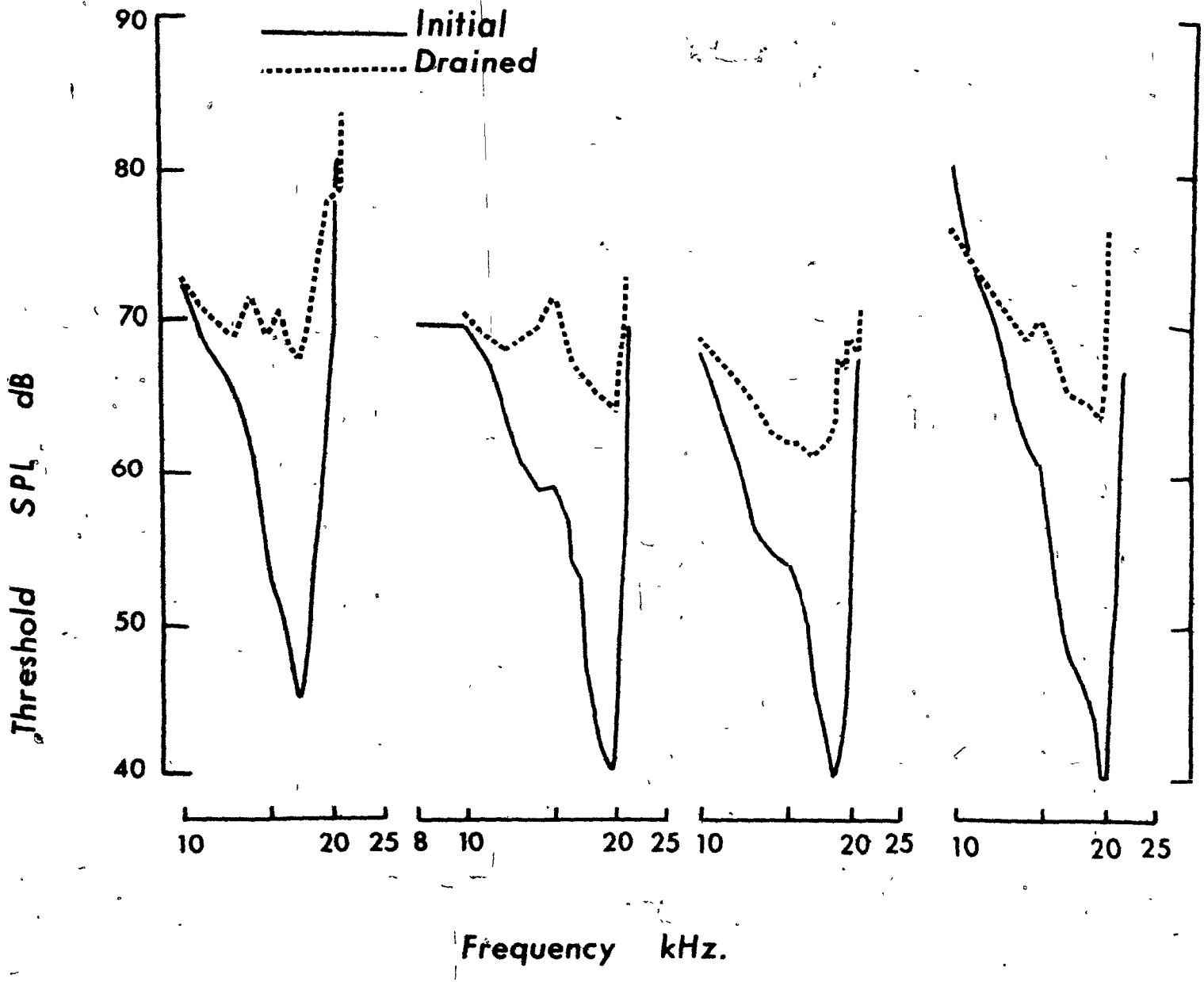
Fig. 3. PST histograms of a single ganglion cell in response to different combinations of T_1 and T_2. T_1 is at the cf. of the neurone. The frequency and intensity of T_2 and the intensity of T_1 are indicated on the histograms.


kHz. , dB
T_1 17.5 , 71
T_2 20 , 50
71
55
71
65
71
70
kHz. , dB
17.5 , 71
9 , 70
71
75
71
80
71
85
T_1
T_2
50ms

effects were only found in those animals in which single unit tuning curves were relatively sharp and sensitive (thresholds at the cf. less than about 50 dB SPL). The broader and less sensitive the tuning, the less apparent was two-tone inhibition. In some animals with very broad ganglion cell tuning curves no two-tone inhibition could be detected despite extensive explorations of different frequency and intensity combinations for both tones.

The close association between the sharpness of neural tuning curves and two-tone inhibition, was further emphasized in fluid drainage experiments. As reported previously (Robertson, 1974) removal of perilymph from the scala tympani causes a loss of sensitivity and a tuning curve change. The effects on the tuning curves of four cells are shown in Fig. 4. In the present study, perilymph removal and replacement had a profound effect on two-tone inhibition in neurones with initially sharp single-tone tuning curves.

Fig. 4. The effect of drainage of perilymph from scala tympani on the tuning curves of 4 ganglion cells. Solid lines; tuning curves in the normal fluid filled condition. Dotted lines; with fluid removed.


Initial
Drained
90
80
70
60
50
40
Threshold SPL dB
10
20
25
8
10
20
25
10
20
25
10
20
25
Frequency kHz.

In all cases, the loss of sensitivity and broadening of the tuning curve caused by complete perilymph removal was accompanied by the disappearance of two-tone inhibition (Fig. 5.). On allowing the perilymph to refill the scala tympani, both the sharp tuning and two-tone inhibition returned. It was not easy to hold the same cell throughout this procedure, nor to keep perilymph from welling up in the scala tympani while the frequency-intensity range was scanned. In 4 cells in 3 animals, fluid was kept out of the scala tympani long enough to verify with certainty that two-tone inhibition was indeed absent in the same cell in which it was previously very strong. Partial confirmation of this result was also obtained from several other cells. In Fig. 5 there appears to be an increase in the background activity during perilymph drainage. Usually however, this was not the case. Fig. 6 illustrates 2 cells in which perilymph drainage caused no systematic change in spontaneous activity even though the cell

Fig. 5. Effect of drainage and replacement of perilymph on two-tone inhibition in a single ganglion cell. In the drained case, as the intensity of T_2 is raised, only an excitatory effect is obtained, whereas in the filled state, inhibition is clearly present. The intensity of T_1 used in the drained case is higher above threshold than in the fluid-filled situation. However, two-tone inhibition was absent in the drained cochlea for all intensities of T_1.

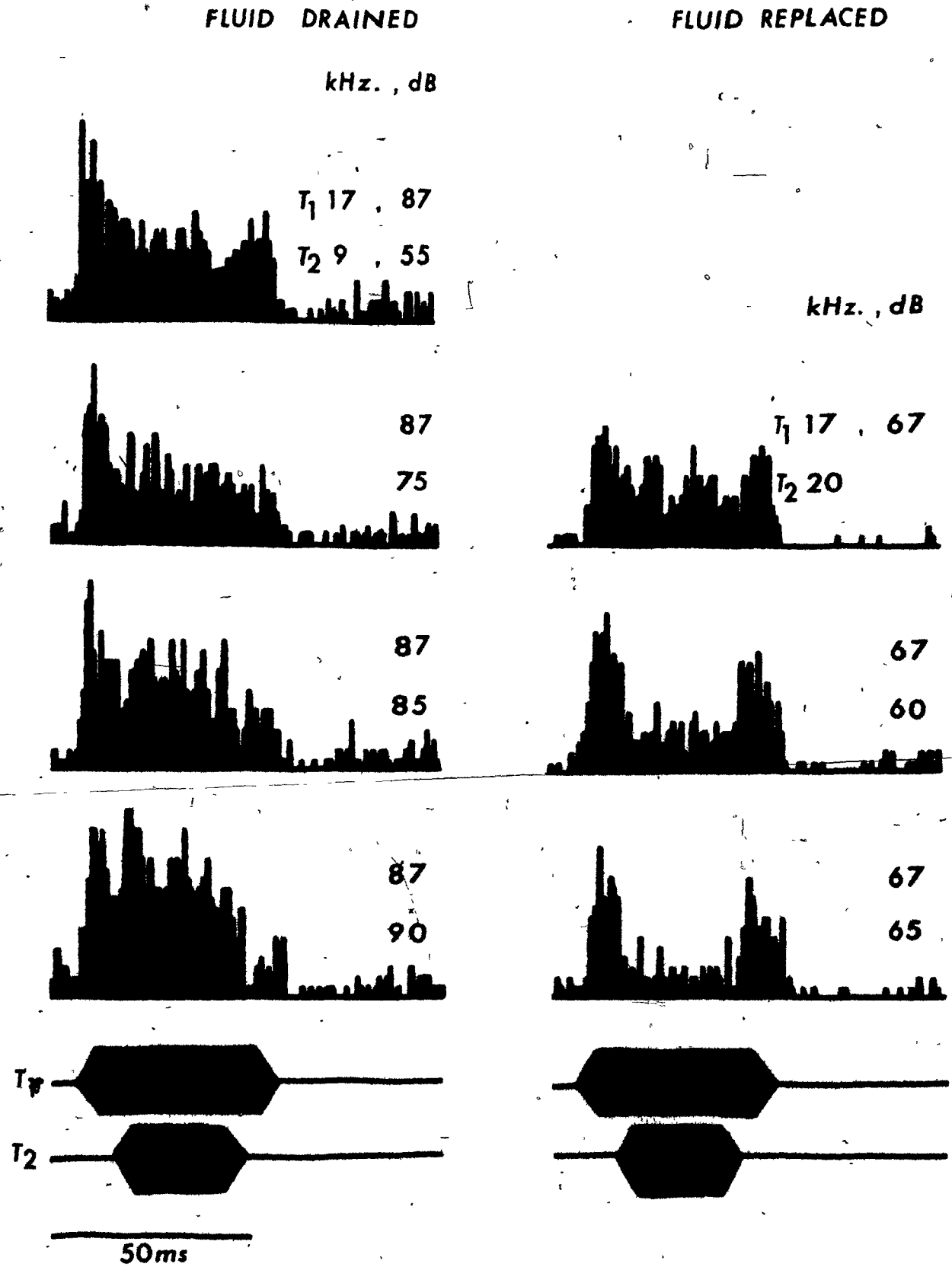
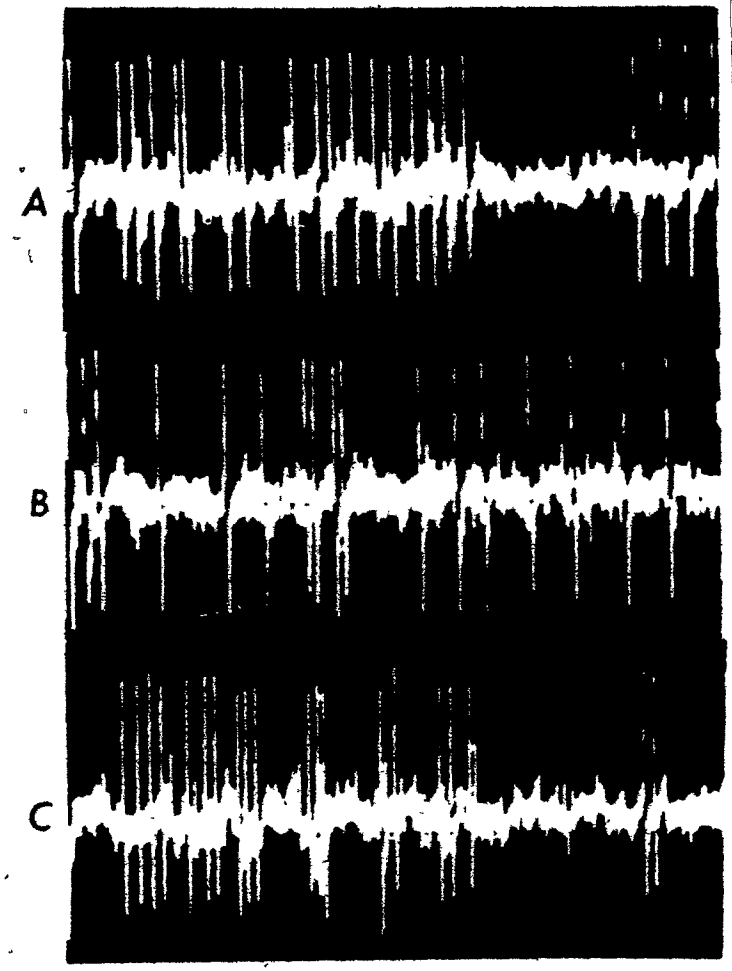

FLUID DRAINED
FLUID REPLACED
kHz., dB
T_1 17 , 87
T_2 9 , 55
kHz., dB
87
75
T_1 17 , 67
T_2 20
87
85
67
60
87
90
67
65
T_1
T_2
50ms

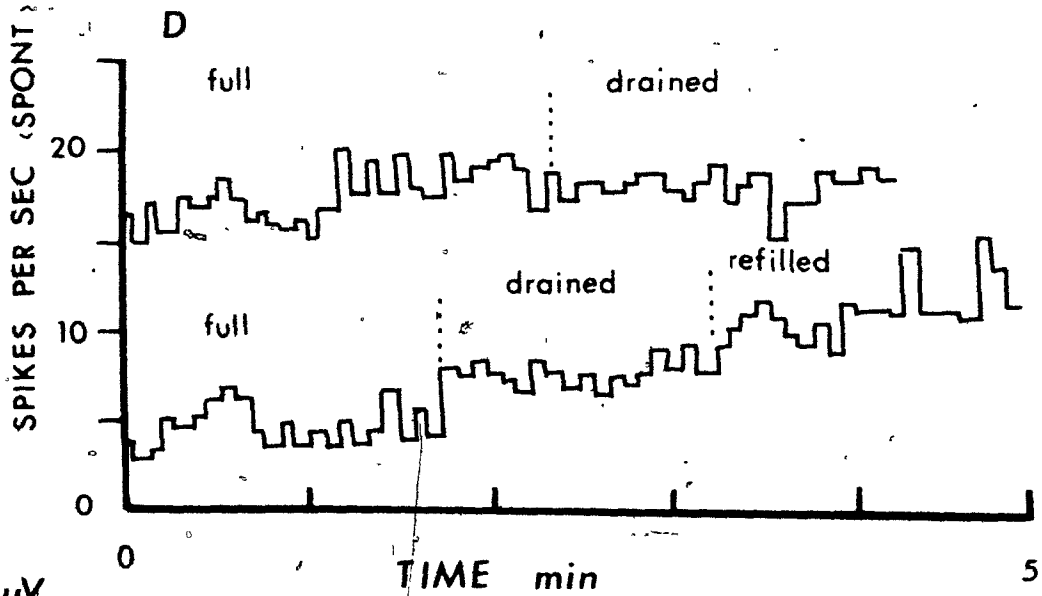
Fig. 6. Effect of drainage on sensitivity and spontaneous activity. A; response of a cell to a tone burst as indicated. B; response to the same tone burst after perilymph removal. C; the response with perilymph replaced. D; the effects of fluid changes on the mean spontaneous activities of 2 ganglion cells. No systematic effects are seen though the cell with lower mean rate shows a gradual increase in rate throughout the drain and refilling treatment.



19 kHz, 57dB

100μV

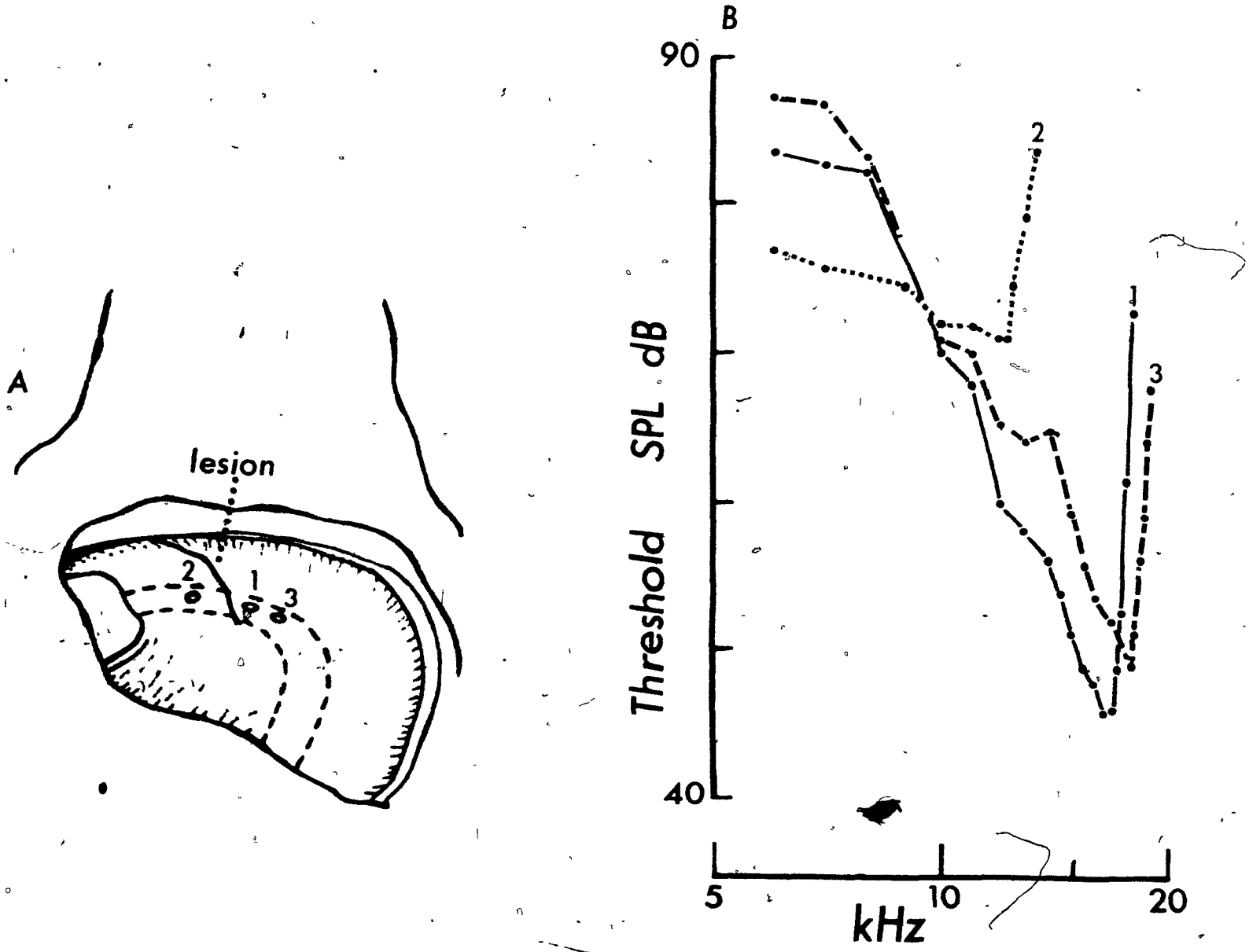
10ms



sensitivity was greatly reduced.

The effects of structural damage were essentially the same as those of fluid drainage, but were irreversible. In several dissections, the delicate spiral lamina close to the inner edge of the basilar membrane was cracked during exposure of the ganglion. In such cases, tuning curves were broad and insensitive and two-tone inhibition was absent. Fig. 7 illustrates an instance in which the effect of damage was localized. At locations basal to the site of damage, single unit tuning curves were sharp and exhibited two-tone inhibition. In the region of the damage, tuning curves were insensitive and broad and were completely lacking in two-tone effects. The sharply tuned cells at location 3 were recorded after those at location 2, showing that the broad tuning curves were not a result of a time-dependent deterioration in the animal's condition.

Fig. 7. Effects of local damage to the spiral lamina, on tuning curves and two-tone inhibition of single ganglion cells. Cells from location 1 were obtained before the damage indicated was created. In making the exposure at location 2 the spiral lamina was cracked. All tuning curves at this location were broad and lacked two-tone inhibition. Subsequently at location 3 all cells were sharply tuned and showed two-tone inhibition.


A
lesion
2
1
3
B
90
40
Threshold SPL dB
2
1
3
5
10
20
kHz

Discussion

Previous studies have shown that the frequency selectivity of sharply tuned primary auditory neurones is labile (Evans, 1972; Robertson and Manley, 1974; Evans and Klinke, 1974; Kiang et al., 1970). A variety of procedures; anoxia, perilymph removal, intravenous furosemide, hair cell damage, all cause a loss of sensitivity and an accompanying broadening of the tuning curve. In the present study, it is shown that such changes are linked also with a loss of two-tone inhibition, at least for the case of perilymph removal and structural damage. In those animals in which broad tuning curves were also found without identifiable cause, two-tone inhibition was weak or absent. It appears likely that all procedures or pathological conditions which produce a loss of sensitivity and broadening of the tuning curve will affect two-tone inhibition in this way.

However, though two-tone inhibition appears to be closely linked to sharp tuning, it would be simplistic

to assume a causal relation between them. Two-tone inhibition differs in several ways from classical lateral inhibition (Arthur et al., 1971). It is thus by no means certain that this inhibitory phenomenon in any way determines the single-tone tuning curves.

It has been suggested by some authors that two-tone inhibition may be an inherent property of the same physiological entities in the organ of Corti which are responsible for the normal sharpness of neural tuning curves (Pfeiffer, 1970; Kim et al., 1973). Thus two-tone suppression may be a result of a single narrow band system, but not a cause of it. The actual nature of this system is still controversial. Non-linear vibrations including two-tone suppression have been observed on the basilar membrane of squirrel monkey (Rhode, 1971, 1973), and it has been suggested that sharp neural tuning curves may also derive from the same mechanical non-linearities (Robertson and Manley, 1974; Kim et al., 1973). Thus the simultaneous loss of two-tone inhibition

and sharp tuning caused by perilymph removal and damage may reflect an alteration in the vibration pattern of the basilar membrane. A mechanical origin for two-tone interaction receives some support from studies on squirrel monkey nerve fibres (Rose et al., 1974) and from the behaviour of cochlear microphonic suppression (Legouix et al., 1973). There is however, considerable disagreement on the question of non-linear basilar membrane behaviour. Several authors believe the basilar membrane vibration to be broadly tuned, linear and not labile (Wilson and Johnstone, 1972, 1973). The discrepancies between the techniques employed by various workers have been discussed in detail (Robertson, 1974; Rhode, 1973). Final resolution of the disagreement may only come with the advent of better basilar membrane measurement techniques (e.g. Dragsten et al., 1974).

If, as some authors maintain, the basilar membrane vibrates linearly and with broad tuning, the

source of sharp neural tuning and two-tone inhibition must lie in an additional mechanism, perhaps at the hair cell level. Synaptic interactions between afferent neurones do not seem to be possible (Moller, 1970; Evans and Wilson, 1973). It has been suggested that an alternative explanation for the effects of fluid removal may be an interference with current spread along the basilar membrane caused by an increased scala tympani resistance (Robertson, 1974; Johnstone, J.R. personal communication). However, the lack of effect of perilymph removal on spontaneous activities of primary afferent neurones (Fig. 6.) may not be consistent with this hypothesis. It is possible that spontaneous activity arises, at least in part from random release of transmitter substance by the hair cells and is modulated by standing current through the hair cells (Konishi et al., 1970). If perilymph drainage significantly increases extracellular resistance in the organ of Corti-to-scala

tympani pathway, one would expect standing current levels through the hair cells to be diminished and thus possibly cause a reduction in spontaneous activity. The fact that this is not found perhaps reduces the likelihood that two-tone inhibition and sharp neural tuning derive from electrotonic spread of current along the basilar membrane. Of course changes in spontaneous activity may be only transient or else spontaneous activity may in fact be a poor indicator of hair cell standing current levels.

There are many problems in deciding on the significance of the above results and it is still not possible to pinpoint the structural and physiological entities responsible for sharp neural tuning and two-tone inhibition in the primary auditory pathway in mammals.

Summary

1. The occurrence of broad insensitive tuning curves in primary auditory neurones of the guinea pig spiral ganglion was associated with a loss of two-tone inhibition in these same cells.

2. This was true whether the tuning curve broadening was produced either by perilymph removal, structural damage, or some unknown pathological condition of the animal.

