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Auditory Nerve Fibre Activity in the Tokay Gecko

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A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the degree of Master of Science.

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Montréal, Canada

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Abstract

Spontaneous and response activity were recorded from single auditory nerve fibres in the lizard, the Tokay gecko. The effect of temperature change on the frequency selectivity (tuning) of the fibres was studied in some detail. Small increments in temperature were found to consistently shift the frequency response curves (or tuning curves) of the animal toward higher frequencies. Cooling had the reverse effect. Temperature was monitored either orally or within the cochlea contralateral to the exposed auditory nerve. The results are compared with temperature effects that have been reported for cochlear potentials and, recently, for auditory nerve fibres in other species. Their implications with respect to auditory tuning mechanisms are discussed. A number of general properties of the fibres' activity were also determined. Computer analysis of these data revealed interesting patterns of both spontaneous and response activity. An attempt is made to relate these patterns to morphology and possible tuning mechanisms in the Tokay's inner ear.

Resûmé

On a enrègistré chez le lézard gecko de Tokay les activités spontanées et provoquées des fibres nerveuses auditives simples. On a étudié en détail les effets de changements de température sur la sélectivité de fréquences (tuning) des fibres.

On a trouvé que de légères augmentations de température élèvent les courbes de réponses aux fréquences (tuning curves) à des fréquences plus hautes. Le refroidissement des fibres produit l'effet contraire. Les températures enregistrées étaient orales ou cochléaires et dans ce dernier cas contralatérales au nerf auditif exposé.

On a comparé les résultats aux effets déjà rapportés de changements de température sur les potentiels cochléaires du lézard gecko, et aussi, plus récemment, sur les fibres auditives d'autres espéces et on a discuté des implications quant aux mécanismes de séléctivité auditive. On a dégagé un certain nombre de propriétés génerales de l'activite des fibres. L'analyse par ordinateur de ces données révèle des schémes intéressants d'activité spontanée et provoquée.

On a tenté d'établir un rapport entre les schèmes d'activité et la morphologie et les mécanismes possibles de sélectivité (tuning) de l'oreille interne du Tokay.

To L.J.

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Preface

This thesis is based on research conducted between June, 1976 and March, 1978. The research is original in that auditory nerve fibre activity has not been previously examined in the Tokay gecko, and has been studied in very few other reptiles. To my knowledge, the effects of temperature change on the activity of single auditory neurons had not been examined in any species prior to this project.

The bulk of the thesis consists of two papers which will be submitted for publication. Because of the complementary nature of these papers, they constitute a two-part report and will be submitted together as such. For this reason a connecting text was not considered necessary. The results reported in these papers were obtained from 44 animals. The data from nine of these animals were collected and analysed by Lorraine Pawson; these results are included in the report on general properties of auditory nerve fibre activity (Paper I of this thesis).

As specified in the thesis guidelines outlined by the Faculty of Graduate Studies and Research of McGill University, the following regulation must be cited:

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I am indebted to Lorraine Pawson for her cheering presence and expert help, particularly in the more tedious aspects of statistical analysis. Thanks are due to Prof. Y.L. Werner for his part in inspiring the temperature effects research, and to Prof. R.R. Capranica for his interest and advice concerning the same. I would like to thank Seth Pullman for his help in assembling the thesis, and for general encouragement. I am especially grateful to Dr. G.A. Manley for his invaluable ideas and generous and enthusiastic support throughout this study.

Introduction

The vertebrate inner ear has successfully resisted efforts to fully understand the mechanisms underlying peripheral frequency analysis. Not surprisingly, these efforts have mostly focussed on the mammalian system. The work of von Békésy (1960) established a mechanical basis for frequency analysis within the mammalian cochlea. von Békésy demonstrated that sinusoidal stimulation of the cochlea produces travelling waves along the elongate basilar membrane supporting the sensory epithelium (organ of Corti). The travelling wave develops slowly from the basal end of the cochlea (adjacent to the stapedial footplate), reaches a maximum amplitude, and decays apically. The distance from the basal end at which the maximum occurs varies with the frequency of the pure tone stimulation. With high frequency stimuli, the displacement of the basilar membrane reaches a maximum relatively early and damps out quickly; thus the apical end is little affected by high frequencies. Lower frequencies displace a greater length of the cochlear partition (basilar membrane plus organ of Corti). The travelling wave rises more slowly to a maximum and decays more apically. Therefore, different frequencies are represented at different positions along the membrane. Furthermore, the progression from high to low frequencies is continuous from base to apex. This distribution of frequencies along the basilar membrane can be fully explained in terms of a baso-apical gradient in its physical properties. It becomes gradually wider and more flexible with increasing distance from the basal end.

