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

Donald Robertson and Geoffrey A. Manley

Department of Biology, McGill University, Montreal, P.Q., Canada

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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.

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 $Q_{10\,dB}$; the cf. divided by the bandwith of the tuning curve 10 dB above threshold at the cf.

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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 1 kHz, 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 as small as was feasible (usually about 700 × 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 5–8 μ 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.

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 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

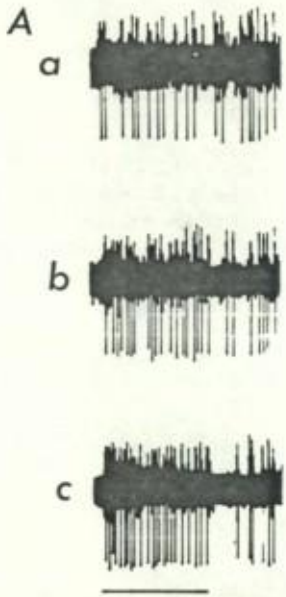

Fig. 1 A—D. Patterns of activity of spiral ganglion cells. A Oscilloscope tracings of activity of a single ganglion cell a) spontaneous activity, b) response to 15 kHz 10 dB above threshold, c) response at 15 dB above threshold. B Interspike interval histogram of the spontaneous activity of a sensitive 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

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. 1 C). 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. 1 D) 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

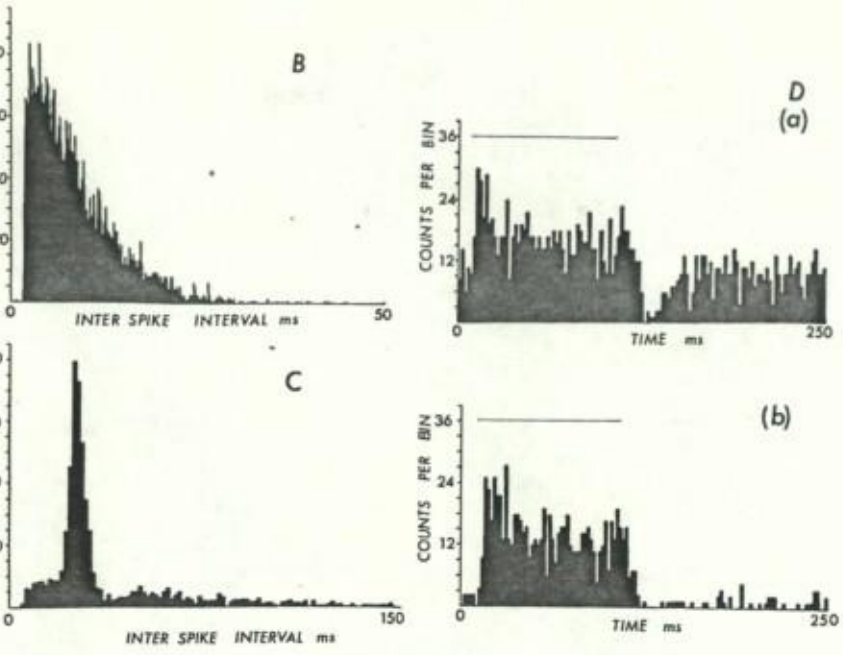

Fig. 1 B—D

ells. The slopes of these functions were very similar for frequencies both n and off the cf. in the majority of cells studies (Fig. 2). The dynamic ange that could be investigated was limited to about 20 dB above hreshold due to the presence of a large cochlear microphonic response t higher intensities that made discrimination of individual spikes ifficult.

b) Distribution of Characteristic Frequencies in the Ganglion

At any given recording location all the cells encountered had the ame cf. This cf. varied systematically with the recording location in he ganglion and, in the accessible area in the basal turn of the cochlea, anged from 11 kHz to 21 kHz. In photographs of the osmium-stained ochleas the myelinated peripheral processes of the bipolar cells could e seen to follow an orderly path from the recording site in the ganglion o the basilar membrane (Fig. 3). Distances from the basal end of the asilar membrane to points on the membrane could be measured from uch 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 ata from 8 animals relating the cf. of cells in the ganglion to the corre-

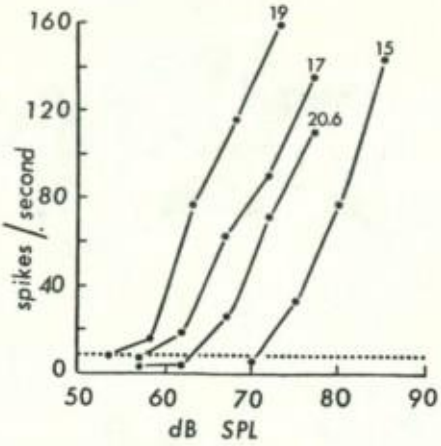

Fig. 2. Rate-intensity functions at different frequencies for a cell with a cf. at 19 kHz. The rate was measured as the number of spikes falling within ten 100 msec tone bursts. Functions are shown at 19, 17, 15 and 20.6 kHz. Sound pressure is relative to 2×10^{-4} dynes cm^{-2}

sponding distance along the basilar membrane. The distribution of frequency along the basilar membrane 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 $Q_{10\,dB}$ 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 a consistent linear relationship between the $Q_{10\,dB}$ 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