3. In the case of perilymph removal, the broadening of tuning and loss of two-tone inhibition were fully reversible.

4. It is suggested that two-tone inhibition is intimately linked to the mechanism responsible for sharp neural tuning curves in the primary neurones. Arguments for the nature of this mechanism are discussed.

Acknowledgments

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References

Arthur, R.M., Pfeiffer, R.R., Suga, N.: Properties of two-tone inhibition in primary auditory neurones. J. Physiol. (Lond.) 212, 593-609 (1971).

Dragsten, P.R, Webb, W.W., Paton, J.A., Capranica, R.R.: Auditory membrane vibrations: measurements at sub-angstrom levels by optical heterodyne spectroscopy. Science. 185, 55-57 (1974).

Evans, E.F., Klinke, R.: Reversible effects of furosemide on the tuning of single cochlear nerve fibres. J. Physiol. (Lond.) C50, Proc. Physiol. Soc. (1974).

Evans, E.F., Wilson, J.R.: The frequency selectivity of the cochlea. In: Basic Mechanisms in Hearing, p. 519-554 (Moller, A.R., Ed.) Acad. Press. (1973).

Evans, E.F.: The frequency response and other properties of single fibres in the guinea pig cochlear nerve. J. Physiol. (Lond.) 226, 263-287 (1972).

Frank, K. Becker, M.C.: Microelectrodes for recording and stimulation. In: Physical techniques in Biological Research. 5 (Nastuk, W.L., Ed.) New York: Acad. Press 1964.

Turhman, G.G., Frishkopf, L.S.: A model of neural inhibition in the mammalian cochlea. J. acous. Soc. Amer. 36, 2194-2201 (1964).

Kiang, N.Y.S., Moxon, E.C., Levine, R.A.: Auditory nerve activity in cats with normal and abnormal cochleas. In: Sensorineural Hearing Loss, p. 241-273 (Wolstenholme, G., Knight, K., Eds.) London: Churchill 1970.

Kiang, N.Y.S.: Discharge patterns of single fibres in the cat's auditory nerve. Cambridge (Mass.) M.I.T. Press 1965.

Kim, D.O., Molnar, C.E., Pfeiffer, R.R.: A system of nonlinear differential equations modelling basilar membrane motion. J. acous. Soc. Amer. 54, 1517-1529 (1973).

Konishi, T., Teas, D.C., Wernick, J.S.: Effects of electrical current applied to the cochlear partition on discharges in individual auditory nerve fibres. I. Prolonged DC polarization. J. acous. Soc. Amer. 47, 1519-1527 (1970).

Legouix, J.P., Remond, M.C., Greenbaum, H.B.: Interference and two-tone inhibition. J. acous. Soc. Amer. 53, 409-419 (1973).

Moller, A.R.: Studies of the damped oscillatory response of the auditory frequency analyzer. Acta physiol. scand. 78, 299-314 (1970).

Nomoto, M., Suga, N., Katsuki, Y.: Discharge pattern and inhibition of primary auditory nerve fibres in the monkey. J. Neurophysiol. 27, 768-787 (1964).

Pfeiffer, R.R.: A model for two-tone inhibition of single cochlear-nerve fibres. J. acous. Soc. Amer. 48, 1373-1378 (1970).

Ratliff, F.: Inhibitory interaction and the detection and enhancement of contours. In: Sensory Communication, (Rosenblith, W.A., Ed.) M.I.T. Press 1961.

Rhode, W.S.: An investigation of post-mortem cochlear mechanics using the Mössbauer effect. In: Basic Mechanisms in Hearing. (Moller, A.R., Ed.) Acad. Press 1973.

Rhode, W.S.: Observations on the vibration of the basilar membrane in squirrel monkeys, using the Mössbauer technique. J. acous. Soc. Amer. 49, 1218-1231 (1971)

Robertson, D.: Cochlear neurones: frequency selectivity altered by perilymph removal. Science 186, 153-155 (1974)

Robertson, D., Manley, G.A.: Manipulation of frequency analysis in the cochlear ganglion of the guinea pig. J. Comp. Physiol. 91, 363-375 (1974).

Rose, J.E., Kites, L.M., Gibson, M.M., Hind, J.E.: Observations on phase sensitive neurones of anteroventral cochlear nucleus of the cat: nonlinearity of cochlear output. J. Neurophysiol. 37, 218-253 (1974).

Sachs, M.B.: Stimulus response relations for auditory nerve fibres: two-tone inhibition. J. acous. Soc. Amer. 44, 1025-1036 (1969).

Sachs, M.B., Kiang, N.Y.S.: Two-tone inhibition in auditory nerve fibres. J. acous. Soc. Amer. 43, 1120-1128 (1968).

Wilson, J.P., Johnstone, J.R.: Basilar membrane correlates of the combination tone $2f_1 - f_2$. Nature 241, 206-207 (1973).

Wilson, J.P., Johnstone, J.R.: Capacitive probe measures of basilar membrane vibration. Symp. on Hearing theory, LPO Eindhoven (1972).

The frequency selectivity of the spiral ganglion cells and its relation to basilar membrane tuning was the principle subject of the previous three papers of this thesis. Many of the issues involved are elaborated on, and a final assessment is made in the discussion at the end of this thesis. The remaining two papers are concerned with other properties of the spiral ganglion cells. In Paper IV, spontaneous activity is examined, and in Paper V electrophysiological and histological evidence on the mode of impulse propogation in these bipolar neurones is presented.

PAPER IV

Patterns of Spontaneous Activity of Primary Auditory Neurones in the Cochlear Ganglion of the Guinea Pig.

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Dept. of Biology, McGill University, Montreal,

Que. CANADA.

Running Title: Spontaneous Activity of Auditory Neurones.

Introduction

The existence of spontaneous activity in single primary neurones of the mammalian auditory pathway is well established (Kiang, 1965; Walsh et al., 1972; Evans, 1972). Spontaneous activity is defined as that occuring in the absence of any controlled stimulus and the extreme sensitivity of the auditory pathway means that the possibility that uncontrolled room noise or loudspeaker "hiss" is the source of such activity must be considered. It has been shown (Kiang, 1965) that such spontaneous activity still exists in sound-proofed chambers in which ambient noise levels are below the threshold of hearing, so that an intracochlear source is most likely. Further proof of this is presented in the present paper which reports extracellular recordings from single neurones in the acoustic ganglion of the guinea pig cochlea.

In the study of spontaneous activity, two levels of analysis have commonly been used. One is the mean rate of firing which constitutes the crudest form of

characterization of firing. The second is the inter-spike interval histogram (TIH)(Rodieck and Kiang, 1962; Gerstien and Kiang, 1960; Bishop et al., 1964; Kiang, 1965). This represents a distribution of the probability of a firing as a function of the time after a previous firing. These two properties of spontaneous activity are reported here for single cochlear ganglion cells of guinea pig and are compared with available data from the fibres of the cochlear nerve. Possible sites of origin of the spontaneous activity are also discussed.

Methods

All experiments were conducted in a sound-proofed, shielded room with the experimenter and electronic equipment located outside the room during single cell recordings. The animals were young pigmented guinea pigs (150-400g), anaesthetized with 35mg/kg of pentobarbital sodium. They were relaxed with Quelecin (Roche) to eliminate movements and muscle noise and were artificially ventilated. Noise from the airflow in the respiration inlets and outlets to the animals' tracheae was below the threshold of human hearing and no single cell activity synchronised to the respiration cycle could be detected.

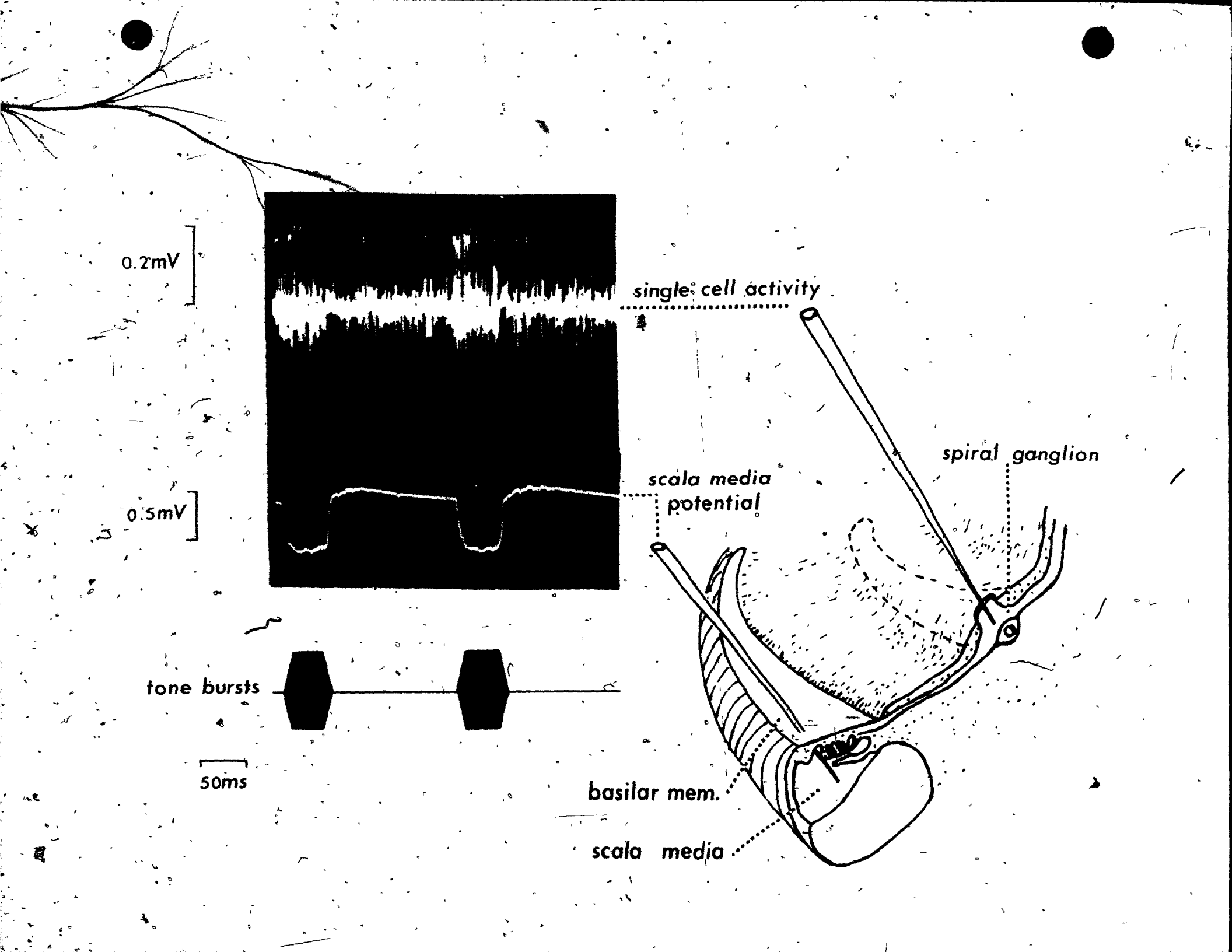
The details of ganglion cell recording from the basal turn of the cochlea have been reported previously (Robertson and Manley, 1974; Robertson, 1974). In Fig. 1 the relation of the recording electrodes to the various intracochlear structures is represented schematically. Single neurone recordings were obtained mainly with metal-

filled microelectrodes (Frank and Becker, 1964) whose gold-platinum ball tip was 5-8 microns in diameter. Some data was also obtained using 3M KCl-filled glass micropipets, of DC resistance 10-20 megohms in 0.9% saline. Currents were sometimes passed between these electrodes and a silver-silver chloride reference in the neck muscles using a GRASS stimulator.

In some experiments, resting and sound evoked potentials inside the scala media were also measured. This was achieved by inserting a 150mM KCl-filled glass micropipet through the basilar membrane into the scala media (Fig. 1). The resting endocochlear potential (+EP), usually 80-100mV with respect to the neck muscles, and the DC sound-evoked potentials (negative summating potential, or -SP) were obtained in this way.

Single cell activity was usually recorded on an AM tape recorder and analysed after the experiment. For analysis of spontaneous activity, TIH were computed with a PDP8 computer. Bin widths ranging from 0.1 to 2.0 ms

Fig. 1. Schematic diagram with oscilloscope tracings of the arrangement for simultaneously recording ganglion cell response and evoked and resting endocochlear potentials. Upper trace shows single primary neurone activity in response to repeated tone bursts, and lower trace shows the -SP recorded inside scala media in response to the same acoustic stimuli. The resting DC level of the endocochlear potential (+80 - +100 mV) was also recorded.


0.2mV
0.5mV
single cell activity
scala media potential
spiral ganglion
tone bursts
50ms
basilar mem.
scala media

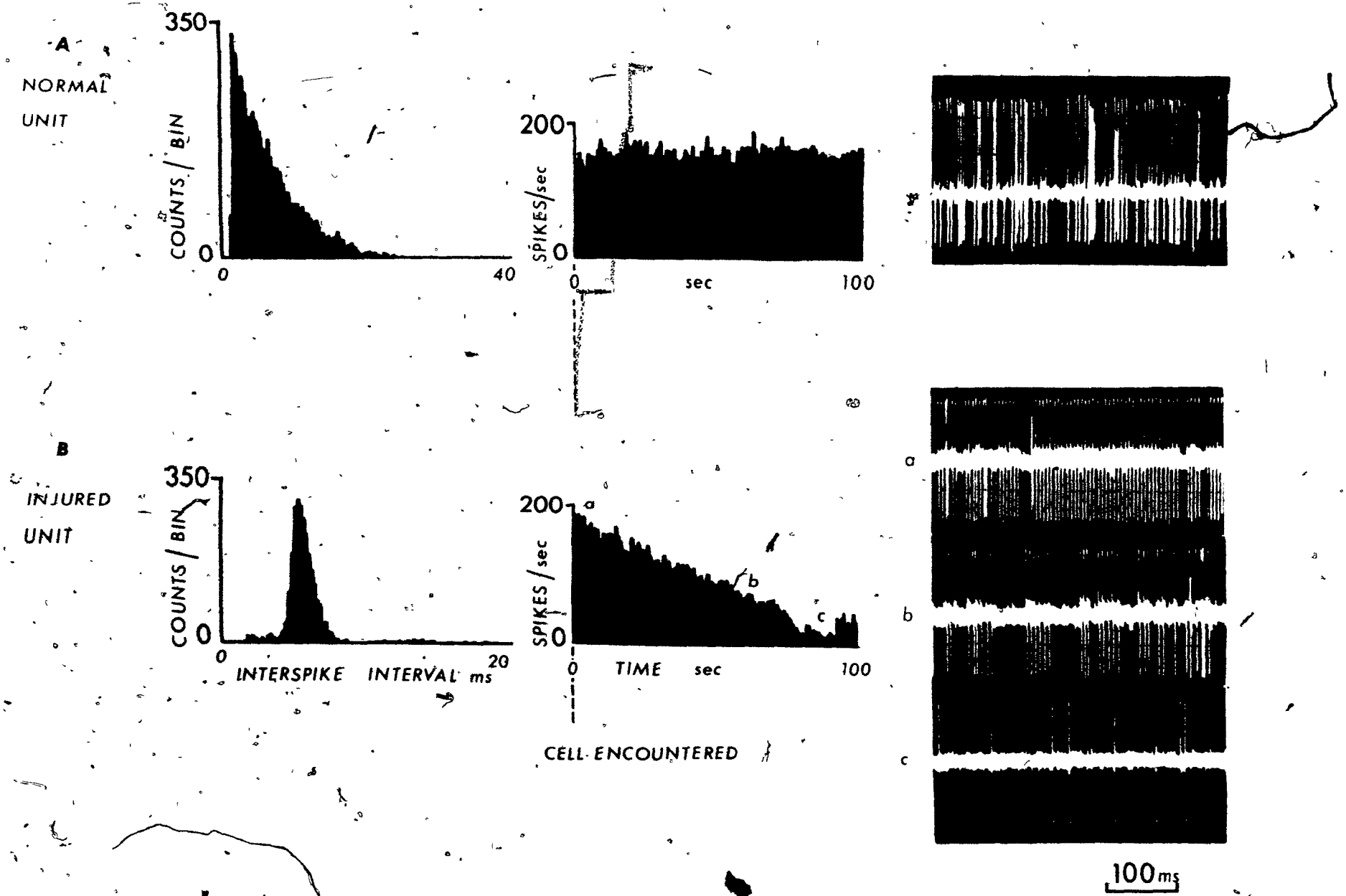
were used and sufficient lengths of taperecorded data were used to accumulate at least 2000 counts per histogram. For units with low rates of spontaneous activity, this necessitated considerable lengths of time in contact with the cell and most TIH data were obtained from units with mean rates of firing greater than 30/sec. In some experiments on the effects of anoxia, a continuous record of mean rate was obtained by connecting the spike data to a rate meter with a pen-recorder printout. The negative summating potentials in response to tone bursts were either measured directly off an oscilloscope screen for moderate to intense stimulation, or were computer averaged on-line for stimulus intensities less than about 65dB sound pressure level.

The physiological condition of the animal was evaluated by measurement of the pure-tone thresholds and the threshold-frequency response curves (Robertson and Manley, 1974).

Results

The most usual pattern of spontaneous activity, which was defined as "normal", was stable over many minutes after the time of initial encountering of the cell (Fig. 2A). Though the mean rate measured over several minutes was constant the fine structure of the discharge showed very irregular intervals between successive spikes. TIH of such spontaneous activity showed an asymmetric shape with a mode of less than 10ms, indicating a greater probability of occurrence of short intervals. The tails of such histograms were exponential (see below). Frequently however, cells were encountered with unstable firing patterns. Initial contact with the cell was accompanied by rapid discharges with very regular intervals between spikes. Thus the TIH of such discharge was symmetric (Fig. 2B). Such "injury" discharge was never maintained for long and firing gradually became intermittent and ceased altogether. Apart from the different stabilities of these two spontaneous firing patterns, another finding

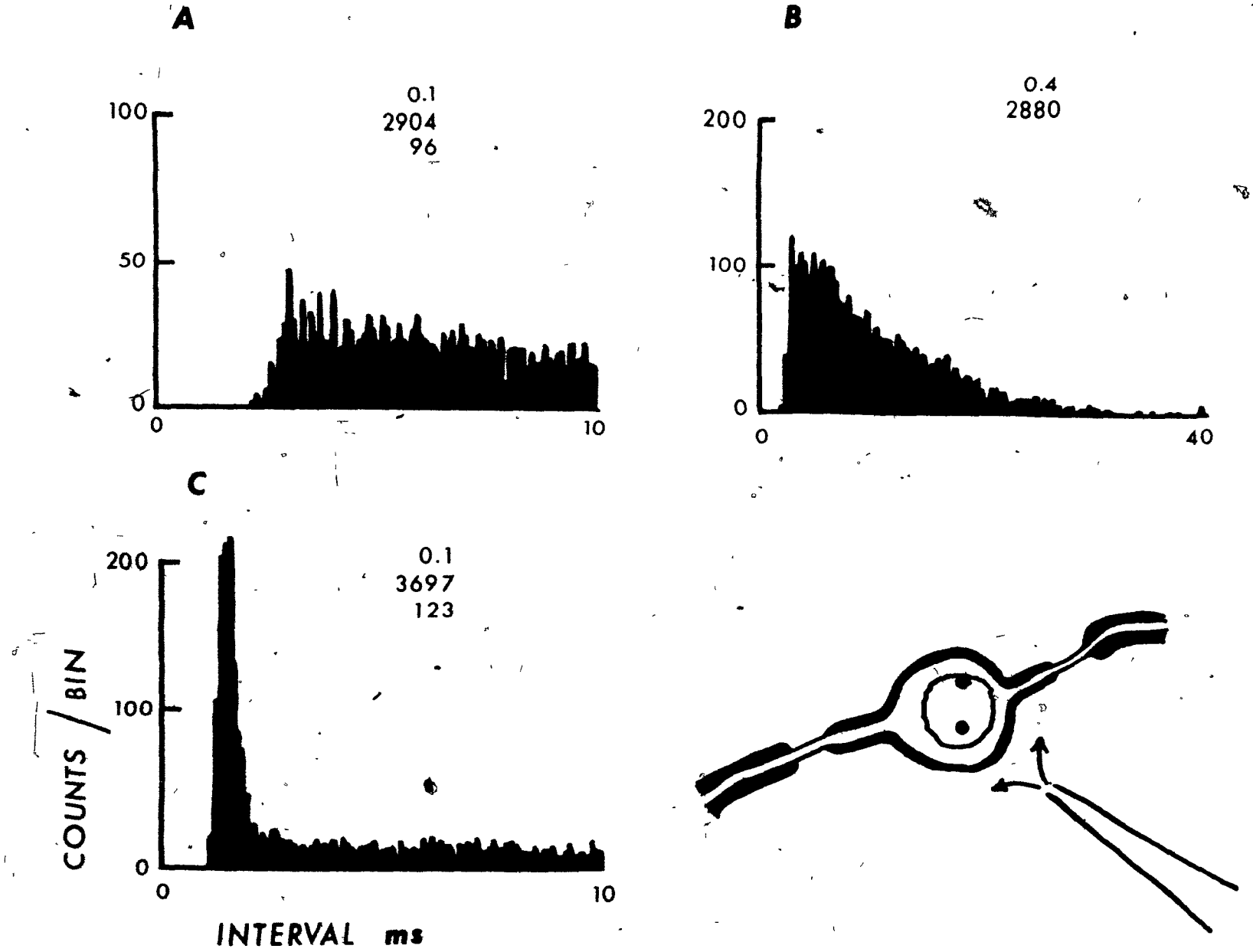
Fig. 2. Spontaneous activity of normal (A) and injured (B) cochlear ganglion cells. For the normal cell, the TIH is asymmetric with an exponential tail. Mean spontaneous rate is constant from the time the cell is encountered. The fine structure of discharge shows irregular interspike intervals. For the injured cell, activity decreases after the time of initial contact. TIH of early discharge is symmetric due to very regular interspike intervals (a). At times b), c), the discharge decreases to very low rates.


A
NORMAL
UNIT
350
COUNTS / BIN
0
0
40
200
SPIKES/sec
0
0
sec
100
B
INJURED
UNIT
350
COUNTS / BIN
0
0
INTERSPIKE INTERVAL ms
20
200
SPIKES / sec
a
b
c
0
0
TIME sec
100
CELL ENCOUNTERED
a
b
c
100ms

suggests that they can be classified as "normal" and "injured". Those units with asymmetric TIH and stable firings usually responded sensitively to acoustic stimulation, while "injured" units, when their initial firing rates had dropped, never gave more than a few infrequent spikes to the most intense stimulation and often failed totally to respond. An interesting clue to the origin of this injury discharge was obtained with KCl-filled pipets. During the application of a positive current through the microelectrode (presumably ejecting potassium from the electrode tip), the firing rate of an initially normal cell rose dramatically (Fig. 3) and the shape of the TIH altered to resemble more closely that of injured units, with a large narrow peak indicating a predominance of regular interspike intervals.

Supporting evidence for an intracochlear origin of the spontaneous firings was obtained from two instances in which the threshold of ganglion cells to acoustic stimulation was elevated by up to 20dB. When the ossicular

Fig. 3. The effect of application of +ve current through a 3MKCl recording microelectrode, on the "spontaneous" discharge of a single ganglion cell. A, B, TIH's of the discharge without current, showing typical asymmetric shape and exponential tail. Mean rate is 96/sec. The numbers in right hand corner of the histograms denote bin width (msec), total number of counts and mean rate. C, the TIH of the same cell during application of the current -- the histogram shows a large symmetric peak, due to the greater predominance of more regular intervals between spikes. Mean rate is 123/sec. during the application of the current.


A
0.1
2904
96
100
50
0
0
10
B
0.4
2880
200
100
0
0
40
C
0.1
3697
123
200
100
0
0
10
COUNTS / BIN
INTERVAL ms

chain of the middle ear was broken, auditory thresholds were 20-30 dB higher than normal, indicating a loss in the transmission of airborne sounds to the basilar membrane. Spontaneous activities of cells in such animals, showed the same distribution of mean rates as in intact, sensitive animals. Several cells in such insensitive preparations had mean rates of spontaneous discharge higher than 90/sec. In addition, it has been reported (Robertson, 1974), that drainage of perilymph from the scala tympani elevates the thresholds of single ganglion cells by about 20dB at their best frequencies, without significant effects on the mean rate of spontaneous activity. Such evidence, together with sound-proofing of the experimental chamber, shows that spontaneous activity in the cochlear neurones cannot arise from uncontrolled sounds and that it is truly intracochlear in origin.

In agreement with findings on VIIIth nerve axons, guinea pig cochlear ganglion cells showed wide differences

in mean rate of spontaneous activity in the same animal. The distribution of mean rates in the pooled data is shown in Fig. 4A. 60% of all cells recorded had spontaneous rates greater than 20/sec. When plotted against the threshold of those cells in which this was measured (at the best frequency), it can be seen that for cells of reasonable to excellent sensitivity, there is no relationship between threshold and mean rate of spontaneous activity. Cells with the same sensitivity at the best frequency vary in mean spontaneous rate from 0 - 140/sec. (Fig. 5). The large peak of cells with low spontaneous rates (0-20/sec.) in Fig. 4A is explained by the tendancy for cells of poor sensitivity to have low rates (Fig. 5). Such cells appear to occur in animals in poor physiological condition (Robertson and Manley, 1974; Evans, 1972) or with extensive hair cell damage (Kiang et al., 1970). Mean rates higher than 160/sec. were registered but these occurred in cells showing clear injury behaviour.

Fig. 4. A; histogram of the distribution of mean spontaneous rates in 219 units. 60% of the cells have mean rates higher than 20/sec.

B; the shapes of TIH of 3 different units of mean rates 36/s, 100/s, 132/s. All histograms show characteristic short mode (<10 msec.) and exponential tails. Vertical scale is linear.

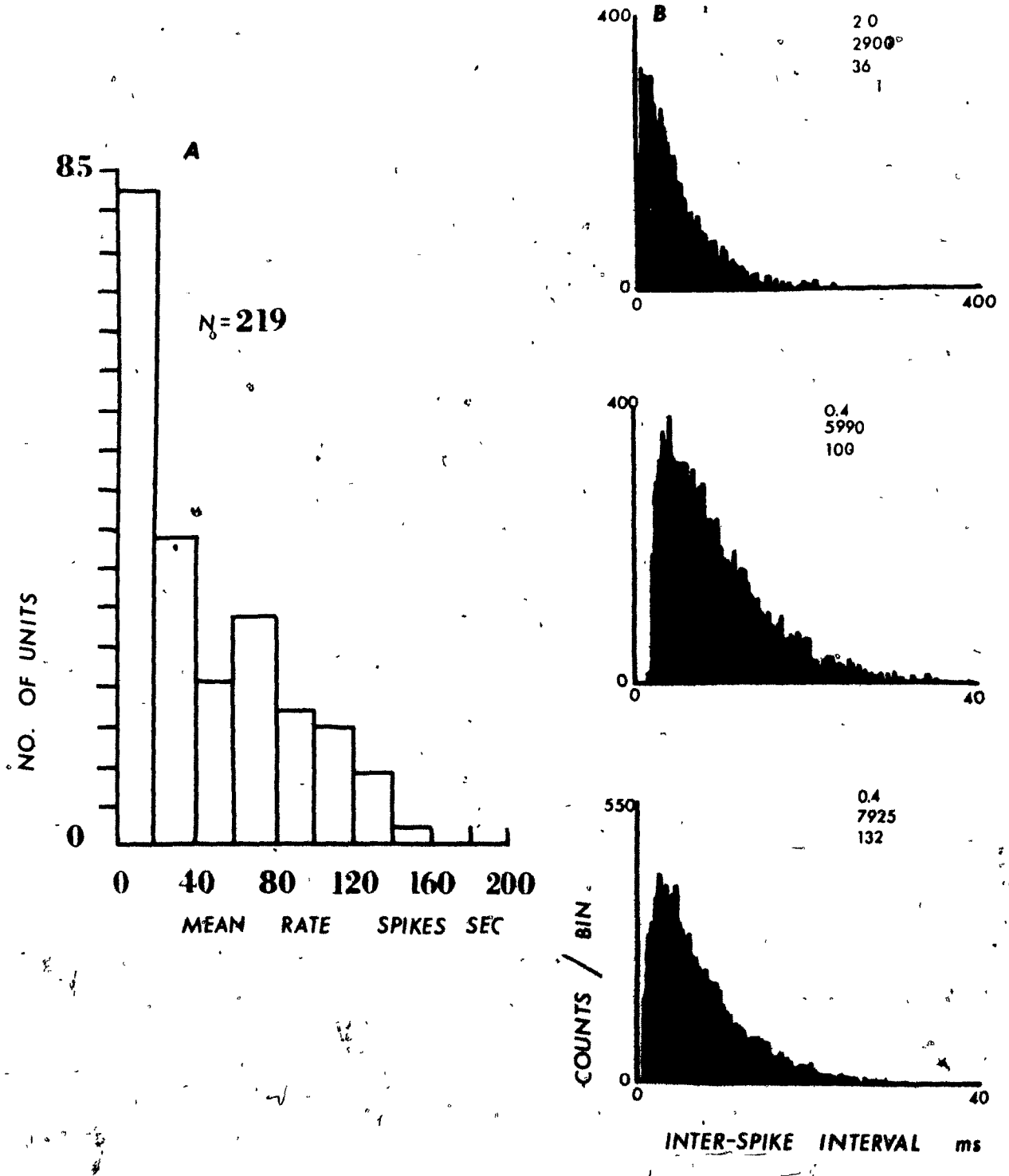
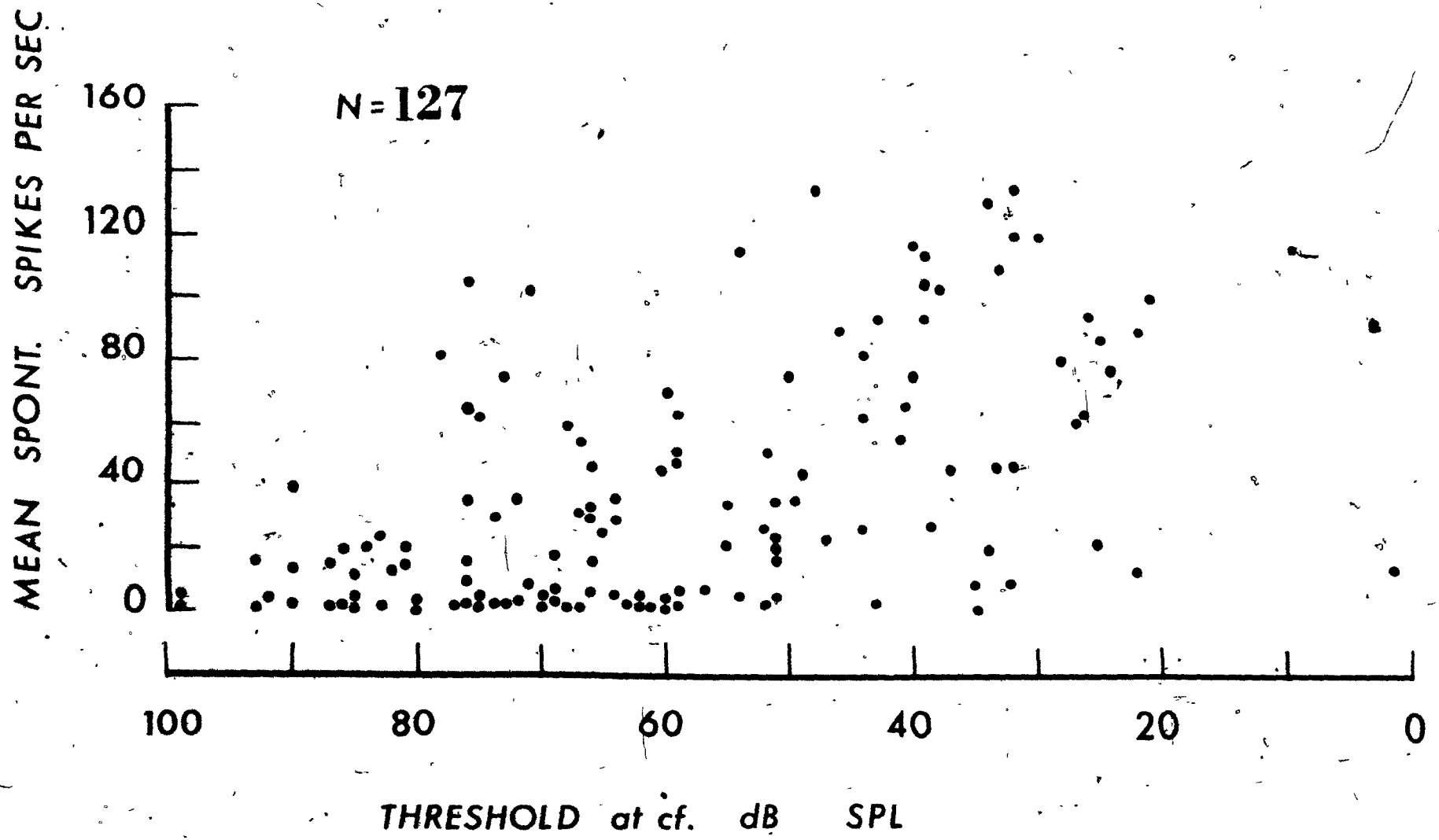

A
85
0
N = 219
NO. OF UNITS
0
40
80
120
160
200
MEAN RATE SPIKES SEC
B
400
0
400
2 0
2900
36
1
400
0
40
0.4
5990
100
550
0
40
0.4
7925
132
COUNTS / BIN
INTER-SPIKE INTERVAL ms

Fig. 5. Relation between mean spontaneous rate and threshold to auditory stimulation at the best frequency, for 127 ganglion cells. For sensitive units, there appears to be no relation between threshold and spontaneous rate.


N = 127
MEAN SPONT. SPIKES PER SEC
160
120
80
40
0
100
80
60
40
20
0
THRESHOLD at cf. dB SPL

As seen in Fig. 4B, cells with very different mean rates showed similar shapes of TIN. In Fig. 6 it can be seen that the modes of TIH in all those cells for which smooth histograms were obtained are less than 10 ms. Differences in mean rates resulted mainly from differences in the slopes of the exponential tails (i.e. variations in the probability of occurrence of longer interspike intervals). In Figs. 2, 3, 4 vertical bin counts are plotted on linear scales, whereas in Fig. 7 a logarithmic scale is used to show the exponential nature of the TIH tails. In Fig. 7B exponential plots for 4 cells of differing mean rates are shown, illustrating the constant mode and varying probability of occurrence of longer interspike intervals. Lines are drawn by eye as shown in Fig. 7A.

One procedure which did alter spontaneous activity rates of single cells in the ganglion was transient anoxia. The effect of anoxia on the sensitivity and threshold-frequency response curves has been

Fig. 6. Modal values of TIH, plotted against mean rate of spontaneous firing for 61 ganglion cells.

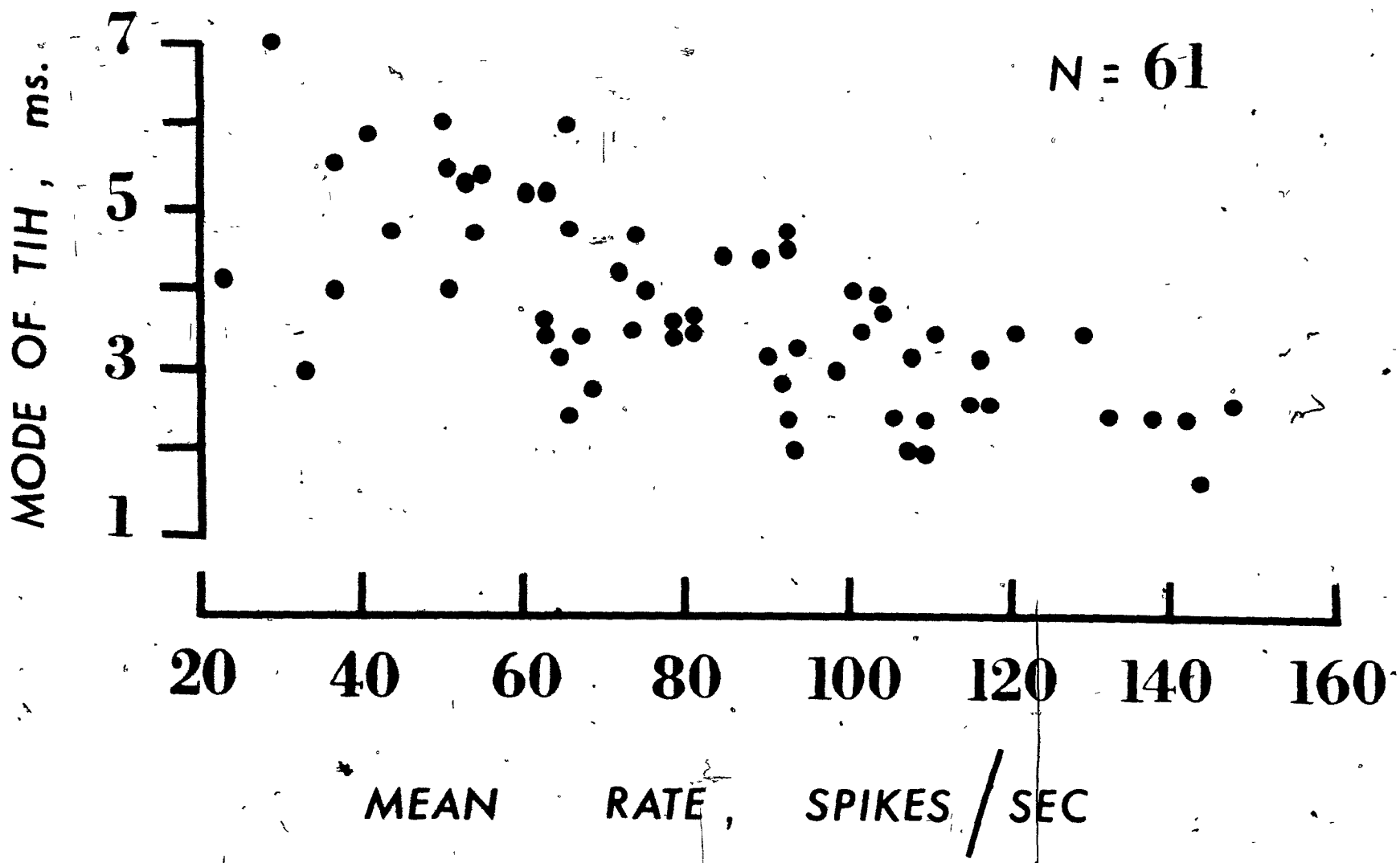
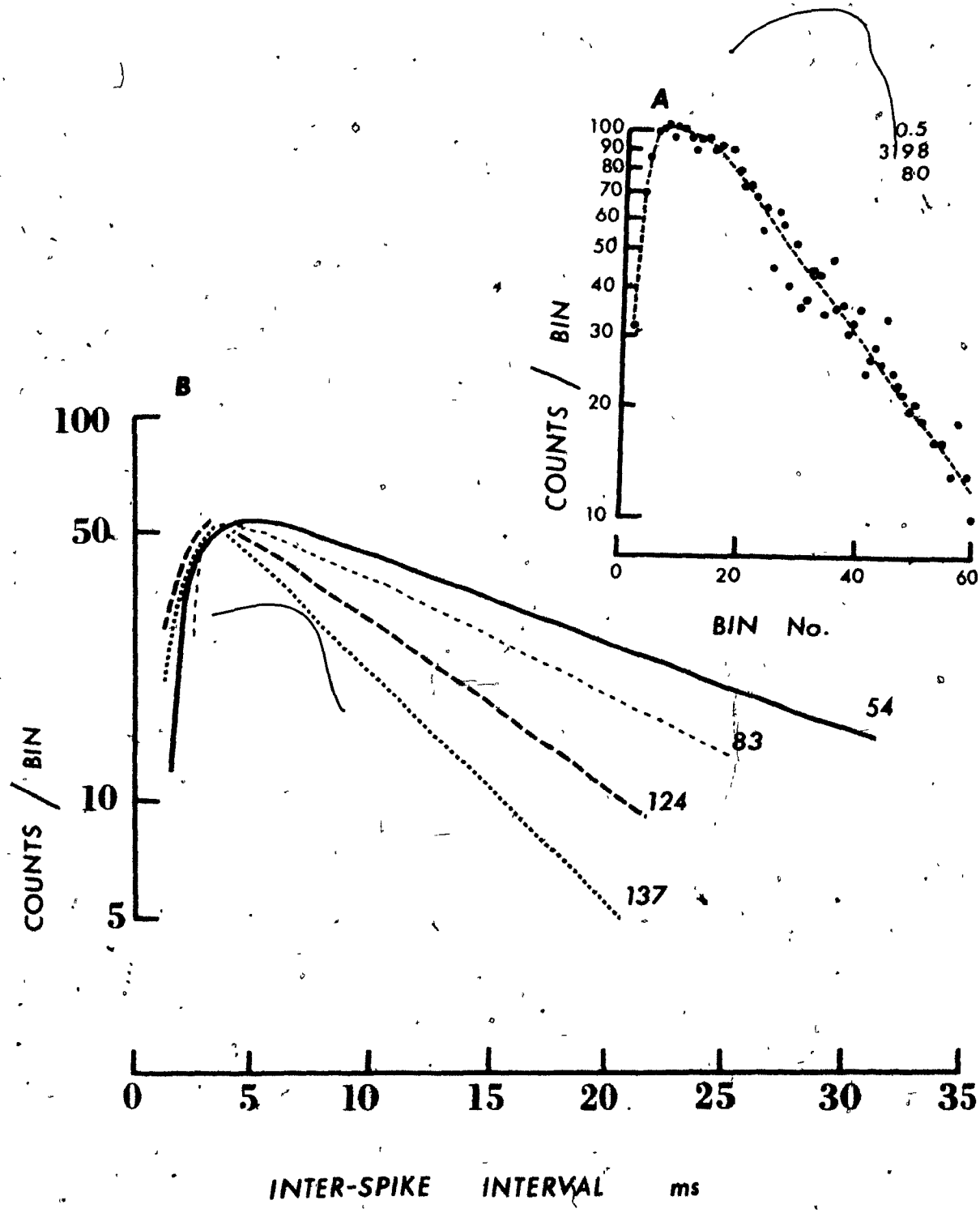
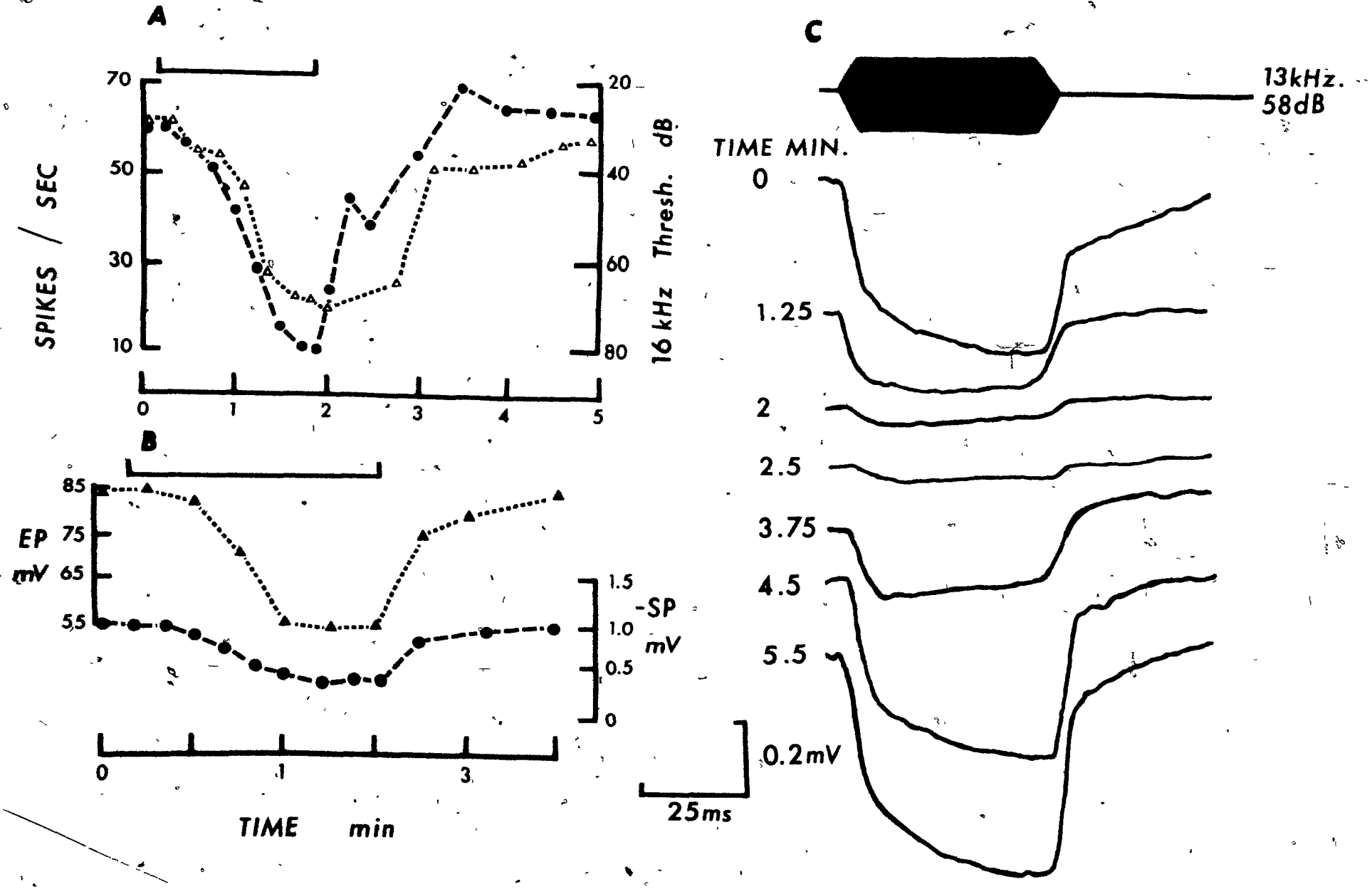

N = 61
MODE OF TIH, ms.
7
5
3
1
20
40
60
80
100
120
140
160
MEAN RATE, SPIKES / SEC

Fig. 7. Illustrating the exponential nature of the tails of TIH's. A; shows line of fit drawn by eye through vertical bin counts. The vertical axis is logarithmic, and a straight line thus denotes exponential fall off. Numbers in right hand corner denote bin width (msec.), total number of counts and mean rate (80/sec.). B; lines of fit drawn as in A, for TIH of four units with different mean rates of 54,.83, 124 and 137 spikes/sec. Vertical scale is logarithmic. The heights of modes have been made to coincide for better comparison. It can be seen that modal values are not very different whereas slopes of tails vary systematically.


A
100
90
80
70
60
50
40
30
20
10
COUNTS / BIN
0.5
3198
80
0
20
40
60
BIN No.
B
100
50
10
5
COUNTS / BIN
54
83
124
137
0
5
10
15
20
25
30
35
INTER-SPIKE INTERVAL ms

documented previously (Robertson and Manley, 1974). Fig. 8A shows the effects of anoxia, produced by slowing the rate of artificial ventilation, on threshold at the best frequency and on mean rate of spontaneous firings for a single ganglion cell. The decrease in cell sensitivity is seen to follow fairly closely the fall in mean spontaneous rate. In this experiment, the scala media summating potential (-SP) to a moderate intensity tone burst (58dB at 13kHz) was also measured by averaging 20 responses. It can be seen from Fig. 8C that the -SP also shows a dramatic decrease at the time that cell sensitivity and spontaneous firing are impaired. In Fig. 8B, the endocochlear potential (+EP) and the -SP are measured during a transient anoxia similar to the one above. Anoxia sufficient to cause almost a 70% reduction in -SP is accompanied by a 30mV fall in the resting EP.

Fig. 8. The effect of a transient anoxia on single cell properties and scala media potentials. A; filled circles are the mean spontaneous rate of a single cochlear ganglion cell, open triangles are the estimated threshold of the same cell to a 16kHz (best frequency) tone. B; the effect of a transient anoxia on the -SP to a fixed intensity tone burst at 13kHz, and on the magnitude of the endocochlear potential (EP). In A and B, the duration of anoxia is denoted by the horizontal bar above each graph. C; the effect of the anoxia shown in A, on the concurrently measured -SP in scala media. The SP is the computer average of 20 repeated responses. Time, corresponding to that in A, is denoted beside each trace. The position of the tone burst is indicated above.