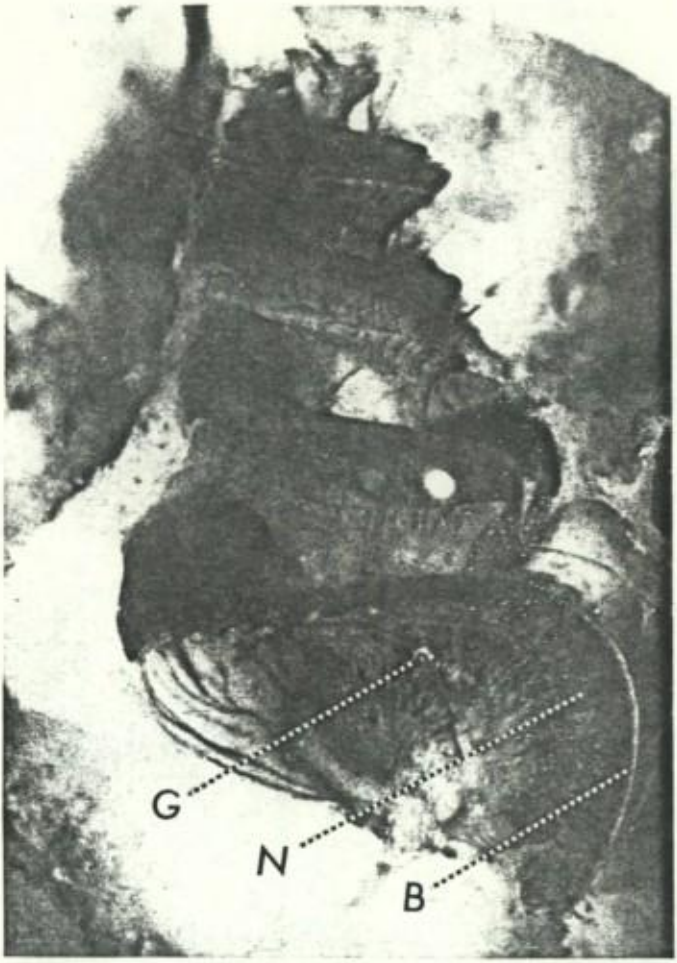

Fig. 3. Photograph of the guinea pig cochlea with bony capsule and stria vascularis removed. The basilar membrane has been stripped from the basal turn. *G* Point of entry of electrode into spiral ganglion. *N* Myelinated peripheral processes of ganglion cells visible through the osseous spiral lamina.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

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.

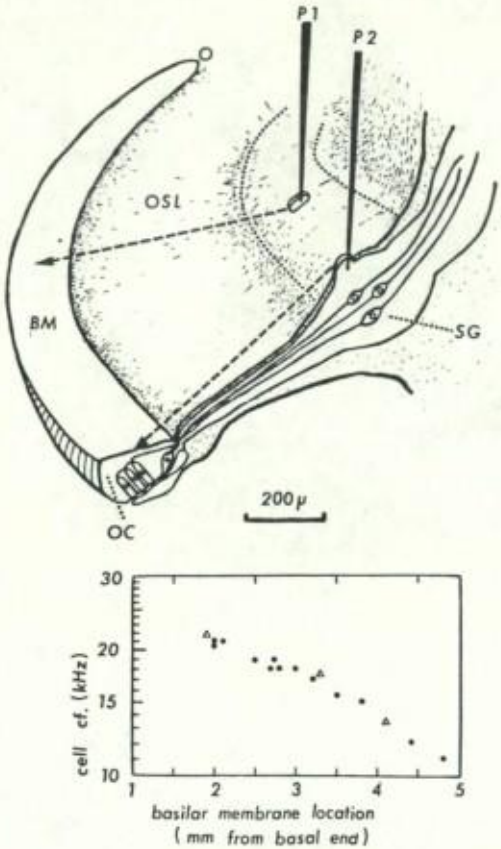

Fig. 4. Relation between characteristic frequency in the ganglion and distanc along the basilar membrane. The drawing shows the method of measuremen *BM* basilar membrane; *OC* organ of Corti; *SG* spiral ganglion; *OSL* osseous spir lamina; *P2* and *P1* two different penetration points in the ganglion.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

On investigating the basis of this variation in thresholds and tunin curves between animals, it was found that tuning curves could b deliberately altered by reducing the rate of artificial ventilation. reduction in respiratory rate from 60–30 strokes/min caused a rise i 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 a elevated but steady threshold at the cf. and a tuning curve for the ce could again be determined. The changes in the tuning curve produce