A
SPIKES / SEC
70
50
30
10
20
40
60
80
16 kHz Thresh. dB
0
1
2
3
4
5
B
EP mV
85
75
65
55
1.5
1.0
0.5
0
-SP mV
0
1
3
TIME min
C
13kHz.
58dB
TIME MIN.
0
1.25
2
2.5
3.75
4.5
5.5
0.2mV
25ms

Discussion

The general properties of the spontaneous discharge of primary cochlear ganglion cells in the guinea pig, which are reported in this study, are similar to those of primary fibres in the cochlear nerve in cat and guinea pig (Kiang, 1965; Walsh et al., 1972; Evans, 1972). Data presented above reinforce the now firmly held view that such spontaneous firing is indeed endogenous to the cochlea and does not result from uncontrolled background stimulation.

A particularly interesting feature of the spontaneous activity is the independence of TIH modes of mean firing rate. The finding of modal values less than 10ms for all cells with mean rates ranging from 20 - 150/sec. is in excellent agreement with data from cat cochlear nerve fibres (Walsh et al., 1972). The highest mean rates reported here for guinea pig are considerably higher than for cat. Some caution should be exercised here as it is possible that the presence of the recording microelectrode

near the bipolar ganglion cells could modify spontaneous firing rate without clear evidence of injury. The stability of normal cell firing in many cases of high discharge rates argues against this. The distribution of mean rates is in substantial agreement with the report by Evans (1972) on guinea pig cochlear nerve fibres.

The asymmetric shape and exponential tail of the TIH also seems to be characteristic of primary auditory units. In contrast to the vestibular system, where both symmetric and asymmetric TIH are found (Walsh et al., 1972), all normal ganglion cells which were investigated showed the asymmetric shape of TIH. In the vestibular nerve, it has been suggested that asymmetric TIH are possibly a property of single fibre-hair cell connections whereas symmetric histograms might arise from multiple innervation of hair cells. Since, in the cochlea, some 90% of afferent fibres innervate single inner hair cells (Spoendlin, 1972) it might be expected

that one would tend to find only one basic shape of TIH. If symmetric histograms had been found with stable firing patterns, it might have been possible to assign these units to outer hair cells, which are innervated in multiple fashion by the remaining 10% of afferent neurones. In agreement with other authors there was no evidence for separating units into two hair cell groups on the basis of mean rates of spontaneous activity or auditory sensitivity. Only abnormally insensitive units showed any correlation between threshold and spontaneous rate. Thus it is possible either that the small population of outer hair cell neurones is being missed by our microelectrode sampling techniques, or that they do not differ from the major inner hair cell population on any of the criteria that have been used. It might be that the use of anaesthetics obscures normal differences between these two populations.

The actual source of spontaneous activity in the auditory nerve is not known. Several models have been

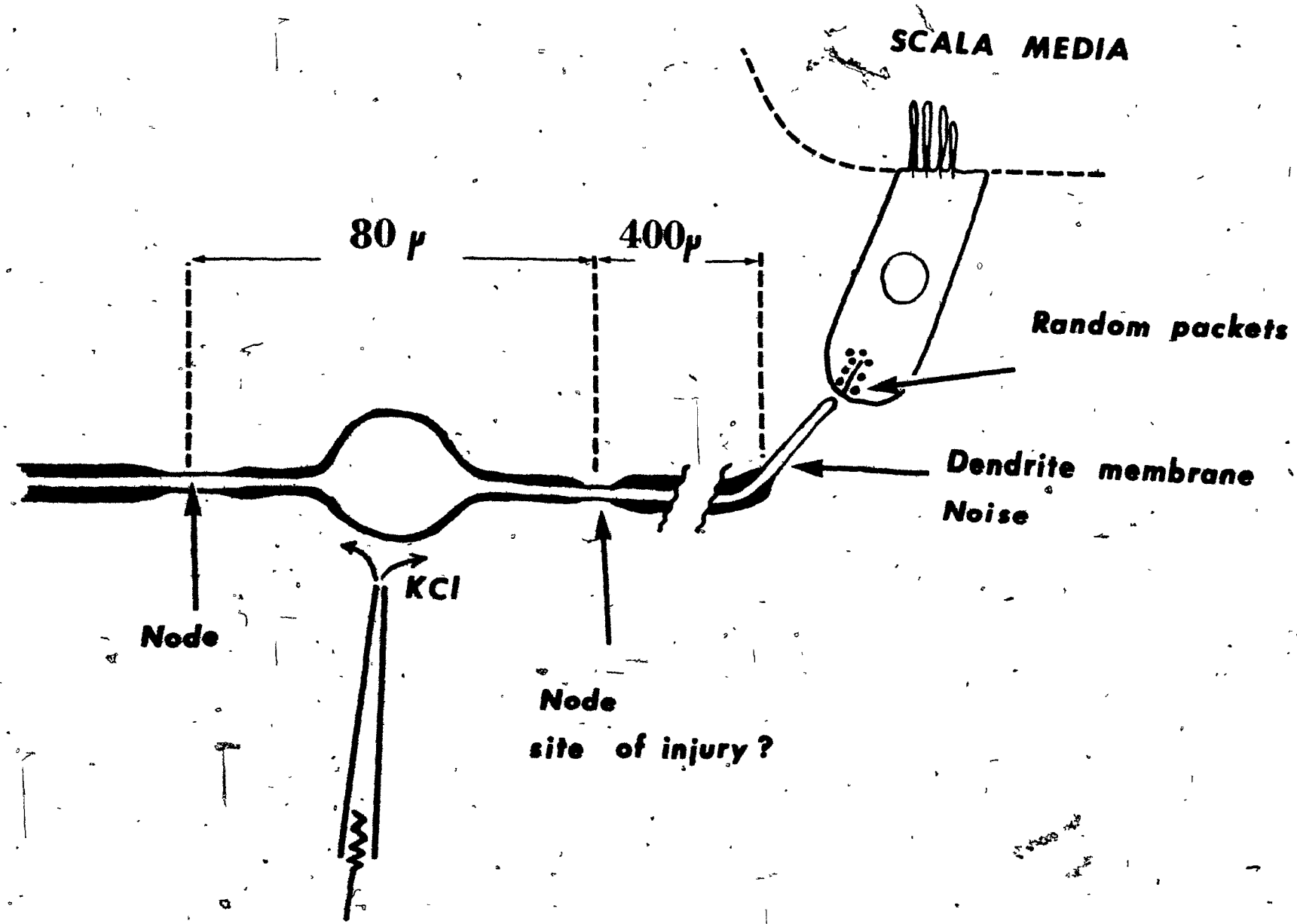
published which mimic fairly well the shapes and characteristics of TIH shown here. For the models with a structural basis, two locations have been chosen as the source of random excitation of the auditory neurones. In the model of Weiss (1966), Gaussian fluctuations in the membrane potential of the dendritic membrane itself coupled with appropriate threshold, spiking and resetting systems, give a realistic result for noise spectra with an upper limit greater than 2kHz. The ability of such noise to produce firings (i.e. exceed threshold) is supposed to depend on fibre size (Verveen, 1963; Verveen and Derksen, 1968). Thus, for nerve endings as small as those within the organ of Corti, small differences in fibre size might explain the wide range of spontaneous rates observed without necessarily producing differences in threshold to acoustic stimulation.

A second model which also yields realistic results (Lummis, 1968) used random (Poisson) release of excitatory transmitter packets from the hair cell as the source of

spontaneous activity. This scheme has a reasonable anatomical and physiological basis (Fatt and Katz, 1952; Flock et al., 1973; Katz, 1966). Differences in mean rate might result from differences in synaptic geometry (Kuno et al., 1971) or some other unknown synaptic property. In such a model, the mean rate of packet release is assumed to depend on the hair cell membrane potential. It is known that a large standing current exists from scala media to scala tympani, through the hair cells (Honrubia et al., 1971; Johnstone et al., 1966) and changes in this standing current might be expected to modulate the rate of transmitter release. This is the basis of the currently held theory of excitation of the auditory nerve fibres by sound (Davis, 1954, 1958, 1965; Honrubia et al., 1971; Honrubia and Ward, 1970; Johnstone et al., 1966). During transient anoxia, the fall in +EP and accompanying drop in the -SP are consistent with a reduction of hair cell standing leakage current. This would be expected to decrease

the mean rate of transmitter release by the above model, and hence cause a reduction in spontaneous activity. This is in fact observed (Fig. 8). Such a scheme receives some support from scala media polarization experiments (Konishi et al., 1970), where it has been shown that spontaneous activities of cochlear nerve fibres can be increased or decreased by passing currents between scala tympani and scala vestibuli. Of course such data is not conclusive, as both anoxia and polarizing currents could equally well affect the sensitivity and membrane properties of the afferent dendrites in the organ of Corti, independently of effects on hair cell transmitter release. Further support comes from the observation (Kiang, 1965) that the firing of single cochlear nerve fibres in response to a click, is reduced below spontaneous rate during the direction of basilar membrane movement corresponding to a decrease in the hair cell standing current. A tentative scheme for spontaneous activity generation is shown in Fig. 9. Two

Fig. 9. A schematic representation obtained from light and electronmicroscopy of the cochlear ganglion cell -- hair cell complex. 2 sites of generation of ~~normal~~ sponto. are indicated within the organ of Corti, and the site of injury, probably within the ganglion itself. Thick black areas on the cell denote myelination. The cell soma is myelinated.


SCALA MEDIA
80 μ
400μ
Random packets
Dendrite membrane
Noise
KCl
Node
Node
site of injury ?

possible sites for the source of spontaneous activity are shown; a) random transmitter release from the hair cell influenced by hair cell leakage currents, and b) membrane noise in the dendrite itself. It may be that both these factors operate together. As shown in the figure, the source of normal spontaneous activity probably lies some 300-400 microns from the recording site in the ganglion itself. The site of generation of injury discharges by mechanical damage by the electrode, or KCl outpouring onto the cell, is thus probably different from the normal location of the spontaneous activity source. Evidence exists that the myelinated ganglion cell soma is of high threshold compared to the nodes of Ranvier a short distance on either side (Robertson, in preparation). It is probable that the symmetric TIH produced by injury is the result of repetitive firing of these nodes in response to suprathreshold depolarization.

One finding, however, raises a further problem

concerning the site of generation of normal spontaneous activity in the primary auditory afferents. It is apparently not possible to show a clear reduction in spontaneous activity in the majority of cochlear nerve fibres, by stimulation of the crossed olivo-cochlear bundle in the medulla (Wiederhold and Kiang, 1970). This efferent system does however cause a marked reduction in sound-evoked activity and presumably acts by shunting of hair cell leakage current away from the excitatory synapse, or by stabilizing the afferent dendrite membrane (Desmedt and Monaco, 1961; Desmedt and Robertson, in press; Fex, 1967). The lack of effect on spontaneous activity is thus surprising, and raises the question as to whether spontaneous firing may be generated at some point more central than within the organ of Corti. The small lengths of bare afferent nerve fibre within the habenula perforata may be sufficiently distant from the efferent endings to escape their stabilizing effect. Such ideas are however in conflict with the evidence

discussed above that the spontaneous activity can be modulated by the changes in hair cell leakage current. There are thus many questions to be answered, and some interesting data might be obtained by studying the effects of localized current applications using the spiral ganglion preparation.

Summary

1. Spontaneous activity of single neurones in the guinea pig spiral ganglion was studied by measurement of mean rates and computation of interspike interval histograms (TIH).

2. The shapes of TIH and the stability of firing patterns allowed cells to be classified as normal or injured. The properties of spontaneous activity of normal cells agree with those of cochlear nerve fibres in cat.

3. Alterations in sensitivity of the neurones by perilymph removal or rupture of the middle ear ossicular chain, did not affect rates of spontaneous firing.

4. Except for animals with very insensitive cells, there was no obvious relation between mean spontaneous rates and thresholds to tone stimuli.

5. The simultaneous effects of respiratory impairment on cell thresholds, spontaneous activity and on cochlear potentials are reported.

6. The results are discussed in relation to the site of generation of spontaneous activity in the primary auditory neurones.

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References

Bishop, P.O., Levick, C. R., Williams, W.O.: Statistical analysis of the dark discharge of lateral geniculate neurones. J. Physiol. (Lond.) 170, 598-612 (1964).

Davis, H.: A model for transducer action in the cochlea. Cold Spring Harb. Symp. Quantit. Biol. 30, 181-189 (1965).

Davis, H.: A mechano-electrical theory of cochlear action. Ann. Otol. Rhin. Laryng. 67, 789-801 (1958).

Davis, H.: The excitation of nerve impulses in the cochlea. Ann. Otol. Rhin. Laryng. 63, 469-481 (1954).

Desmedt, J.E., Monaco, P.: Mode of action of the efferent olivo-cochlear bundle on the inner ear. Nature 192, 1263-1265 (1961).

Evans, E.F.: The frequency response and other properties of single fibres in the guinea pig cochlear nerve. J. Physiol. (Lond.) 226, 263-287 (1972).

Fatt, P., Katz, B.: Spontaneous subthreshold activity at motor-nerve endings. J. Physiol. (Lond.) 117, 109-128 (1952).

Fex, J.: Efferent activity in the cochlea related to hair cell DC activity. J. acous. Soc. Amer. 41, 666-675 (1967).

Frank, K., Becker, M.C.: Microelectrodes for recording and stimulation. In: Physical Techniques in Biological Research. 5 (Nastuk, W.L., Ed.) New York: Acad. Press (1964).

Flock, A., Jorgensen, M., Russell, I.: The physiology of individual hair cells and their synapses. In: Basic Mechanisms in Hearing. p. 273-302 (Moller, A.R., Ed.) Acad. Press (1973).

Gerstein, G.L., Kiang, N.Y.S.: An approach to the quantitative analysis of electrophysiological data from single neurones. Biophys. J. 1, 15-28 (1960).

Honrubia, V., Strelioff, D., Ward, P.H.: The mechanism of excitation of the hair cells of the cochlea. Laryngoscope 79, 1719-1725 (1971).

Honrubia, V., Ward, P.H.: Mechanism of production of cochlear microphonics. J. acous. Soc. Amer. 47, 498-503 (1970).

Johnstone, B.M., Johnstone, J.R., Pugsley, I.D.: Membrane resistance in the endolymphatic walls of the first turn of the guinea pig cochlea. J. acous. Soc. Amer. 40, 1398-1404 (1966).

Katz, B.: Nerve, Muscle and Synapse. New York: McGraw-Hill, 1966.

Kiang, N.Y.S.: Discharge patterns of single fibres in the cat's auditory nerve. Cambridge (Mass.): M.I.T. Press, 1965.

Kiang, N.Y.S., Moxon, E.C., Levine, R.A.: Auditory nerve activity in cats with normal and abnormal cochleas. In: Sensorineural Hearing Loss, p. 241-273 (Wolstenholme, G., Knight, K., Eds.) London: Churchill 1970.

Konishi, T., Teas, D.C., Wernick, J.S.: Effects of electrical current applied to the cochlear partition on discharges of individual auditory nerve fibres. I. Prolonged DC polarization. J. acous. Soc. Amer. 47, 1519-1527 (1970).

Kuno, M., Turkanis, S.A., Weakley, J.N.: Correlation between nerve terminal size and transmitter release at the neuromuscular junction of the frog. J. Physiol. (Lond.) 213, 545-556 (1971).

Lummis, R.C.: The secret code of hearing. Bell Lab. Record Sept. 1968 p. 261-266.

Robertson, D.: Cochlear neurones: frequency selectivity altered by perilymph removal. Science 186, 153-155 (1974).

Robertson, D., Manley, G.A.: Manipulation of frequency analysis in the cochlear ganglion of the guinea pig. J. Comp. Physiol. 91, 363-375 (1974).

Rodieck, R.W., Kiang, N.Y.S., Gerstein, G.L.: Some quantitative methods for the study of spontaneous activity of single neurones. Biophys. J. 2, 351-368 (1962).

Spoendlin, H.: Innervation densities of the cochlea. Acta Otolaryng. 73, 235-248 (1972).

Verveen, A.A., Derksen, H.E.: Fluctuation phenomena in nerve membrane. Proc. IEEE. 56, 906-916 (1968).

Verveen, A.A.: Axon diameter and fluctuations in excitability. Acta morph. neerl.-scand. 5, 79-85 (1963).

Walsh, B.T., Miller, J.B., Gacek, R.R., Kiang, N.Y.S.: Spontaneous activity in the eighth cranial nerve of the cat. Int. J. Neurosci. 3, 221-236 (1972).

Weiss, T.F.: A model of the peripheral auditory system. Kybernetik. 3, 153-175 (1966).

Wiederhold, M.L., Kiang, N.Y.S.: Effects of electrical stimulation of the crossed olivo-cochlear bundle on single auditory nerve fibres in the cat. J. acous. Soc. Amer. 48, 950-965 (1970).

PAPER V

The Relation Between Structure and Spike Shapes of Neural Elements in Guinea Pig Cochlear Ganglion.

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Running Title: Spike Shapes of Acoustic Ganglion Neurons.

SUMMARY

A two-component positive single unit spike could be recorded from neural elements in the acoustic ganglion of the guinea pig cochlea. Over 80% of units recorded with metal or glass electrodes had predominantly positive spikes. Light and electron microscopy showed that the bipolar afferent neurones and their processes are myelinated except at nodes of Ranvier positioned on either side of the cell body, about 40 microns from the point of emergence of the myelinated processes. This evidence suggests that the soma of these bipolar neurones is not excited during the passage of an action potential, and the properties of the two-component spike suggest that it arises from successive activation of the nodes of Ranvier on either side of the cell body. The advantage of such a conduction system is discussed.

INTRODUCTION

The acoustic, or spiral ganglion in the vertebrate cochlea contains mainly the bipolar cell bodies of the primary afferent auditory neurones. These cells send one process peripherally, where they receive excitation from the hair cells of the organ of Corti, and the other to the internal auditory meatus and thence the cochlear nucleus. The ultrastructure of these cells has been intensively studied in several species (9, 14, 15, 17, 21). Both processes of the cells are myelinated and the somas of approximately 90% of the cells in the ganglion are also covered in myelin. The distal processes do not lose their myelin until the point of entry into the organ of Corti, some 400 microns from the ganglion. Little attention has been paid to the location of nodes of Ranvier on the bipolar ganglion cells. One study of the acoustic ganglion of the goldfish (15), comments on the presence of nodes of Ranvier about 20 microns from the cell

bodies. In this paper, the location of nodes on the bipolar ganglion cells of the guinea pig spiral ganglion are examined, as well as the distribution of myelin on the cell bodies and processes. The shapes of extracellularly recorded spikes from single nerve cells in the ganglion are also reported and the relation between the cell anatomy and the extracellularly recorded waveforms is discussed.

METHODS

a) Electrophysiology:

The surgical exposure used and the technique of recording from single units in the spiral ganglion in guinea pigs, have been described previously (13). The experimental animals were pigmented guinea pigs (150 - 400 g). They were anaesthetized with 35mg/kg of pentobarbital, relaxed with Quelecin (Roche) and artificially ventilated. Single unit extracellular recordings were obtained mainly with metal-filled glass micropipets (3). The diameter of the gold-platinum ball on the tip of these electrodes was 5-8 microns. Similar electrodes have recently been used in an analysis of extracellular spike shapes in frog olfactory epithelium (6). The electrodes were connected to a Grass P15, AC preamplifier with bandpass 10-3000 Hz, and records were photographed from an oscilloscope screen with Polaroid film. The frequency response of this system to a square wave at the electrode tip, is shown in Fig. 2D. Less frequently,

DC recordings were obtained with 3M KCl glass micropipets connected to a WPI high impedance probe. The initial DC resistances of these electrodes was 10-20 megohms in 0.9% saline. Movements of the microelectrode were effected by a remote hydraulic microdrive calibrated in microns. No intracellular recordings were made. The spatial relationship between the spiral ganglion, Organ of Corti and recording microelectrode is shown in Fig. 1.

b) Histology:

Cochleas were fixed according to the method reported by Ross (15) and portions of the ganglia were dissected out and embedded in Spurr plastic (18). 2 micron serial sections for light microscopy were cut with glass knives and stained for ½ minute with 1% toluidine blue. Ultrathin silver-gold sections were cut from the same blocks and mounted on 200 mesh copper grids or on Formvar-coated slotted grids. They were

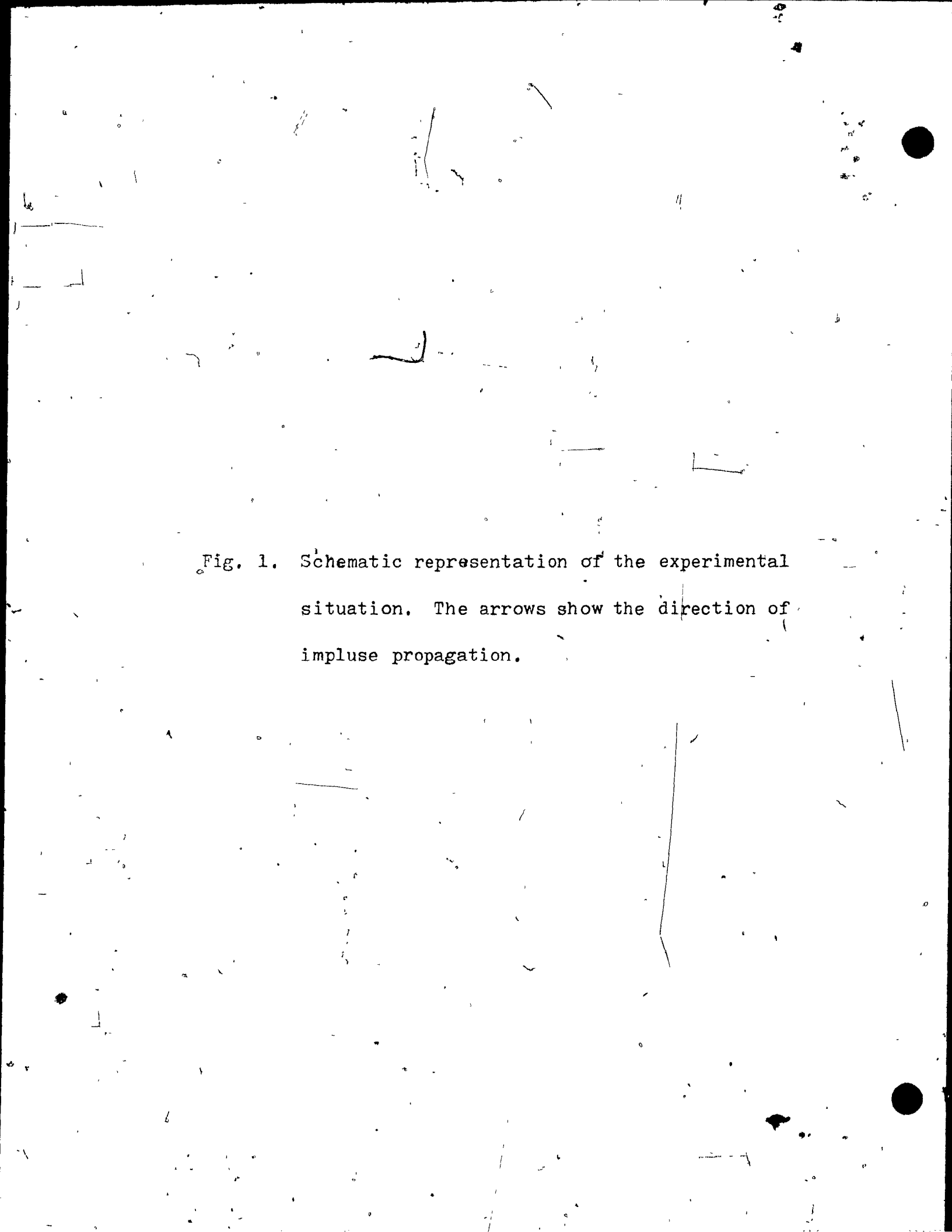
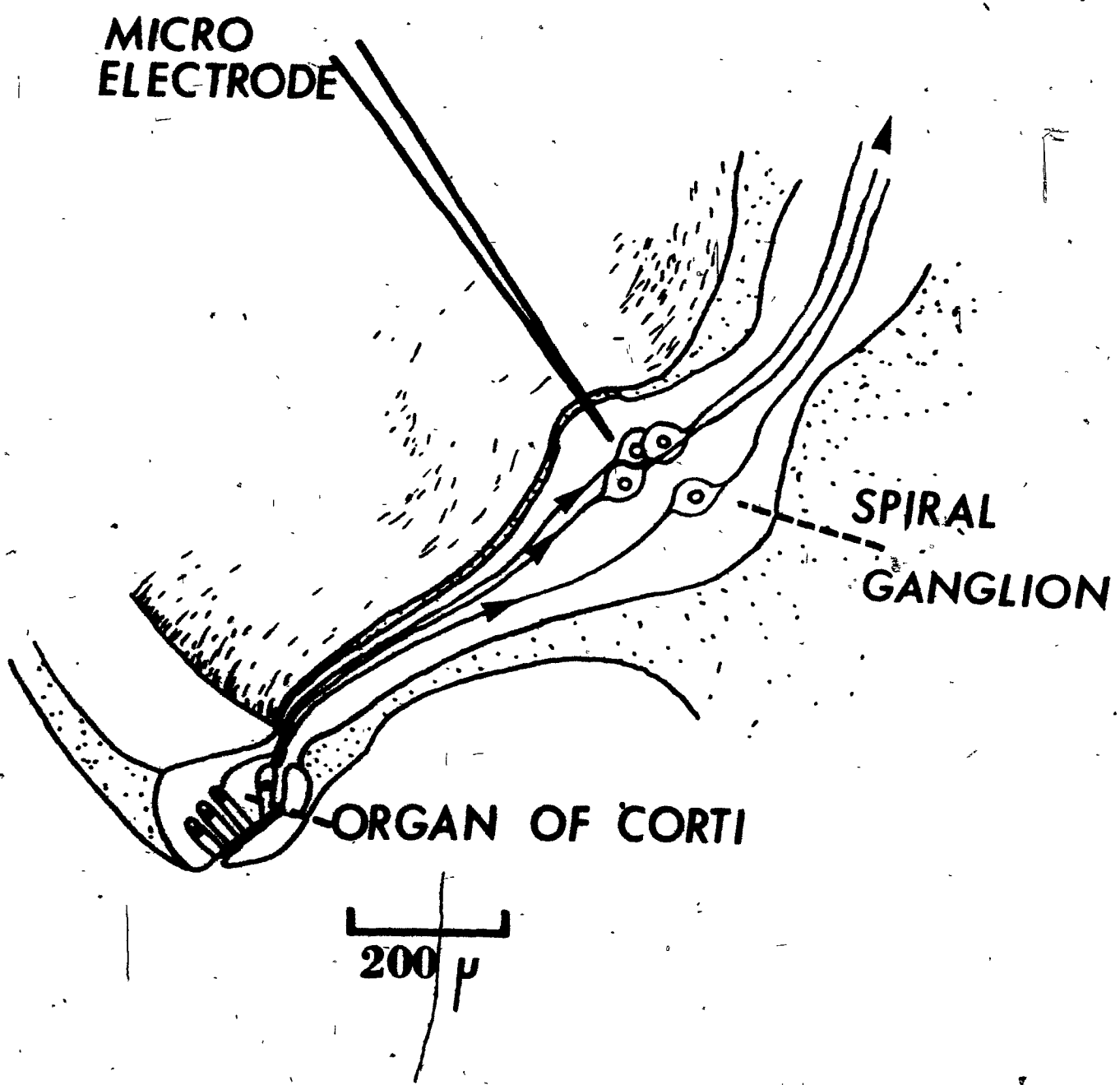


Fig. 1. Schematic representation of the experimental situation. The arrows show the direction of impluse propagation.


MICRO
ELECTRODE
SPIRAL
GANGLION
ORGAN OF CORTI
200 μ

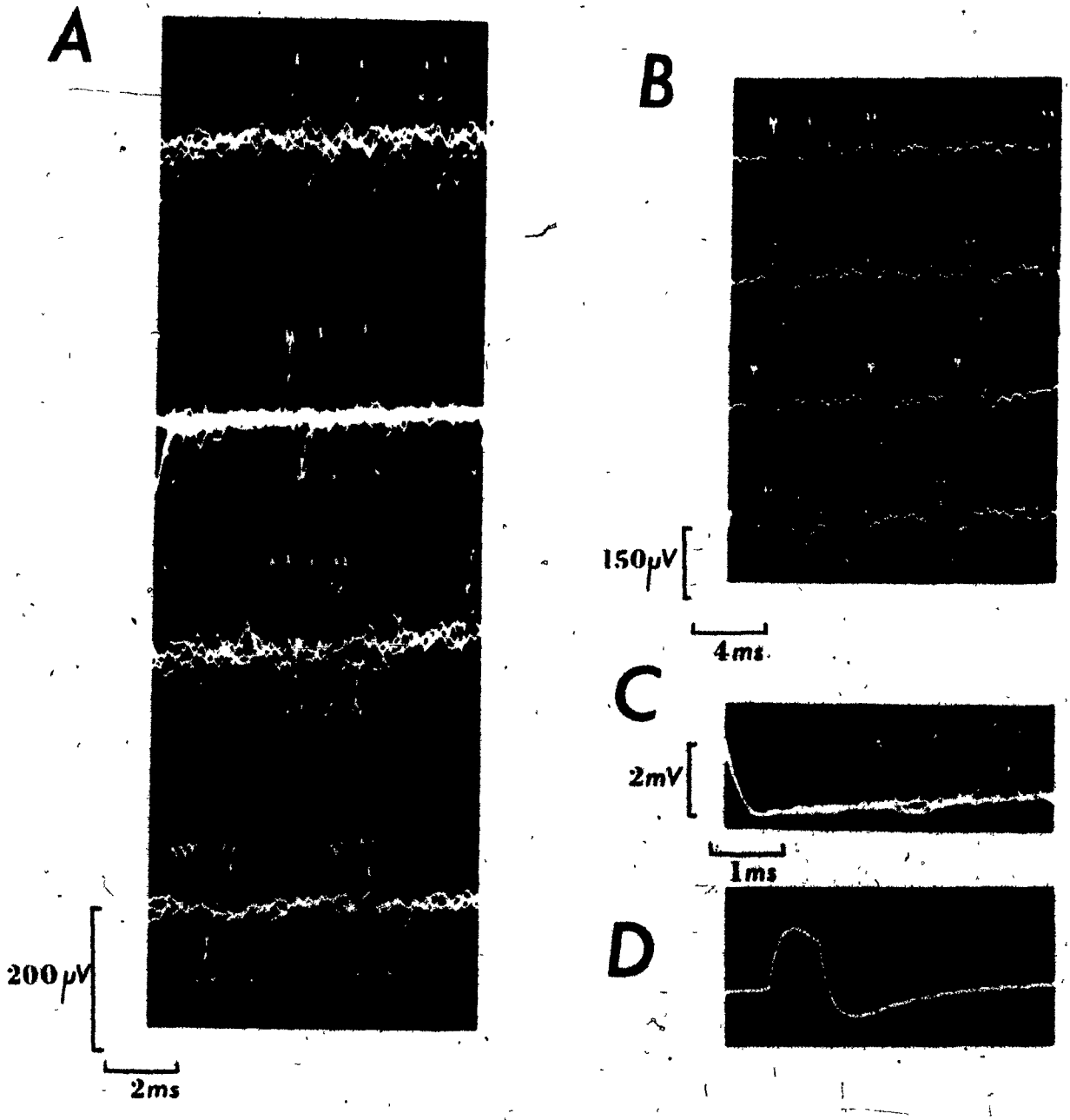
stained with lead citrate, and examined in a Zeiss EM-9 electronmicroscope, at magnifications from 1,700 to 18,000.

RESULTS

a) Spike waveforms:

Reliable recordings with a good signal to noise ratio were obtained from 190 units in 50 guinea pigs. Except where noted, all activity reported here is spontaneous in nature (10, 13). The vast majority (over 80%) of single unit spike shapes recorded with both metal and KCl-filled electrodes showed a predominantly positive phase. A certain amount of later negativity was seen in AC-coupled recordings, due to the band pass of the recording system (Fig. 2D). The largest spikes recorded with metal electrodes were 350 microvolts peak-to-peak. With KCl-filled pipets however, the spikes could sometimes reach 4mV in size (Fig. 2C). The most complex spike shape was found most frequently with metal electrodes and consisted of a two-

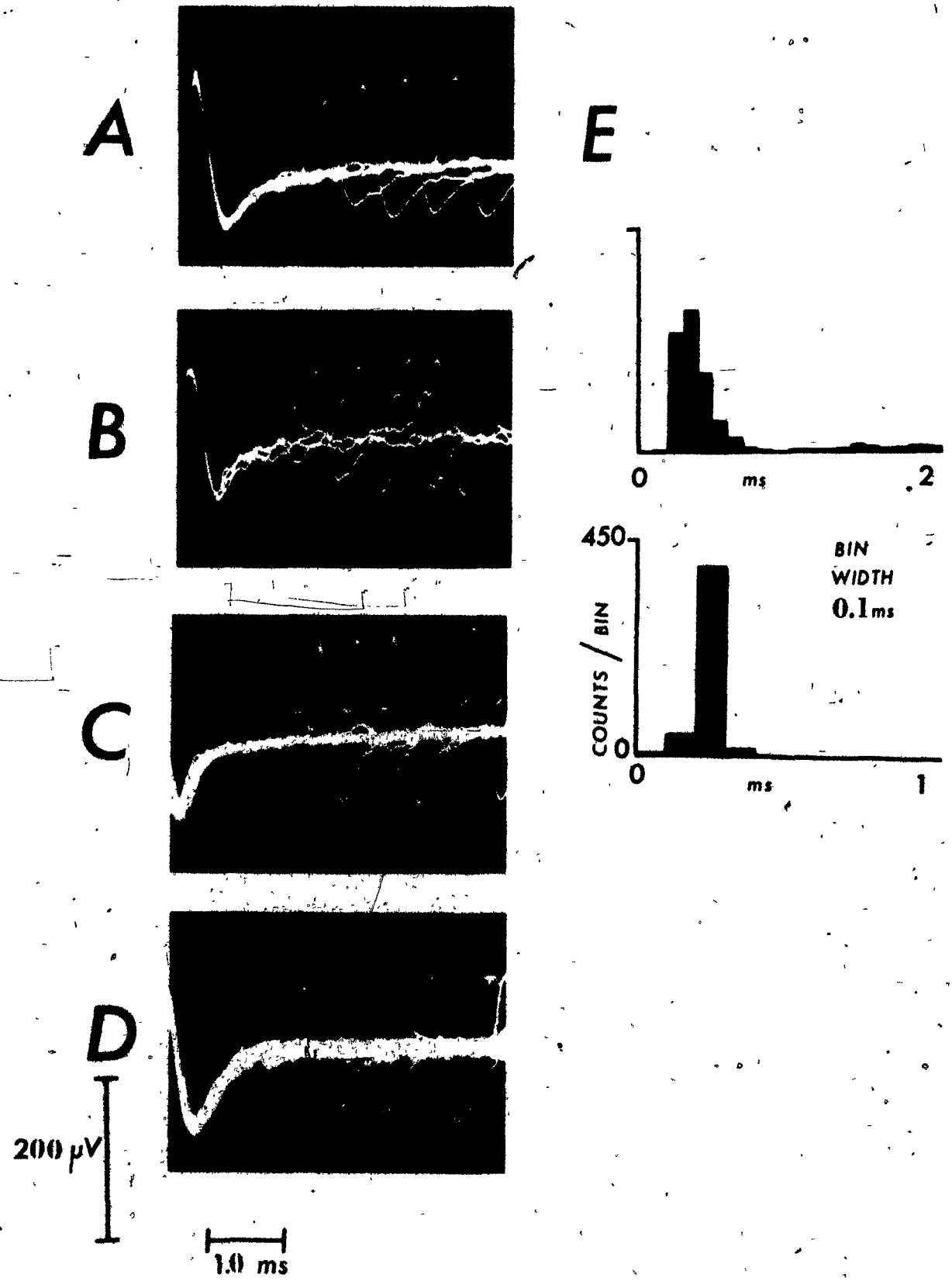
Fig. 2. Oscilloscope tracings of single unit spontaneous activity. Positive deflections are upwards in this and all other figures. A, spike shapes of four units in different animals. The first component is indicated by a dotted line. B, successive tracings of one unit, showing two component shape, and the occasional dropping out of one component (dotted line). C, large, positive spikes with no first component. The trace is a DC record, obtained with KCl-filled electrode. D, response of system to a 2ms square pulse applied to the electrode tip.


A
200 μV
2ms
B
150μV
4ms
C
2mV
1ms
D

component positivity (Fig. 2A, B). Of the 114 units recorded with metal electrodes, 38% could definitely be classified as having such a two-component waveform. With glass micropipets, the percentage was somewhat smaller (20% of 76 units). In a single unit, there were small variations in the delay between the first and second components (Fig. 3E). Between different units there was also a variation in the position and magnitude of the initial phase relative to the second (Fig. 2A). In some cells the initial phase was quite small and almost lost in the baseline noise, whereas other units, often recorded in the same pass of the electrode, showed very large positive double peaks (Fig. 2B). That the two components derive from different units is unlikely, because of the consistency of the waveform and its persistence for long periods of time (up to 1 1/2 hrs. of recording).

In Fig. 3, spike shapes from 4 different units in 4 animals are shown. Successive sweeps of the

Fig. 3. A-D, superimposed sweeps of spontaneous activity, in four different units, triggered as described in the text. Where the differentiation of the first component is not clear, it is indicated by a dotted line. E, histograms of the delay between the first and second components for two different units not the same as in A-D.


A
B
C
D
E
200 μV
1.0 ms
0
ms
.2
450
BIN
WIDTH
0.1 ms
COUNTS / BIN
0
0
ms
1

oscilloscope were triggered on the largest positive component of the spikes. It can be clearly seen that the delay observed between the two positive spike components depends on the interspike interval. Those spikes which occur soon after the triggering spike show a wide separation (up to 0.5msecs) between the two components, whereas those spikes occurring later, show less separation. In Fig. 3D, the spikes occurring at long interspike intervals show a simple, unimodal shape apparently derived from a fusion of the components which are clearly visible at the shorter intervals. Those units which showed a very distinct differentiation between the two components (Fig.2B) still exhibited this basic shape at the longest interspike intervals. In such cases, the second component could occasionally fail to appear, leaving a spike shape similar in size and time course to the first component alone (Fig. 2B).

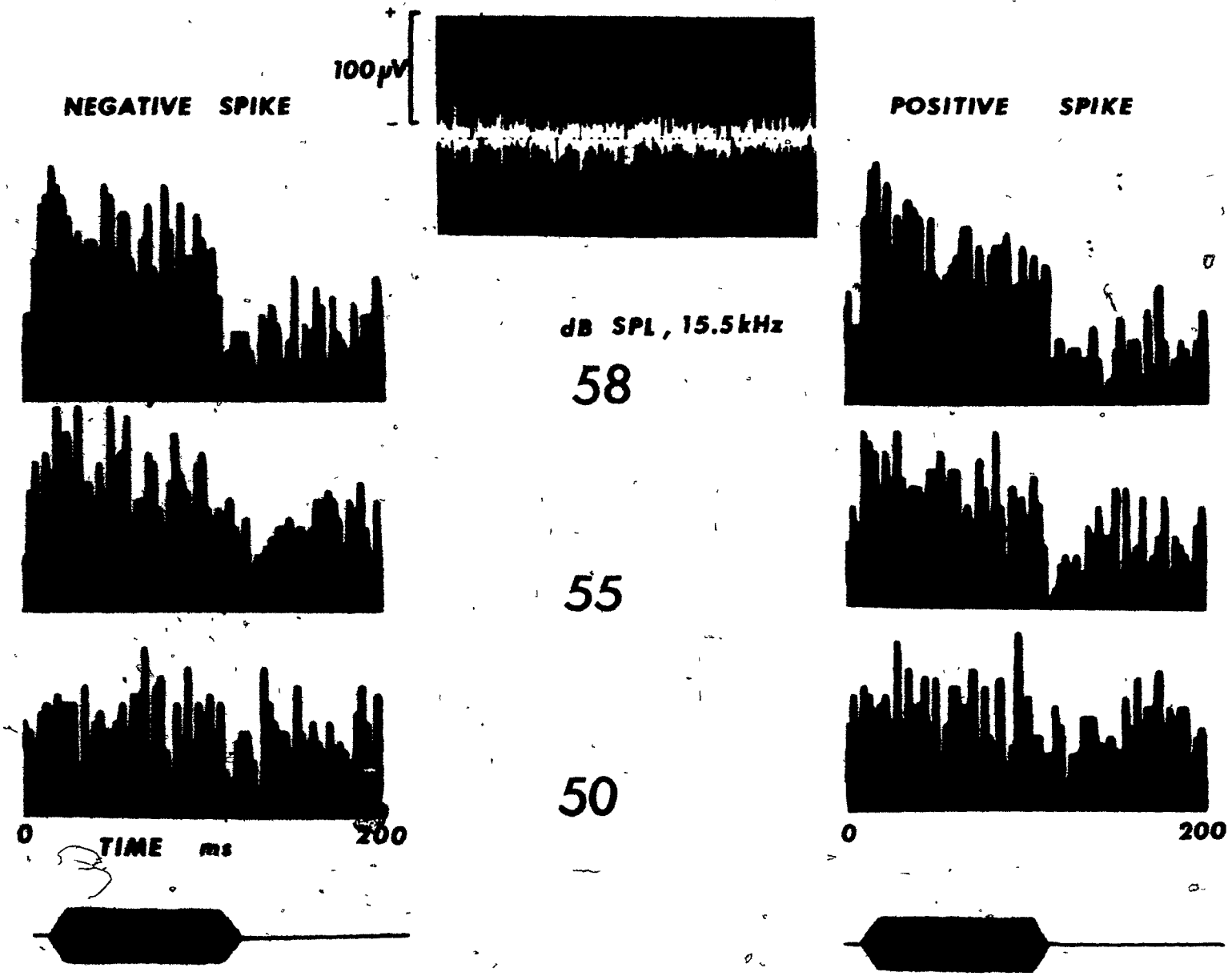
A second spike waveform also encountered showed a monophasic, positive shape which never showed a two-

component waveform (Fig. 2C). These spikes comprised 74% of the units recorded with KCl-filled pipets and 43% of those recorded with metal electrodes. They were easily lost, either spontaneously or by advances of the electrode microdrive of less than 10 micra. In contrast, the two-component spikes were usually only lost by deliberate movements of the microelectrode through 50 micra, or more.

The third spike shape was found least often (19% with metal electrodes and 6% with glass), and showed a predominantly negative waveform with very little positivity. These unit spikes rarely exceeded 50 microvolts in size and were easily lost.

There was no suggestion that the different spike shapes corresponded to units with different physiological properties. All three types of unit showed responses to tone stimuli within the normal range (13). Frequently 2 units of different waveform could be recorded simultaneously with the same electrode (Fig. 4) with no sig-

Fig. 4. Post-stimulus histograms of a positive and negative unit recorded simultaneously with the same electrode. It can be seen that the thresholds of both units to a tone burst are practically identical. The spontaneous activities of both units had mean rates of 70/sec.


+
100 μV
-
NEGATIVE SPIKE
POSITIVE SPIKE
dB SPL, 15.5 kHz
58
55
50
0
TIME ms
200
0
200

nificant differences in their pure-tone thresholds. Spontaneous activities varied greatly between units, but no one type of spike shape showed a tendency to exhibit particularly high or low mean rates.

b) Histology:

Examination of 2 micron serial sections in the light microscope revealed the presence of a node of Ranvier on the central and peripheral processes of the myelinated ganglion cells. The nodes were consistently situated about 1 cell body length from the point of emergence of the processes from the cell soma (Fig. 5A). In toluidine blue-stained sections, the region of axon on the side of the node furthest from the cell body showed a darker staining of the myelin sheath than on the cell body or the emerging processes.

Examination of the above structures in the electron microscope confirmed the general picture obtained with light microscopy. Nodes showed a typical unfolding of the axonal myelin, exposing the underlying axonal

Fig. 5. A, light micrograph of a toluidine blue stained ganglion cell. Arrow indicates the location of a node of Ranvier. Calibration line is 10 microns. B, shows both compact axonal myelin (at top of figure), running adjacent to loose myelin on a cell body (lower part). C, loose myelin on the emerging axon of a cell. D, a nodal region, with bare axonal membrane and typical unfolding of myelin. Calibration mark for all electron micrographs is 1 micron.

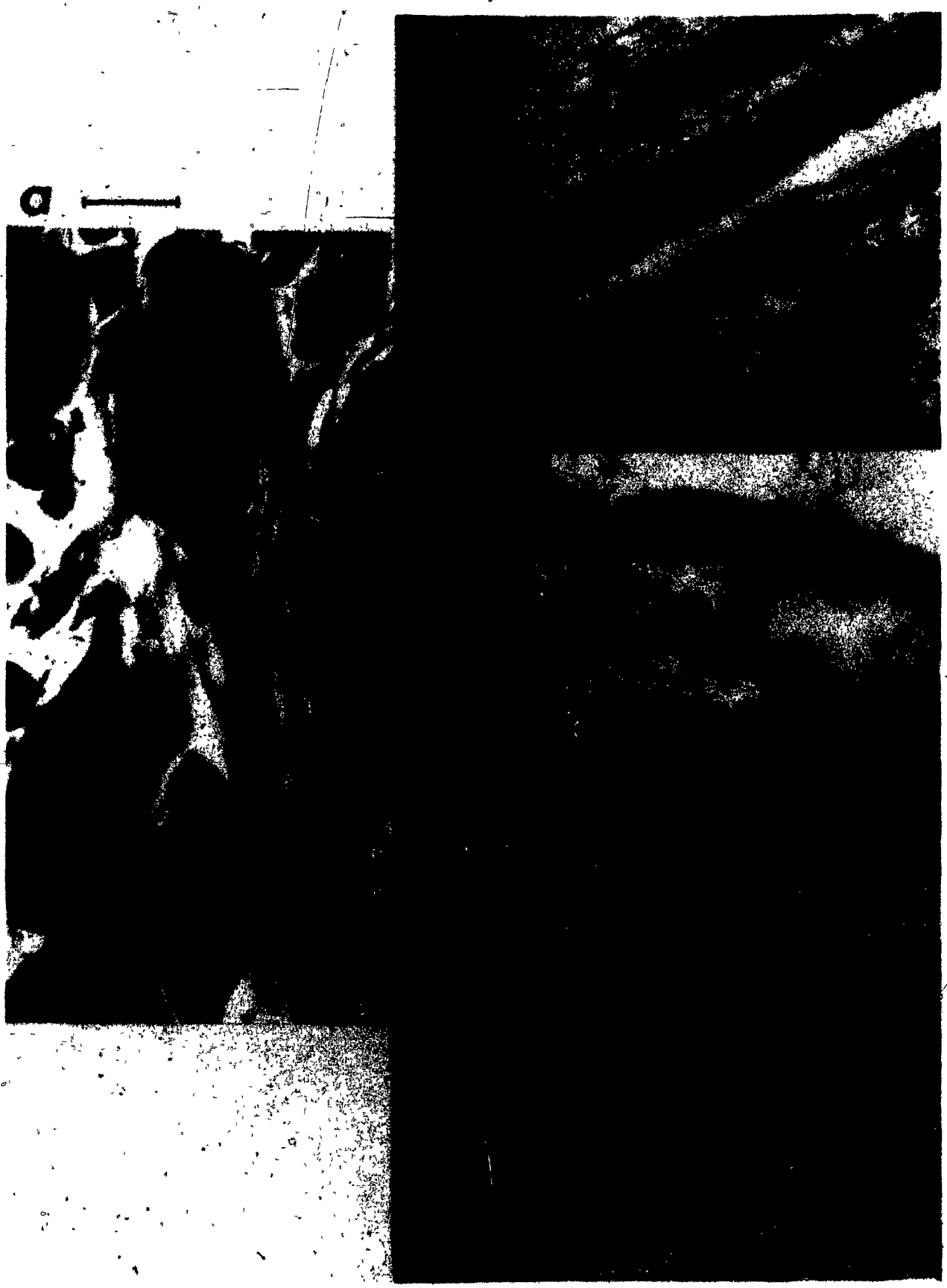
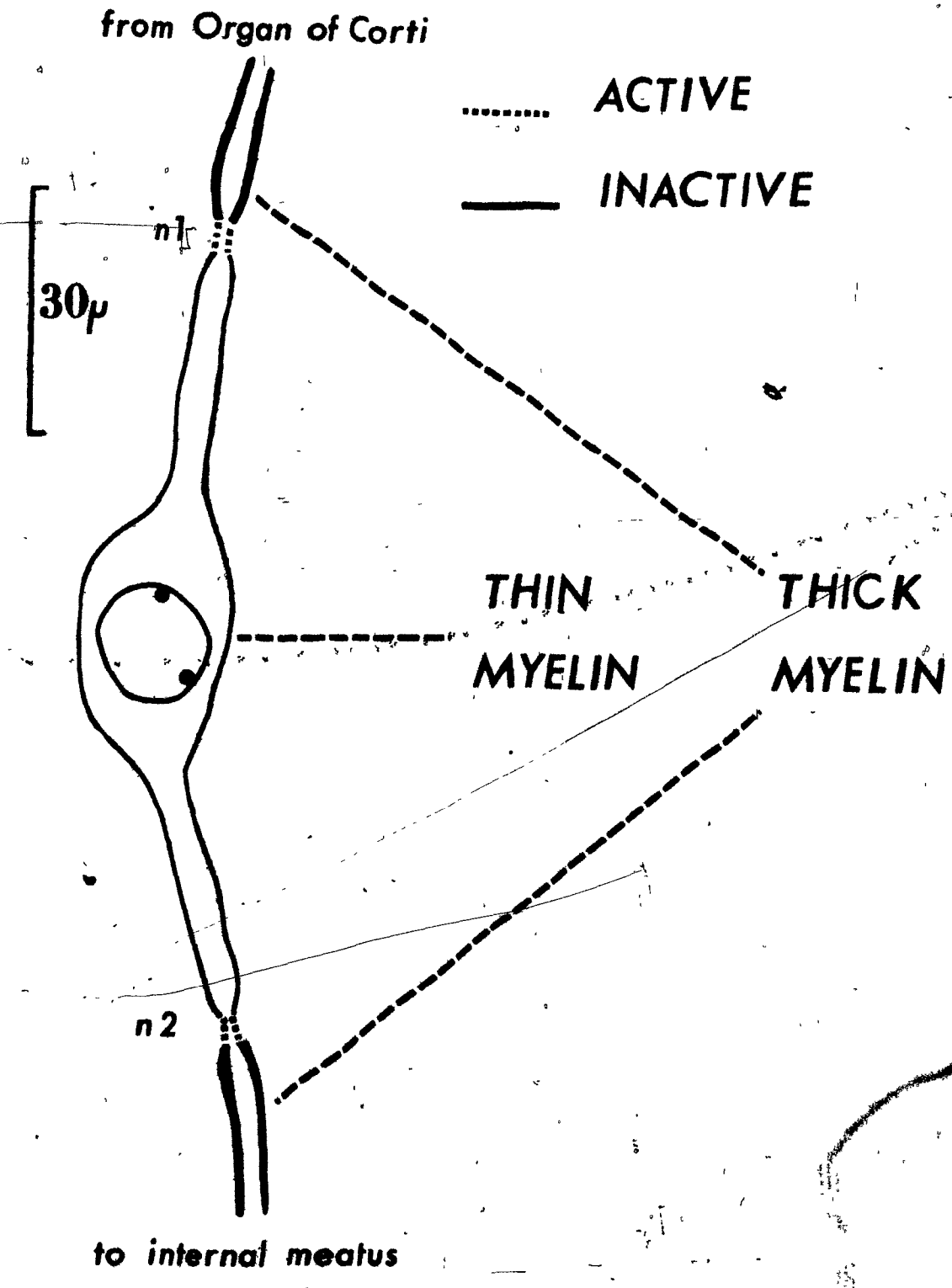