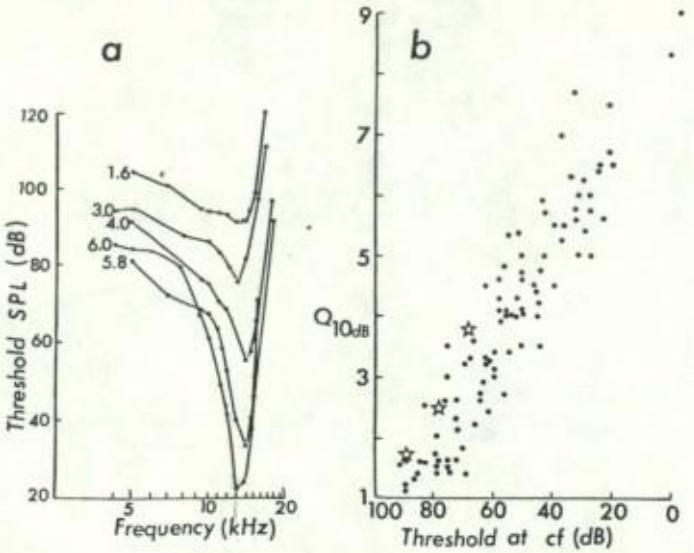

Fig. 5a and b. Variations in Q_{10dB} and thresholds. a) Representative tuning curves each from a different animal showing the gradation in sensitivity and sharpness of the tuning curves. b) Pooled data from 94 cells showing the close relationship between Q_{10dB} and the threshold at the cf. The range 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

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 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.

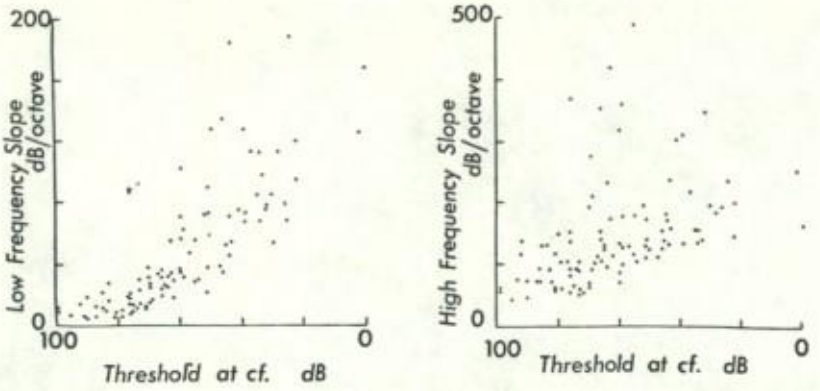

Fig. 6. Low and high frequency slopes of the tuning curves of the 94 units of Fig. 3 B. The method of measurement of slopes is discussed in the text. The slopes are presented plotted against threshold sound intensity at the cf.

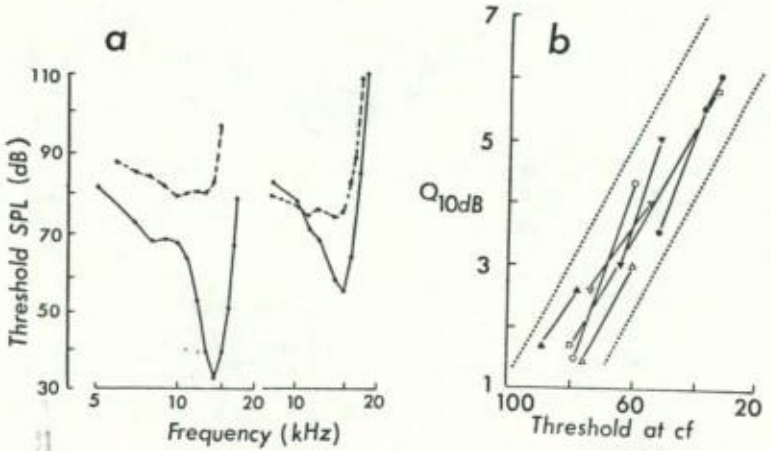

Fig. 7 a and b. The effect of deliberate slowing of the ventilation on tuning curves and sensitivity. a) Tuning curves for 2 cells in different animals. Solid lines: before slowing ventilation, broken lines: after slowing respiration and with a steady elevated threshold at the cf. b) The effect in 7 cells (including the 2 shown in (a) of slowing ventilation. Lines join the $Q_{10\,dB}$ 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. 5 b

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 occurrence 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 $Q_{10\,dB}$ on sensitivity. However, it has not been previously demonstrated that there is a close relationship between $Q_{10\,dB}$ 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 70 dB 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 100 dB/octave respectively. In a later report (Johnstone and Yates, 1973) the slopes were reported as 15 and 300 dB/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 $Q_{10\,dB}$ 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 $Q_{10\,dB}$ 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 this hypothetical second filter is still a matter for discussion.

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 cochleas. 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)

Donald Robertson
Geoffrey A. Manley
Department of Biology
McGill University
P.O. Box 6070
Station A
Montreal, P.Q., Canada, H3C 3G1