a

Fig. 6. Schematic representation of the typical bipolar afferent ganglion cell in the guinea pig cochlea. Only the nodal regions are active; the cell soma and its immediate processes have thinner myelin than on the axons beyond the nodes.


from Organ of Corti
ACTIVE
INACTIVE
n1
30μ
THIN
MYELIN
THICK
MYELIN
n2
to internal meatus

membrane (Fig. 5D). The myelin sheaths on the cell body and its emerging processes contained only 7-12 lamellae, whereas, beyond the nodes, myelin on the axons was much more compact, and contained over 20 lamellae (Fig. 5B, C). A schematic representation of a single cell and its processes is shown in Fig. 6. This general arrangement was found in every myelinated ganglion cell investigated, though variations in the position of the nodes was frequent. The longest distance found between cell soma and node was 40 micra and the shortest was 25 micra.

DISCUSSION

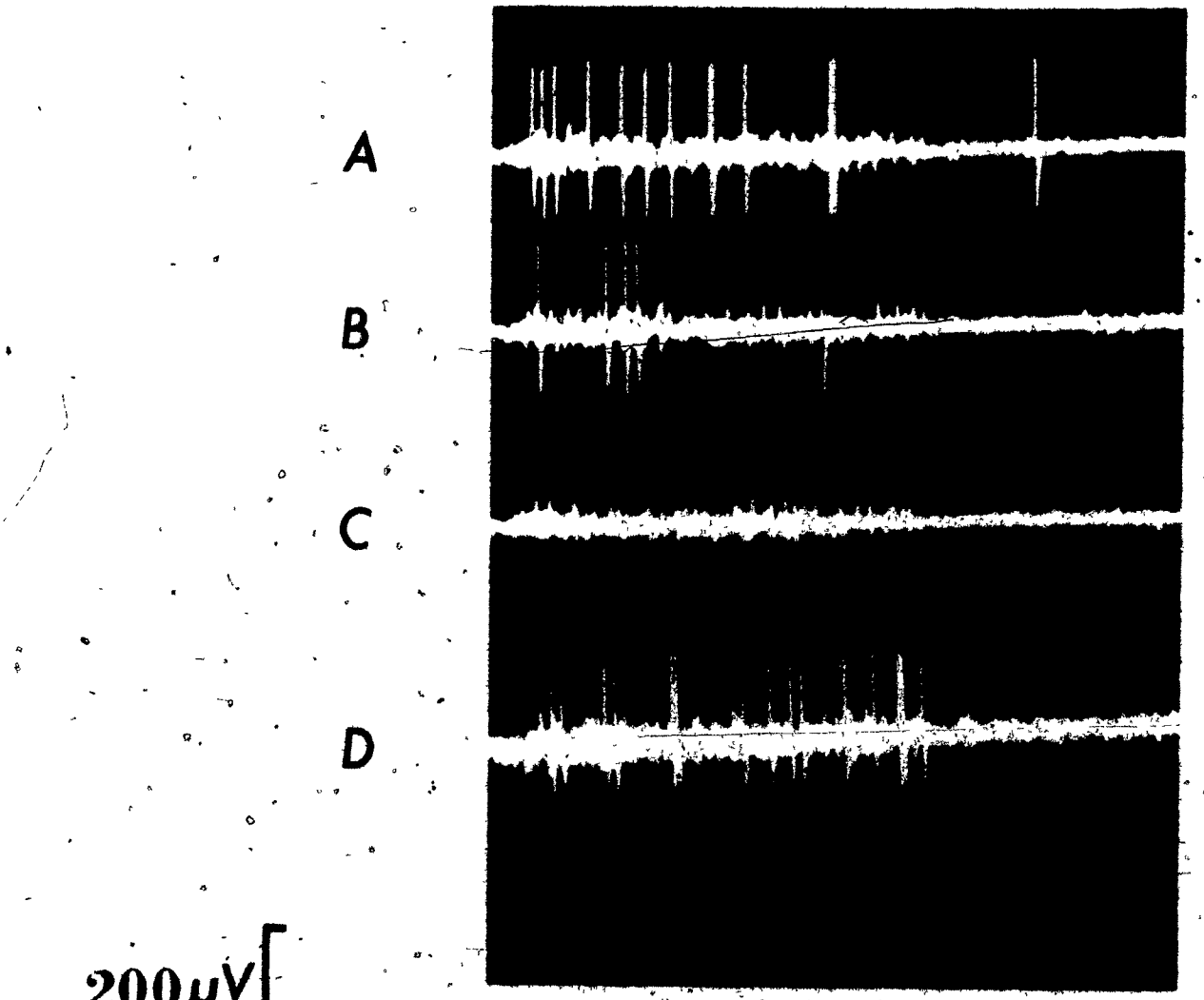
It is generally held that the myelin sheath, by reducing cell capacitance and increasing transmembrane resistance, acts to reduce the loss of action potential current through the cell membrane. Areas of nerve cells covered with myelin have much higher thresholds to externally applied current than the unmyelinated nodal regions (18). On this basis, the only active regions

of the myelinated spiral ganglion cells might be expected to be at the nodes of Ranvier on either side of the cell soma. Positive extracellular potentials have been interpreted as evidence that the region in the vicinity of the electrode is acting as a source of current (8, 5, 1, 4). In addition the absence of a large negative phase implies that the area recorded from is inexcitable, at least by action potential currents (4, 5). The fact that in nearly all cases, spikes recorded with both metal and glass electrodes in the cochlear ganglion are positive and lack a large negative component, is consistent with the myelination of both the cell bodies and their processes in the ganglion. The two positive components of the complex spikes could therefore be explained as the successive firing of first the peripheral, and then the central node, on either side of the inactive cell soma. A bimodal time course of positive current has been reported in the internodal regions of single myelinated peripheral nerve fibers (19).

These observations are therefore consistent with the hypothesis that the soma of the spiral ganglion cell behaves like an internodal region. Presumably, the placement of nodes on either side of the cell body, together with myelination of the soma, reduces the risk of conduction block by the cell body, as it would represent an area of lowered action current density, directly in the conduction pathway. It has been reported in an unmyelinated invertebrate sensory neuron that the bipolar cell body can cause conduction block and hence considerable modification of impulse patterns in the sensory pathway (11). Though the myelin sheath on the soma of the spiral ganglion neurons is not as compact as true internodal myelin (Fig. 5), there is evidence from avian ciliary ganglia that such loose myelin can provide a quite effective insulation (7). On the basis of the above scheme, the variations in the relative sizes of the two positive components in different units, can perhaps be explained as differences in the electrode

position relative to the incoming and outgoing nodes. The variations in delay between components seen in a single unit (Fig. 3E), could be the result of the stochastic behaviour of the nodal membranes (2, 21, 18). Random fluctuations in nodal excitability would result in variations in the time of initiation of the second node action potential in response to action current from the firing of the first node. As shown in Fig. 3, part of the variations in nodal excitability may be ascribed to the effects of previous firing. The effect seen in Fig. 2B where a single component alone sometimes appears, suggests that in some cases, myelination of the cell soma and the strategic placement of nodes is still insufficient to prevent occasional block of excitation of the second node by current leakage through the cell body. In such cases, the presence of the recording microelectrode may in some way be altering the normal excitability of the spike initiation areas. This is supported by the observation (Fig. 7) that the incoming

Fig. 7. The effect of movement of the electrode on the response to a constant intensity tone burst stimulus. A, initial response. B, response after 20 microns advancement of the electrode. C, after 40 microns, a complete cessation of response. D, after withdrawal of the electrode to the original location. The tracings are of 4 superimposed sweeps.


A
B
C
D
200μV
25ms
TONE ON

response to a tone burst stimulus can be reversibly blocked by advance of the microelectrode.

It remains to explain the large monophasic positive spikes frequently encountered with KCl-filled pipets. These spikes never separate into two components, and their duration is shorter than that of the two-component spikes. The properties of these spikes; the fact that they are easily lost, and that they are best recorded with high resistance electrodes, are similar to those reported for single myelinated nerve fibres in peripheral nerve trunks (9). They are therefore probably recorded in close proximity to the compactly myelinated axonal processes. The rarely seen spike waveform having a major negative component, is also easily lost by electrode movement. They are perhaps recorded from the small areas of excitable nodal membrane, where the sinks of the action currents are situated. The possibility cannot be discounted that these negative spikes are produced by the small population (10%) of unmye-

linated cell bodies in the ganglion. These are now believed to be connected to outer hair cells (17). As stated, however, there was no evidence that these negative units had different physiological properties.

The use of only extracellular recordings in this study does not permit the positive identification of structures recorded from. However the presence of a clearly two-component spike correlates well with the anatomical findings on the acoustic ganglion cells. As has been pointed out, the bipolar spiral ganglion cell appears to be an example where the strategic placement of nodes and myelination of the cell body reduces the risk of conduction block in a pathway which must reliably transmit large amounts of information.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bishop, P.O., Burke, W. and Davis, R. The interpretation of the extracellular response of single lateral geniculate cells. J. Physiol. 162: 451-472, 1962

2. Blair, E.A. and Erlanger, J. Responses of axons to brief shocks. Proc. Soc. Exptl. Biol. 29: 926-927, 1932.

3. Dowben, R.M. and Rose, J.E. A metal-filled microelectrode. Science 118: 22-24, 1953.

4. Freygang, W.H. and Frank, K. Extracellular potentials from single spinal motoneurons. J. Gen. Physiol. 42: 749-760, 1959.

5. Freygang, W.H. An analysis of extracellular potentials from single neurons in the lateral geniculate nucleus of the cat. J. Gen. Physiol. 41: 543-563, 1958.

6. Getcheld, T.V. Analysis of unitary spikes recorded extracellularly from frog olfactory cells and axons. J. Physiol. (Lond.): 234, 533-545, 1973.

7. Hess, A., Pilar, G. and Weakly, J.N. Correlation between transmission and structure in avian ciliary ganglion synapses. J. Physiol. 202: 339-354, 1969.

8. Huxley, A.F. and Stämpfli, R. Evidence for saltatory conduction in peripheral myelinated nerve fibres. J. Physiol. 108: 315-339, 1949.

9. Kellerhals, B., Engström, H. and Ades, H.W. Die morphologie des ganglion spirale cochleae. Acta otolaryng. Suppl. 226, 1967.

10. Kiang, N.Y.S. Discharge patterns of single fibers in the cat's auditory nerve. M.I.T. Press: Cambridge (Mass.), 1965.

11. Preston, J.B. and Kennedy, D. Integrative synaptic mechanisms in the caudal ganglion of the crayfish. J. Gen. Physiol. 43: 671-681, 1960.

12. Robertson, D. Cochlear neurons: frequency selectivity altered by perilymph removal. Science 186: 153-155, 1974.

13. Robertson, D. and Manley, G.A. Manipulation of frequency analysis in the cochlear ganglion of the guinea pig. J. Comp. Physiol. 91: 363-375, 1974.

14. Rosenbluth, J. The fine structure of acoustic ganglia in the rat. J. Cell, Biol. 12: 329-359, 1962.

15. Rosenbluth, J., Palay, S.L.: The fine structure of nerve cell bodies and their myelin sheaths in the eighth nerve ganglion of the goldfish. J. Biophys. and Biochem. Cytol. 9: 853-877, 1961.

16. Ross, M.D. and Burkel, W. Multipolar neurons in the spiral ganglion of the rat. Acta otolaryng. 76: 381-394, 1973.

17. Spoendlin, H. Innervation densities of the cochlea. Acta otolaryng. 73: 235-245, 1972.

18. Spurr, A.R. A low viscosity embedding medium for electron microscopy. J. Ultrastr. Res. 26: 3-43, 1969.

19. Tasaki, I. Nervous transmission. Charles C. Thomas: Springfield, 1953.

20. Tasaki, I. and Takeuchi, T. Weitere studien über den aktionstrom der markhaltigen nervenfaser. Pflügers Arch. ges. Physiol. 245: 274-284, 1942.

21. Thomsen, E. The ultrastructure of the spiral ganglion in the guinea pig. Acta otolaryng. Supp. 224, 1966.

22. Verveen, A.A. and Derksen, H.E. Fluctuation phenomena in nerve membrane. Proc. IEEE 56: 906-916, 1968.

FINAL DISCUSSION

In this section the critical evidence from the present studies and from the results of other workers are discussed. The ideas contained in manuscripts I and II are reconsidered, since these papers were written and published some time before the completion of this thesis. In particular, the emphasis of these papers on the mechanical nonlinearity of Rhode will be reassessed in the light of discussions with workers in the field at the University of Western Australia in November and December of 1974.

The role of inner and outer hair cells will not be discussed at length here. Though data on tuning curves, sensitivity, spontaneous activity rates and spike shapes were obtained from over 200 ganglion cells in the present study, there was no indication that the cells could be divided into two populations on the basis of any of these criteria. Variations in sensitivity were small between cells in the same animal, and the tuning curves of successive cells within the ganglion could often be superimposed. Spontaneous activities showed no clear correlation with sensitivity in normal animals. This conflicts somewhat with other authors, who have shown that there is a tendency for units in the cochlear nerve with higher spontaneous rates, to be more sensitive (Geisler et al., 1974; Kiang et al., 1970). As this trend appeared continuous, neither of these authors attempted to assign these units to inner and outer hair cells. One of the drawbacks of the ganglion preparation is the small number of units obtained per animal in contrast to recordings

from the axons of the ganglion cells in the cochlear nerve trunk.

It has been suggested that the neurones attached to the outer hair cells should be more sensitive than inner hair cell radial neurones (Dallos, 1973b). The position of the outer hair cells on the more flexible part of the basilar membrane, and the fact that their neurones probably receive summated inputs from several hair cells makes this a plausible hypothesis. However, the lack of single unit evidence for this concept has meant that most of the supporting evidence is derived indirectly from studies on the receptor potentials. The pitfalls of such studies (especially those employing ototoxic drugs) have already been mentioned in the thesis introduction.

Another suggestion is that the outer hair cell fibres should tune more broadly, since they innervate a greater length of basilar membrane (Billone and Raynor, 1973). Again, this has not been verified. One would also expect the outer hair cell neurones to be most

sensitive to higher frequencies than the inner hair cells radial fibres, since the outer spiral fibres travel basalwards along the basilar membrane before making synaptic contact. The ganglion preparation offers a unique opportunity to test this, but once again no evidence was found. No cells were found at any ganglion location with best frequencies different from other cells at that spot. It may be that the very small number of outer hair cell neurones means that they are simply not detected by our microelectrode techniques. Alternatively, the right questions are not being asked.

At this point, let us suggest another role for the outer hair cells. It may be possible that the large number of outer hair cells can exert a significant shunting of the excitatory current available to inner hair cells. This might have the effect of extending the dynamic range of the inner hair cell radial afferents. Though this dynamic range is only 20-40dB (Sachs and Abbas, 1974) there is evidence that in some situations it might be even less.

In the cochlear nerve of some species of reptiles with very few hair cells and nothing like the mammalian arrangement, the dynamic range of neurones is significantly less than 20dB (Manley, personal communication). This evidence is certainly suggestive but this tentative hypothesis will not be discussed further.

The findings in the first three manuscripts which are relevant to the problem of neural frequency selectivity can be summarized as follows.

1. The best frequencies of cells in the ganglion are consistent with the location of displacement maxima which have been reported on the basilar membrane.

2. The normal sharpness of tuning curves of ganglion cells is labile. As the sensitivity at the cf. falls, the Q_{10dB} drops until eventually both high and low frequency slopes resemble the reported mechanical data for the basilar membrane. This lability, which results in a strong correlation between the Q_{10dB} and the sensitivity at the cf. can be investigated in the same cell by deli-

berate respiratory impairment, but some unknown physiological variables also contribute to differences in sharpness of tuning from one animal to another.

3. Removal of perilymph bathing the basilar membrane results in three changes in the behaviour of initially sharply tuned ganglion cells. a) A lost of sensitivity of about 20dB at the cf., b) a dramatic broadening of the tuning curve, c) a total loss of two-tone inhibition. All three effects are reversible and are accomplished without significant changes in mean rate of spontaneous activity.

4. Cells which are initially broadly tuned because of some unknown pathological condition of the animal show only weak or no two-tone inhibition. Damage to intracochlear structures, such as cracking of the spiral lamina, causes broadening of tuning and a loss of two-tone inhibition.

As is pointed out in the papers the interpretation of these findings requires a decision to be made between two possible mechanisms of neural frequency selectivity

in the cochlea. The first explanation is that basilar membrane tuning at threshold sound intensities is as sharp as the neural tuning curves; these are simply a faithful reflection of the basilar membrane displacement pattern. The foundation of this view is the basilar membrane data of Rhode. His nonlinear basilar membrane behaviour could also conceivably explain the occurrence of two-tone inhibition and the generation of distortion products such as $2f_1 - f_2$. The second hypothesis which stems in large measure from the failure of other workers to confirm Rhode's findings, is that the basilar membrane vibration is broadly tuned at all intensities, vibrates linearly and is not directly responsible for the generation of two-tone inhibition and $2f_1 - f_2$ in the neural output of the cochlea. Instead, the sharp portions of neural tuning curves are postulated to derive from a second, sharp filter at the hair cell level, which is also closely linked to the generation of neural distortion products. Some of the arguments for and against these mechanisms

have been already presented. Let us here try to make a final assessment of the available evidence.

The effects of perilymph removal cast some doubt on some of the basilar membrane measurements which report linear behaviour. Thus it is surmised that Rhode's effect is not found by others because of technical limitations. However, in personal discussion, J.R. Johnstone and myself came to the final conclusion that the amount of fluid that has to be removed to produce the effect on neural tuning curves reported in paper II is probably greater than was required for the capacitive probe measurements of Wilson and Johnstone (1972, 1973). Johnstone is therefore firmly of the opinion that his basilar membrane measurements are quite valid and that the effect of perilymph removal is caused by an increase in the scala tympani resistance and an effect on current shunting of some sort or another along the basilar membrane. That is, an effect on the operation of a second filter. The difficulty of fitting the spontaneous activity findings

into this theory have been noted in papers II and III, though we know so little about the generation of spontaneous activity (paper IV) that this may not be damning evidence. On the basis of the Wilson and Johnstone hypothesis, the effects of fluid removal on two-tone inhibition are explained by the second filter being also intimately linked to the generation of two-tone inhibition.

However, I feel it would be incautious to minimize the possibly adverse effects of surgical intervention on basilar membrane mechanics. Rhode (1971) has noted that the nonlinearity he observed was fragile and was absent in cochleas in which minor damage had occurred to supporting tissues near the basilar membrane. The exposure of the basilar membrane in the guinea pig certainly requires a larger opening of the scala tympani than in the squirrel monkey (B.M. Johnstone, personal communication; Rhode, 1973). The basilar membrane results in the guinea pig which show the highest reported slope values (Johnstone and Yates, 1973), were apparently obtained using

a minimal opening of the scala tympani similar to that for the spiral ganglion preparation (Yates, personal communication).

Taking an overall view of the 110 guinea pigs used in the present studies, it is remarkable how many "bad" animals can occur with broad insensitive tuning curves, and yet which otherwise appear normal. Though this was not systematically investigated, the subjective impression gained was that the smaller the scala tympani opening, the better the chances of obtaining sharp tuning curves from the spiral ganglion cells. If excessive bleeding occurred from the edges of the scala tympani opening, resulting in blood clots in the scala tympani, broad insensitive tuning curves were usually obtained.

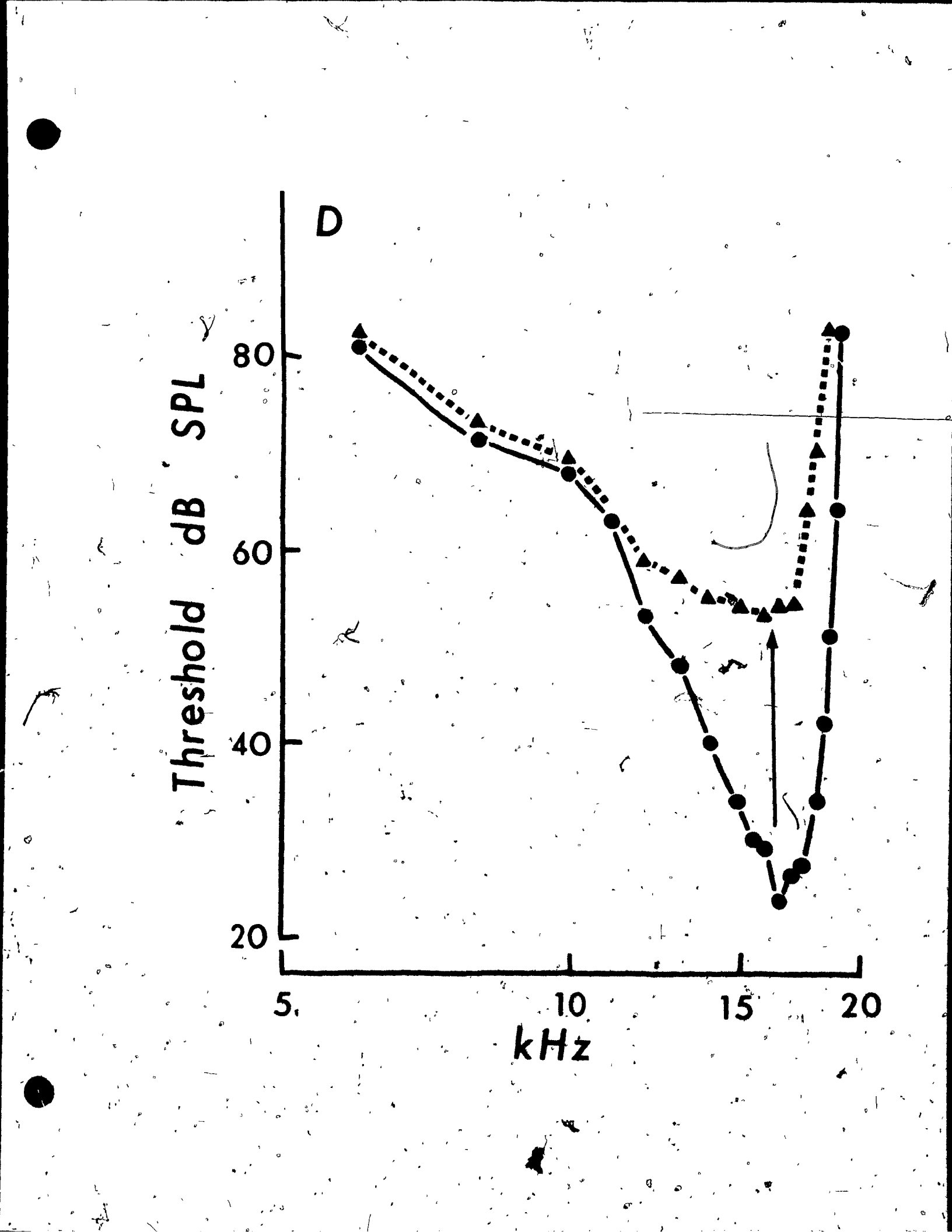
Wilson and Johnstone (1972) used the click-evoked N_1 response to monitor the viability of their preparation, and they report that this was not significantly affected by opening and draining of the basal turn scala tympani. This is probably a poor measure of basal turn activity

alone (particularly when measured with a single electrode on the bulla). It should be noted that N_1 does lose sensitivity with time after extensive opening of the scala tympani (J.R. Johnstone, personal communication; Wilson and Johnstone, 1972), and it is not clear whether tests of linearity were obtained by these authors before significant decreases in N_1 had occurred.

I feel that the spiral ganglion preparation offers a unique opportunity to critically investigate this problem. With this preparation simultaneous basilar membrane and single ganglion cell tuning curves could be obtained. If linearity is tested, it can be determined with certainty whether broad basilar membrane tuning does truly occur together with sharp neural tuning, in the same animal, from the same location, at the same time and subject to the same surgical invasion. Though the techniques are now available, as far as I am aware, this most critical control experiment has not been done.

At first sight, the effects of anoxia are consis-

Fig. 1. The effect of anoxia on the tuning curve of a single ganglion cell obtained as described in Paper I. The solid line is the initial tuning curve, the dotted line is during respiratory impairment. Note particularly that the sensitivity of the cell on the low frequency tail is not appreciably affected.


D
Threshold dB SPL
80
60
40
20
5.
10
15
20
kHz

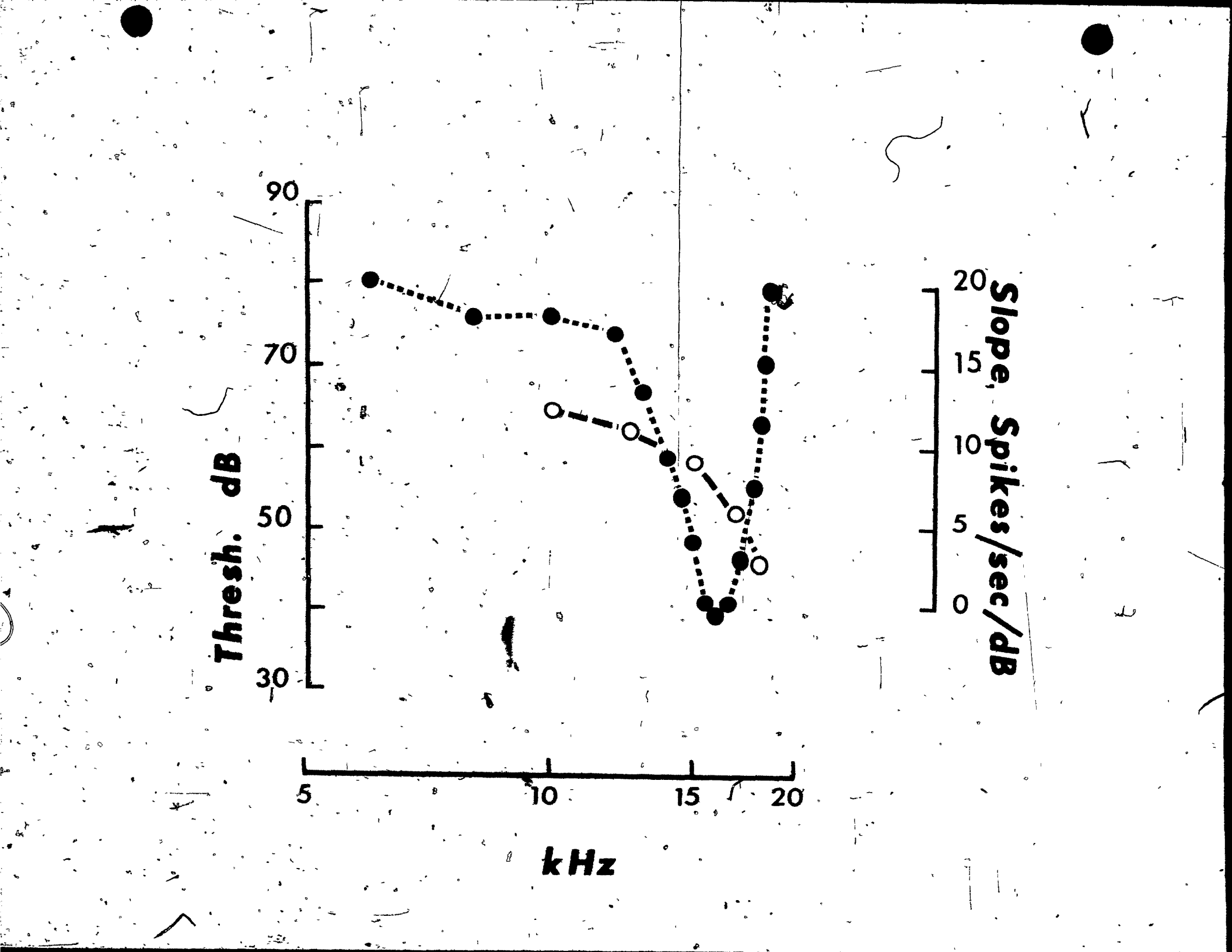
tent with the basilar membrane behaviour described by Rhode. However, there is a problem. In some cases, though the tuning curves are broader, there is no significant loss of sensitivity at points removed from the characteristic frequency (see Fig. 7a of paper I and Fig. 1 of this discussion). Certainly, the model based on Rhode's data would predict that for a loss of sensitivity in the hair cell or the neurone, more sound pressure has to be put in at the peak of the tuning curve to regain threshold than on the portions of the curve where behaviour is linear. But how can there be up to 40dB lost at the cf. and yet, in some cases, no loss at all when the tail of the tuning curve or the very last high frequency points are examined? How can a simple effect of anoxia such as we have postulated, together with nonlinear basilar membrane vibration explain a frequency selective sensitivity change of this magnitude? It certainly looks as if a physiologically vulnerable filtering mechanism responsible for the sharp neural tuning only in the region of the cf. is being re-

duced in its effectiveness by the anoxia. A similar difficulty applies when one considers the effects of COCB stimulation on the shapes of tuning curves of single cochlear nerve fibres (Kiang et al., 1970; Wiederhold, 1970). Once again there is hardly any change in the sensitivity at frequencies far removed from the cf. and large changes in the sharp portion of the tuning curve. There does not seem to be any way for such a coarse and diffuse system as the COCB to produce this frequency selective effect unless it is acting on a second filter operating in the sharp region of the curve. It would seem that a simplistic application of non-linear basilar membrane mechanics as found by Rhode cannot explain these results. The absence of two-tone inhibition in initially broad tuning curves and its disappearance in fluid-drained cochleas would suggest that this filter is also linked to two-tone inhibition. On the credit side of the non-linear mechanical hypothesis it should be noted that a Rhode-like non-linear basilar membrane can apparently produce two-tone

inhibition (Kim et al., 1973)

What is the evidence that a Rhode-type non-linearity is reflected in the behaviour of normally operating cochlear nerve fibres? On this point there is again considerable disagreement. Pfeiffer et al., (1973) have recently documented nonlinear behaviour of cochlear nerve fibres in the cat which they believe to be consistent with nonlinear basilar membrane mechanics. They also report that at intensities close to threshold, these fibres show a smooth transition into linear behaviour in accordance with the results of various models of non-linear basilar membrane motion (Kim et al., 1973; Littlefield et al., 1973; Hall, 1974). These authors are strong proponents for a basilar membrane mechanical explanation for almost all primary auditory neurone properties. Some findings on the slopes of rate versus intensity functions of cochlear nerve fibres also suggest that the sort of saturating nonlinearity found by Rhode is in part reflected in the output of neurones. Sachs and Abbas (1974)

Fig. 2. Solid circles and dotted line show the tuning curve of a single ganglion cell. The open circles and broken line are the slopes of the rate versus intensity functions at each indicated frequency.


90
70
50
30
Thresh. dB
5
10
15
20
kHz
20
15
10
5
0
Slope, Spikes/sec/dB

and Pfeiffer et al. (1973) report thatthe slopes of rate versus intensity functions decrease systematically across the sharp portion of the tuning curves, especially on the high frequency slope. In Paper I of this thesis it was stated that these functions are parallel at all frequencies. This must now be amended, since this statement was based on curves generated with insufficient data points and at only a small number of frequencies. In fact the effect reported by these other authors can be seen Fig. 1 of Appendix II of this thesis and Fig. 2 of the present discussion. The slopes of the rate functions vary almost exactly as described by Sachs and Abbas (1974). As they discuss, this is consistent with the flattening of the input-output curves of the basilar membrane in the region of the cf. as reported by Rhode (see Fig. 6 of the thesis introduction). This does not however, necessarily imply that the non-linearity will produce a sufficient sharpening to explain the neural tuning curves and the

arguments discussed previously must be borne in mind. It might be as easily stated by the proponents of linear basilar membrane mechanics that such slope changes must be a property of the additional filtering mechanism which they consider to be necessary. The data on the nonlinear behaviour of cochlear nerve fibres (Pfeiffer et al., 1973) is in part contradicted by other workers who claim that the filter properties themselves, as measured in cochlear nerve fibres, are independent of input level (de Boer, 1969; Evans and Wilson, 1973; Geisler et al., 1974). The grating technique used by Evans and Wilson appears to be particularly insensitive to nonlinear phenomena since they failed to find two-tone inhibition using their technique. However, as shown by Geisler et al. (1974), iso-rate contours for cochlear nerve fibres in the squirrel monkey do not appear to change very much in sharpness as stimulus level is raised. The apparent broadening of iso-intensity contours as intensity is

raised (Rose et al., 1971) has been pointed out by Evans (1973) to be a result of the saturation of the input-output curve of the neurones at about 20 - 30 dB above threshold. The actual intensity range which can be investigated in such studies is small (20 - 30 dB) owing to the rapid saturation of the output of cochlear nerve fibres. Since the basilar membrane model of Kim et al. (1973) shows a transition into linearity close to threshold, this may be a factor in the failure of some authors to detect certain nonlinearities.

The question of the combination tone $2f_1 - f_2$ has been discussed in the thesis introduction where it has been pointed out that there are several discrepancies between the psychophysical findings and cochlear microphonics on the one hand and cochlear microphonics and basilar membrane findings on the other. There are some reservations about this lack of correspondence which should be stated here. Firstly, the presence of nonlinearities in the neural output has only been investi-

gated in man, cat and squirrel monkey, while CM data on this question are from guinea pig. Similarly, though the absence of $2f_1 - f_2$ on the basilar membrane comes from the work on guinea pig (Wilson and Johnstone, 1973), there is no neural data on the presence of combination tone responses in the output of the cochlea in this animal. Even if these species differences are not important, there is still another difficulty. Presumably, the neural and psychoacoustical data reflect the output of the inner hair cell radial nerve fibres, as these greatly predominate in the cochlea. Yet the cochlear microphonic measurements probably reflect mainly the output of the outer hair cells. Thus, discrepancies between CM and psychophysical data on neural output may not be important since we do not know the behaviour of the inner hair cell microphonic.

Such difficulties aside, the most critical piece of evidence is that all the psychoacoustical and single neural data shows that $2f_1 - f_2$ is frequency analysed at

the same place on the basilar membrane where a pure tone of the same frequency would be located. Yet cochlear microphonic measurements of this distortion product show that it peaks at the location of the primaries and behaves in other respects very differently from the neural distortion product. Wilson and Johnstone (1973) take this as evidence that the neural distortion product is generated by interaction of receptor currents within the hair cell. Thus, it is not seen in the microphonics to any large extent, but is present in the neural output. To explain the fact that the distortion product is frequency analysed in the same way as pure tones they postulate that a sharp additional filter is also present within the hair cell and only allows the generated distortion product to excite the nerve fibres at the appropriate place on the basilar membrane. This hypothesis is also strongly advocated by Evans and Wilson (1973). The essentials of this hypothesis could be summarized as follows:

a) The basilar membrane results of Wilson and Johnstone (1972, 1973) are correct. So too are those of other authors who find broad tuning and linearity on the membrane. Even if a nonlinearity does exist (Rhode, 1971), it is not important since it cannot explain the puzzle of the combination tones.

b) The nonlinearity responsible for combination tone generation is subsequent to both the basilar membrane and the mechano-electrical transduction of CM.

c) Linked to the nonlinearity is a second filter centred about the peak of the basilar membrane tuning curve (since the basilar membrane maxima and best neural frequencies show the same basilar membrane locations). This filter is responsible for the sharp portion of the neural tuning curve, and also confers on the combination tones the property that they are analysed at their appropriate place.

In view of the reservations explained above, this particular hypothesis cannot be unequivocally supported

at the moment. On balance however, the results in this thesis and the data from other workers seem to suggest that a unified explanation of neural behaviour in the cochlea based on nonlinear basilar membrane mechanics alone, will not suffice. The Organ of Corti is a complex mechanical structure and there is scope for fine mechanical motions which may stimulate hair cells without being reflected in either the CM or the basilar membrane motion. Modelling can only give a hazy idea of the possibilities, as our knowledge of the mechanical properties of structures such as the pillar cells, stereocilia and tectorial membrane is limited. Unknown too is the role of the outer hair cells, whose sparse afferent innervation and dense efferent synapses are in such sharp contrast to the inner hair cells. The possibility of interaction between these two sets of receptor cells by electrical means should not be discounted. Thus, though the second filter hypothesis deserves serious consideration, the structural and physiological

entities which might comprise such an additional mechanism are at present mysterious.

Appendix I

Sound stimuli used in these experiments were tone bursts produced by a General Radio 1900A and a Hewlett Packard 200 oscillator, and gated by commercially available timers and electronic switches (Grason Stadler). The rise-fall time and the duration of the tone bursts could be varied and their intensity regulated in 1 dB or 5 dB steps. The stimuli were amplified by a Sony 200 F preamplifier and a Marantz power amplifier. As only the high-frequency basal turn of the cochlea was being investigated, a high frequency driver was used. This was a tweeter removed from an AR-3 loudspeaker unit.

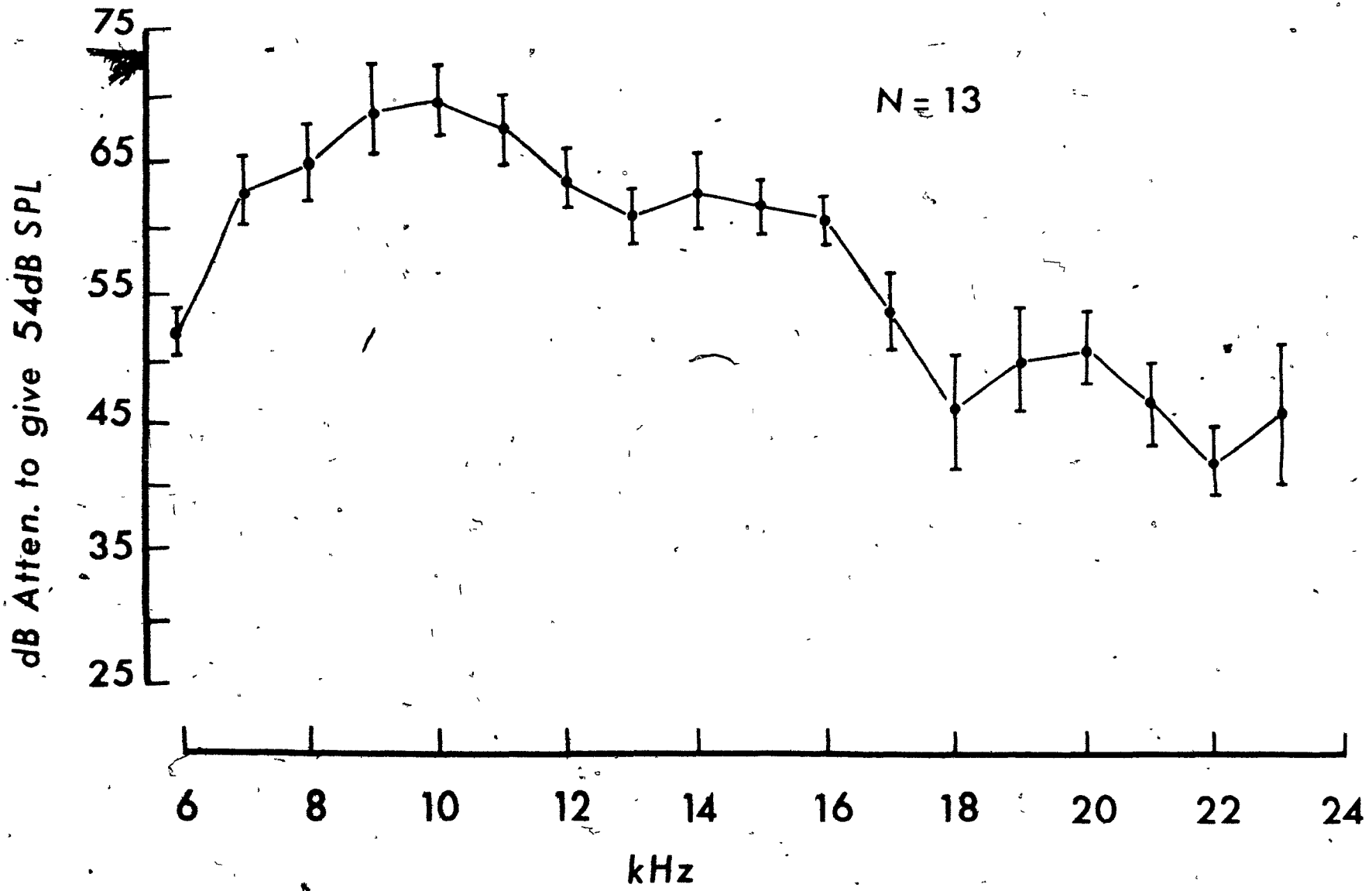
The tweeter was connected by 1 cm length of Plexiglass tubing to a metal sound delivery system which incorporated a 1 mm diameter probe and microphone for sound calibration during an experiment. The output of the microphone was measured with a General Radio 1900A tracking wave analyser which also supplied a sinusoidal input to the speaker. The tympanic membrane was exter-

iorized to eliminate resonances in the outer ear canal (Manley and Johnstone, 1974). A transparent Plexiglass tip to the sound-delivery system allowed the tip of the probe tube and the tympanic membrane to be easily visualized so that the system could be sealed over the tympanic ring of the bulla with reproducible placement of the probe tube in relation to the tympanic membrane. The tip of the probe was positioned 1 mm from the umbo of the manubrium of the malleus.

Calibration of the probe tube was effected by placing the same delivery system with probe tube over a tympanic ring dissected from an experimental animal. The tympanic membrane was replaced by a ¼ inch condensor microphone sealed in its place and in the same position relative to the tip of the probe tube. This ensured that the volume of the small cavity at the end of the delivery system was practically identical in the experimental animal and the calibrating coupler. The dB differences between the readings of the ¼ inch and probe

tube microphones thus served as a calibration to convert probe tube readings during an experiment into sound pressure levels at the tympanic membrane. Owing to the limitations of the tweeter, only frequencies between 4 and 25 kHz were normally used. This meant that the hook region of the ganglion, containing cells with best frequencies higher than about 21 kHz, could not be investigated. Neither could the low frequency tails of single cell tuning curves be investigated below 4 kHz. In Fig. 1, the frequency response of the system, obtained by using the coupler calibration, is shown. Mean values and standard deviations are from 13 animals in which great care was taken to keep all factors constant. The standard deviations of the readings become appreciably larger for frequencies above about 16 kHz. This agrees well with the finding by Johnstone and Taylor (1969), that probe tube readings become increasingly sensitive to small variations in probe position above 15 kHz. It has also been pointed out

Fig. 1. Calibration curve obtained from 13 animals using the probe tube microphone and the probe tube calibration described in the text of the Appendix. The points are the mean values for 13 animals, and the heights of the vertical bars indicate ± one standard deviation.


75
65
55
45
35
25
dB Atten. to give 54dB SPL
N = 13
6
8
10
12
14
16
18
20
22
24
kHz

(Dallos, 1973b) that the use of a high impedance condenser microphone in place of the tympanic membrane can lead to differences in sound pressure generated in the coupler and in the real animal. Since the tweeter used did not have a flat frequency response and a closed delivery system had to be used because of the widely opened bulla, the use of a probe tube microphone, with its inherent inaccuracies is an unavoidable complication. An absolute calibration of the output of both condenser microphones was obtained using a General Radio 1562 sound-level-calibrator.

Appendix II

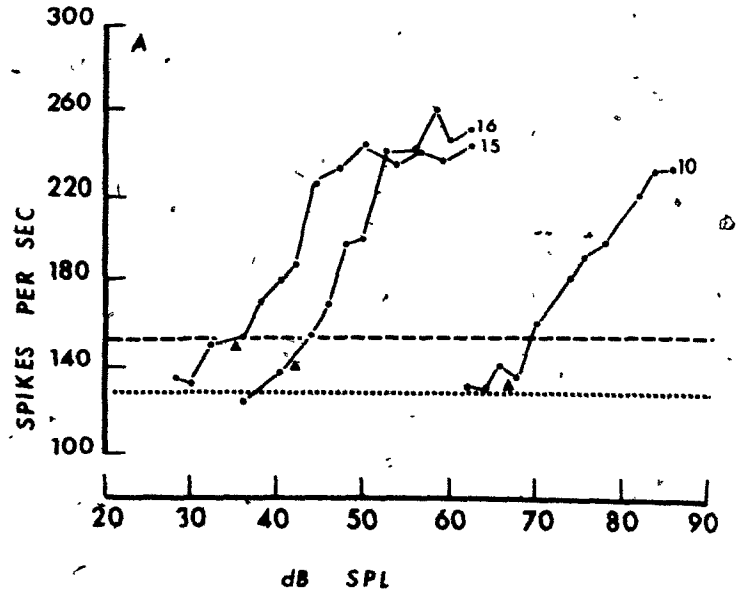
Single cell threshold estimations

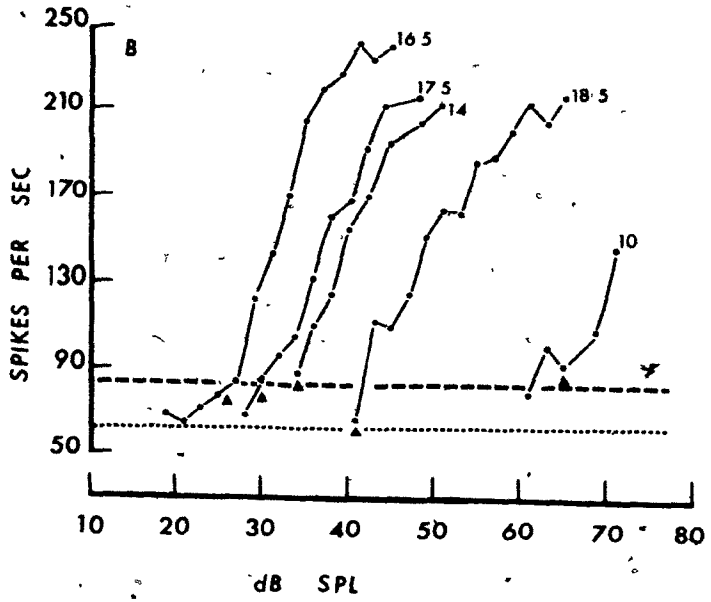
The thresholds of single ganglion cells were estimated by the method described by Kiang (1965) and Evans (1972). 50 ms tone bursts with 5 ms rise-fall time were presented to the animal at a rate of 4/sec. The single unit activity was monitored visually on an oscilloscope and was also played through headphones worn by the experimenter. The intensity of the tone bursts was then raised in 1 or 5 dB steps and threshold was designated when an increase in spike rate above spontaneous firing could be first detected by audio-visual methods, reliably locked to the tone bursts. With practice this procedure became quite easy even for cells with very high rates of spontaneous activity. It has been shown that for cochlear nerve fibres with low cf. this estimate of threshold is suspect, since fibres show phase locking to the stimulus at sound pressure levels up to 20 dB below those at which an increase in

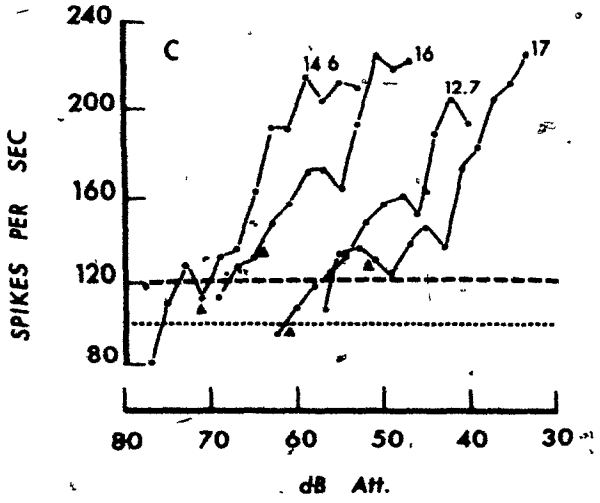
overall firing rate occurs (Rose et al., 1971). For basal turn ganglion cells however, this is not a problem since phase locking does not occur above about 4 kHz (Rose et al., 1967). A more objective estimate of threshold might be obtained by designating a certain per cent increase of firing above the background rate. Though this would be more rigorous there is no reason for it to be more meaningful than the criterion used here. An increase in firing rate of say 20% for a cell with a spontaneous rate of 100/ sec. would represent an average of one additional spike/50 ms tone burst, but for a cell with a spontaneous activity of 10/ sec., it would mean one extra spike/ two tone bursts. As the audiovisual criterion is rapid and easy and is used by other workers it was regarded as sufficient for the present work.

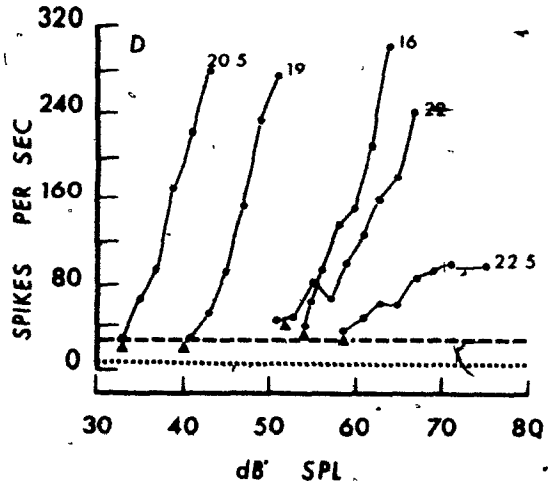
In an attempt to assess just what the audiovisual criterion corresponded to in quantitative terms, input-output curves at different frequencies were com-

Fig. 2. Rate versus intensity functions for 4 sensitive spiral ganglion cells, with different mean rates of spontaneous activity. The dotted lines are the mean spontaneous rates. Broken lines represent an increase in rate of 1 spike per 50 ms tone burst. Arrows indicate the thresholds estimated at each frequency by audiovisual criteria. Numbers on each graph indicate the frequency of stimulation in kHz. In Fig. 2 C, stimulus intensity is plotted as dB attenuation, to obtain a clearer separation of the 4 curves.


A
300
260
220
180
140
100
SPIKES PER SEC
16
15
10
20
30
40
50
60
70
80
90
dB SPL


B
250
210
170
130
90
50
SPIKES PER SEC
16 5
17 5
14
18 5
10
10
20
30
40
50
60
70
80
dB SPL


C
240
200
160
120
80
SPIKES PER SEC
14 6
16
12.7
17
80
70
60
50
40
30
dB Att.


D
320
240
160
80
0
SPIKES PER SEC
20 5
19
16
22 5
30
40
50
60
70
80
dB SPL

puted with the PDP8 computer for numerous cells. Firing rates were obtained by counting the number of spikes occurring in 20 repeated 50 ms tone bursts. In Fig. 1 it can be seen that the thresholds estimated by audio visual criteria show a certain degree of variation compared to these rate functions. Relative to a rate corresponding to one additional spike/tone burst, thresholds are very accurate for cells with mean spontaneous rates lower than about 60/sec. (Fig. 1B, D). At higher rates of spontaneous activity, the threshold estimations vary by about 3 dB above and below this line. Since errors in the estimation of sound pressure level at the eardrum are probably greater than this (Appendix I) the audio visual criterion can be regarded as a good working method of threshold estimation.

Bibliography

1. Angelborg, C., Engstrom, H. 1973: The normal Organ of Corti: In: Basic Mechanics in Hearing, p. 185-183 (Moller, A.R., Ed.) Acad. Press.

2. Bekesy, G. Von. 1960: Experiments in Hearing. New York: McGraw Hill.

3. Bekesy, G. Von. 1953: Description of some mechanical properties of the Organ of Corti. J. Acous. Soc. Amer. 25, 770-785.

4. Bekesy, G. Von. 1952: DC resting potentials of the cochlear partition. J. acous. Soc. Amer. 24, 72-79.

5. Billone, M., Raynor, S. 1973: Transmission of radial shear forces to cochlear hair cells. J. acous. Soc. Amer. 54, 1143-1156.

6. Bosher, S.K., Warren, R.L. 1971: A study of the electrochemical and osmotic relationship of the cochlear fluids in the neontal rat at the time of development of the endocochlear potential. J. Physiol. (Lond.) 212, 729-754.

7. Dallos, P. 1973a: Cochlear potentials and cochlear mechanics. In: Basic Mechanisms in Hearing, p. 335-376 (Moller,A.R., Ed.) Acad. Press.

8. Dallos, P. 1973b: The auditory periphery. Acad. Press.

9. Dallos, P. 1971: Comments on "Correspondence between cochlear microphonic sensitivity and behavourial threshold in the cat". J. acous. Soc. Amer. 50, 1554.

10. Dallos, P. 1969: Comments on the differential electrode recording technique. J. acous. Soc. Amer. 45, 999-1007.

11. Dallos, P. 1968: On the negative potential within the Organ of Corti. J. acous. Soc. Amer. 44, 818.

12. Dallos, P., Billone, M.C., Durrant, J.D., Wang, C-Y., Raynor, S. 1972: Cochlear inner and outer hair cells: functional differences. Science. 177, 356-358.

13. Dallos, P., Durrant, J.D. 1972: On the derivative relationship between stapes movement and cochlear microphonic. J. acous. Soc. Amer. 52, 1263-1265.

14. Dallos, P., Sweetman, D.H. 1969: Distribution pattern of cochlear harmonics. J. acous. Soc. Amer. 45, 37-46.

15. Dallos, P., Schoeny, Z.G., Worthington, D.W., Cheatham, M.A. 1969: Cochlear distortion. Effect of direct current polarization. Science 164, 449-451.

16. Davis, H. 1965: A model for transducer action in the cochlea. Cold Spring Harb. Symp. Quantit. Biol. 30, 181-190.

17. Davis, H. 1958: A mechano-electrical theory of cochlear action. Ann. Otol. Rhin. Laryng. 63, 789-801.

18. Davis, H. 1954: The excitation of nerve impulses in the cochlea. Ann. Otol. Phin. Laryng. 63, 469-481

19. de Boer, E. 1969: Initiation of nerve impulses in the ear. Proc. kon. ned. Akad. Wet. 72, 129-151.

20. Densert, O. 1974: Adrenergic innervation in the rabbit cochlea. Acta otolaryng. 78, 345-356.

21. Desmedt, J.E., Robertson, D. 1975: Ionic mechanism of the efferent olivo-cochlear inhibition studied by cochlear perfusion in the cat. J. Physiol. (Lond.) in press.

22. Engstrom, H. Ades, H.W., Anderseh, A. 1966: Structural pattern of the Organ of Corti. Stockholm: Almgvist and Wiksell.

23. Engstrom, H., Ades, H.W., Hawkins, J.E. 1962: Structure and functions of the sensory hairs of the inner ear. J. acous. Soc. Amer. 34, 1356-1363.

24. Engstrom, H., Wersall, J. 1958: Structure and innervation of the inner ear sensory epithelia. Int. Rev. Cytol. 7, 535-585.

25. Evans, E.F. 1974: The effects of hypoxia on the tuning of single cochlear nerve fibres. J. Physiol. (Lond.) 238, 65 P.

26. Evans, E.F. 1973: In discussion In: Basic Mechanisms in Hearing, p. 589 (Moller, A.R., Ed.) Acad. Press.

27. Evans, E.F. 1972: The frequency response and other properties of single fibres in the guinea pig cochlea. J. Physiol. (Lond.) 226, 263-287.

28. Evans, E.F. 1970: Narrow tuning of cochlear nerve fibre responses in the guinea pig. J. Physiol. (Lond.) 206, 14-15P.

29. Evans, E.F., Wilson, J.P. 1973: The frequency selectivity of the cochlea. In: Basic Mechanisms in Hearing, p. 519-554 (Moller, A.R., Ed.) Acad. Press.

30. Fernandez, C. 1952: Dimensions of the guinea pig cochlea. J. acous. Soc. Amer. 24, 519-525.

31. Fernandez, C. 1951: The innervation of the cochlea. Laryngoscope 61, 1152-1165.

32. Flock, A. 1933: The tectorial membrane: position and ion content studied with the electron probe. Proc. 85th Meeting Acous. Soc. Amer. p. 48.

33. Flock, A., Jorgensen, M., Russell, I. 1973: The physiology of individual hair cells and their synapses. In: Basic Mechanisms in Hearing, (Moller, A.R., Ed.) p. 273-306. Acad. Press.

34. Flock, A. Kimura, R., Lundqvist, P.G., Wersall, J. 1962: Morphological basis of directional sensitivity of the outer hair cells in the Organ of Corti. J. acous. Soc. Amer. 34, 1351-1360.

35. Furukawa, T., Ishii, Y. 1967: Neurophysiological studies on hearing in goldfish. J. Neurophysiol. 30, 1377-1403.

36. Geisler, C.D., Rhode, W.S., Kennedy, D.T. 1974: Responses to tonal stimuli of single auditory nerve fibres and their relationship to basilar membrane motion in the squirrel monkey. J. Neurophysiol. 37, 1156-1172.

37. Goldstein, J.L. 1967: Auditory nonlinearity. J. acous. Soc. Amer. 41, 676-689.

38. Goldstein, J.L., Kiang, N.Y.S. 1968: Neural correlates of the aural combination tone $2f_1 - f_2$. Proc. IEEE 56, 981-992.

39. Guinan, J.J., Peake, W.T. 1967: Middle ear characteristics of anaesthetized cats. J. acous. Soc. Amer. 41, 1237-1261.

40. Hall, J.L. 1974: Two-tone distortion products in a nonlinear model of the basilar membrane. J. acous. Soc. Amer. 56, 1818-1828.

41. Held, H. 1926: Die cochlea der sauger und vogel ihre entwicklung und ihre bau. In: Handbuch der Normalen und Pathologischen Physiologie p. 467-526 (Bethe, A., Ed.) Berlin: Springer.

42. Honrubia, V., Strelioff, D., Ward, P.H. 1971: The mechanism of excitation of hair cells in the cochlea. Laryngoscope 31, 1719-1725.

43. Honrubia, V., Ward, P.H. 1970: Mechanism of production of cochlear microphonics. J. acous. Soc. Amer. 47, 498-503.

44. Honrubia, V., Ward, P.H. 1969: The dependence of the cochlear microphonic and summating potentials on the endocochlear potential. J. acous. Soc. Amer. 46, 388-392.

45. Iurato, S. 1967: Submicroscopic Structures of the inner ear. Oxford: Pergamon Press.

46. Iurato, S. 1962: Efferent fibres to the sensory cells of Corti's organ. Exp. Cell Res. 27, 162-164.

47. Johnstone, B.M. 1967: Genesis of the cochlear endolymphatic potential. In: Current Topics in Bioenergetics. 2, 335-352. New York: Acad. Press.

48. Johnstone, B.M., Yates, G. 1973: Basilar membrane tuning. Proc. 85th Meeting Acous. Soc. Amer.

49. Johnstone, B.M., Sellick, P.M. 1972: The Peripheral auditory apparatus. Quart. Rev. Biophys. 5, p. 1-58.

50. Johnstone, B.M., Taylor, K.J., Boyle, A.J. 1970: Mechanics of the guinea pig cochlea. J. acous. Soc. Amer. 47, 504-509.

51. Johnstone, B.M., Taylor, K.J. 1969: Use of probe microphones to measure sound pressures in the ear. J. acous. Soc. Amer. 46, 1404-1405.

52. Johnstone, B.M., Boyle, A.J. 1967: Basilar membrane vibration examined with the Mössbauer technique. Science 158, 389-390.

53. Johnstone, J.R., Johnstone, B.M. 1966: Origin of summating potential. J. acous. Soc. Amer. 40, 1405-1413.

54. Johnstone, B.M., Johnstone, J.R., Pugsley, I.D. 1966: Membrane resistance in endolymphatic walls of the first turn of the guinea pig cochlea. J. acous. Soc. Amer. 40, 1398-1404.

55. Johnstone, C.G., Schmidt, R.S., Johnstone, B.M. 1963: Sodium and potassium in vertebrate endolymph as determined by flame microphotometry. Comp. Biochem. Physiol. 9, 335-341.

56. Kellerhals, B., Engstrom, H., Ades, H.W. 1967: Die morphologie des ganglion spirale cochleae. Acta oto-laryng., Suppl. 226.

57. Kiang, N.Y.S. 1965: Discharge patterns of single fibres in the cat's auditory nerve. Cambridge (Mass): M.I.T. Press.

58. Kiang, N.Y.S., Moxon, E.C., Levine, R.A. 1970: Auditory nerve activity in cats with normal and abnormal cochleas. In: Sensorineural Hearing Loss, p. 241-273 (Wolstenholme, G., Knight, K., Eds.) London: Churchill.

59. Kim, D.O., Molnar, C.E., Pfeiffer, R.R. 1973: A system of nonlinear differential equations modelling basilar membrane motion. J. acous. Soc. Amer. 54, 1517-1529.

60. Klinke, R. 1974: Efferent innervation of vestibular and auditory receptors. Physiol. Rev. 54, 316.

61. Kohllofell, L.U.E. 1973: Observations of the mechanical disturbances along the basilar membrane with laser illumination. In: Basic Mechanisms in Hearing. p. 95-117 (Moller, A.R., Ed.) Acad. Press.

62. Kohllofell, L.U.E. 1971: Studies of the distribution of cochlear potentials along the basilar membrane. Acta oto-laryng. Suppl. 288.

63. Kohllofell, L.U.E. 1970: Longitudinal amplitude and phase distribution of the cochlear microphonic (guinea pig) and spatial filtering. J. Sound Vib. 11, 325-334.

64. Kurokawa, S. 1965: Experimental study on electrical resistance of basilar membrane in guinea pig. Japan. J. Otol. 68, 1177-1195.

65. Laszlo, C.A., Milsum, J.H., Gannon, R.P. 1972: Modelling and simulation of the cochlear potentials of the guinea pig. J. acous. Soc. Amer. 52, 1648-1972.

66. Lim, D.J. 1972: Fine morphology of the tectorial membrane. Its relationship to the Organ of Corti. Arch. otolaryng. 96, 199-215.

67. Littlefield, W.M., Kim, D.O., Molnar, C.E., Pfeiffer, R.R. 1973: Exploration of the range of linear response activity of cochlear nerve fibres. J. acous. Soc. Amer. 54, 283A.

68. Llinas, R., Nicholson, C. 1969: Electrophysiological analysis of alligator cerebellum: a study of dendritic spikes. In: Neurobiology of Cerebellar Evolution and Development. p. 431-465 (Llinas, R., Ed.) Chicago: Amer. Med. Assoc.

69. Lorente de No, R. 1937: The neural mechanism of hearing - b. the sensory endings in the cochlea. Laryngoscope 47, 373-377.

70. Manley, G.A., Johnstone, B.M. 1974: Middle-ear function in the guinea pig. J. acous. Soc. Amer. 56, 571-576.

71. Mulroy, M.J., Altmann, D.W., Weiss, T.F., Peake, W.T. 1974: Intracellular electric responses to sound in a vertebrate cochlea. Nature. 249, 482-485.

72. Nomoto, M., Suga, N., Katsuki, Y. 1964: Discharge patterns and inhibition of primary auditory nerve fibres in the monkey. J. Neurophysiol. 27, 768-787.

73. Pappas, G.D., Bennett, M.V.L. 1966: Specialized junctions involved in electrical transmission between neurones. Ann. N.Y. Acad. Sci. 137, 495-508.

74. Pappas, G.D., Nakajima, Y., Bennett, M.V.L. 1965: The fine structure of electrically transmitting junctions in the CNS. Anat. Rec. 151, 397-402.

75. Patton, M.L., Kater, S.B. 1972: Electrotonic conduction in the optic nerves of planorbid snails. J. Exp. Biol. 56, 695-702.

76. Pfeiffer, R.R., Kim, D.O. 1973: Considerations of nonlinear response porperties of single cochlear nerve fibres. In: Basic Mechanisms in Hearing. p. 555-591 (Moller, A.R., Ed.) Acad. Press.

77. Reinecke, M. 1967: Electronenmikroskopische untershungen am ganglion spirale des meerschweinchens. Arch. klin. exp. Ohren.-, Nas.-, Kehlk. Heilk. 189, 158-167.

78. Rhode, W.S. 1973: An investigation of post-mortem cochlear mechanics using the Mössbauer effect. In: Basic Mechanisms in Hearing p. 49-67 (Moller, A.R.; Ed.) Acad. Press.

79. Rhode, W.S. 1971: Observations of the vibration of the basilar membrane on squirrel monkeys using the Mössbauer technique. J. acous, Soc. Amer. 49, 1218-1231.

80. Rhode, W.S., Geisler, C.D. 1967: Model of the displacement between opposing points on the tectorial membrane and reticular lamina. J. acous. Soc. Amer. 42, 185-190.

81. Rose, J.E., Kites, L.M., Gibson, M.M., Hind, J.E. 1974: Observations on phase sensitive neurones of anteroventral cochlear nucleus of the cat: nonlinearity of cochlear output. J. Neurophysiol. 37, 218-253.

82. Rose, J.E., Hind, J.E., Anderson, D.J., Brugge, J.F. 1971: Some effects of stimulus intensity on response of auditory nerve fibres in the squirrel monkey. J. Neurophysiol. 34, 685-699.

83. Rose, J.E., Brugge, J.F., Anderson, D.J., Hind, J.E. 1969: Phase-locked response to low-frequency tones in single auditory nerve fibres in the squirrel monkey. J. Neurophysiol. 30, 769-793.

84. Rosenbluth, J., Palay, S.L. 1961: The fine structure of nerve cell bodies and their myelin sheaths in the eighth nerve ganglion of the gold-fish. J. Biophys. Biochem. Cytol. 9, 853-877.

85. Ross, M.D. 1974: The tectorial membrane of the rat. Am. J. Anat. 139, 449-462.

86. Ross, M.D. 1973: Multipolar neurones in the spiral ganglion of the rat. Acta otolaryng. 76, 38-49.

87. Sachs, M.B., Abbas, P.J. 1974: Rate versus intensity functions for auditory nerve fibres in cats: tone-burst stimuli. J. acous. Soc. Amer. 56, 1835-1847.

88. Sando, I. 1965: The anatomical interrelationships of the cochlear nerve fibres. Acta otolaryng. 59, 417-436.

89. Shaw, S.R. 1972: Decremental conduction of the visual signal in barnacle lateral eye. J. Physiol. (Lond.) 220, 145-175.

90. Smith, C.A., Haglan, B.J. 1973: Golgi stains of the cochlear nerve fibres. J. acous. Soc. Amer. 54, 283-284.

91. Smith, C.A. 1967: Innervation of the Organ of Corti. In: Submicroscopic Structure of the Inner Ear. p. 107-131 (Iurato, S., Ed.) New York: Pergammon Press.

92. Smoorenburg, G.F. 1972: Combination tones and their origin. J. acous. Soc. Amer. 52, 615-632.

93. Spoendlin, H. 1973: The innervation of the cochlear receptor. In: Basic Mechanisms in Hearing. p. 185-234 (Moller, A.R., Ed.) Acad. Press.

94. Spoendlin, H. 1972: Innervation densities of the cochlea. Acta otolaryng. 73, 235-248.

95. Spoendlin, H. 1971: Degeneration behaviour of the cochlear nerve. Arch. klin. Exp. Ohr.-, Nas.-, Kehlk. Heilk. 200, 275-291

96. Spoendlin, J. 1969: Inervation patterns in the Organ of Corti of the cat. Acta otolaryng. 67, 239-254

97. Spoendlin, H. 1966: The Organization of the Cochlear Receptor Basel-New York: Karger.

98. Steele, C.R. 1973: A possibility for sub-tectorial membrane fluid motion. In: Basic Mechanisms in Hearing. p. 69-93 (Moller, A.R., Ed.) Acad. Press.

99. Stone, J. 1973: Sampling properties of micro-electrodes assessed in cat's retina. J. Neuro-physiol. 36, 1071-1082.

100. Sweetman, D.H., Dallos, P. 1969: Distribution pattern of cochlear combination tones. J. acous. Soc. Amer. 45, 58-71.

101. Tasaki, I., Davis, H., Eldredge, D. 1954: Exploration of cochlear potentials in guinea pig, with a microelectrode. J. acous. Soc. Amer. 26, 756-764.

102. Thomsen, E. 1967: The ultrastructure of the spiral ganglion in the guinea pig. Acta otolaryng. Suppl. 224.

103. Wersäll, J. 1973: Problems and pitfalls in studies of cochlear hair cell pathology. In: Basic Mechanisms in Hearing. p. 235-256 (Moller, A.R., Ed.) Acad. Press.

104. Wersäll, J., Flock, A., Lundquist, P.G. 1965: Structural basis for directional sensitivity in cochlear and vestibular sensory receptors. Cold Spring Harb. Symp. Quantit. Biol. 30, 115-132.

105. Whitfield, I.C., Ross, H.F. 1965: Cochlear microphonics and summating potentials, and the outputs of individual hair-cell generators. J. acous. Soc. Amer. 38, 128-130.

106. Wiederhold, M.L. 1970: Variations in the effects of electrical stimulation of the crossed olivocochlear bundle on cat single auditory nerve fibre responses to tone bursts. J. acous. Soc. Amer. 48, 966-977.

107. Wilson, J.P., Johnstone, J.R. 1973: Basilar membrane correlates of the combination tone $2f_1 - f_2$. Nature 241, 206-207.

108. Wilson, J.P., Johnstone, J.R., 1972: Capacitive probe measure of basilar membrane vibration. Symp. on Hearing theory. p. 172-181 LPO Eindhoven, Holland.

109. Yates, G., Johnstone, B.M. 1975: Localized cochlear microphonics recorded from the spiral lamina. J. acous. Soc. Amer. in press.