Clearly the mechanical tuning of the basilar membrane must determine the distribution of frequencies in the overlying organ of Corti. Confirmation of this is supplied by recordings from primary afferents emanating from different positions along the cochlear partition (eg. Robertson and Manley, 1974). However, there appears to be an important difference between the tuning of the basilar membrane and the tuning of primary afferents. Although there is some controversy surrounding the techniques that have been used to study basilar membrane tuning, the available data indicate that it is much broader than the tuning of primary auditory neurons (eg. von Békésy, 1960; Johnstone et al.; 1970, Kohlloffel, 1973; Rhode, 1971). The term 'second filter' was coined to describe a hypothetical process between basilar membrane vibration and the primary neuronal response which is capable of sharpening the broad mechanical tuning (Wilson and Johnstone, 1972). The nature of this cochlear sharpening process has been the subject of considerable speculation. Numerous models have been proposed but as suggested by Manley (1977), some are untestable and others are incompatible with the present data. For instance, lateral inhibition and other forms of primary fibre interaction (eg. Zwislocki, 1975) have been virtually ruled out by the lack of anatomical evidence for inter-fibre contacts (Spoendlin, 1975), and by recent intracellular evidence that the hair cell receptor potential is sharply tuned (Russell and Sellick, 1977). That the second filter is metabolically labile is suggested by recordings from primary auditory neurons when the cochlea has been subjected to mechanical damage, ototoxic drugs, or hypoxia (Evans, 1975; Robertson & Manley,

1974). Under these conditions the sharpness of tuning in the primary neurons is reduced to the extent that their tuning curves[1] approach the resonance curves of the basilar membrane. The implication is that the amount by which the normal sharp tuning is reduced represents the contribution of a metabolically labile second filter.

The potential value of a comparative approach to the question of frequency selectivity has been generally overlooked. Primary auditory afferents in teleosts (Fay, 1978; Furukawa and Ishii, 1967), anurans (eg. Capranica, 1976), lizards (Manley, 1977; Weiss et al., 1976; this thesis), caiman (Klinke and Pause, 1977) and birds (Manley and Leppelsack, 1977) have all been shown to have fairly simple, approximately V-shaped tuning curves, as do mammalian cochlear fibres. This does not, of course, necessarily imply a common tuning mechanism. Although many vertebrate ears have similar features in fundamental design, large differences also exist. Among terrestrial vertebrates, the anurans have evolved an auditory periphery that deviates from trends discernible in reptiles, birds and mammals (Manley, 1973). There are two anuran auditory organs, both of which rest against the wall of the otic capsule. The cochlear ducts of reptiles and birds have a design more comparable to the mammalian cochlea. For instance, the basilar papilla - homologous in these animals to the organ of Corti - overlies a basilar membrane that is at least partly suspended in fluid, and hence susceptible to pressure differences on either side.

[1] A tuning curve of an auditory neuron is a plot of the stimulus intensity required to evoke a threshold response to a pure tone against stimulus frequency.

In contrast to the relative uniformity of the mammalian cochlea across different species, lizard inner ear morphology has diversified to an extraordinary degree. Each family has evolved its own basic design; variations in details occur among species within a family. Family patterns are so well defined that classification of a lizard according to family is possible on the basis of cochlear duct anatomy alone (Miller, 1966). The more differentiated lizard cochlear ducts have tended toward: 1) elongation of the basilar membrane and papilla; 2) elaborate accessory structures; 3) well defined receptor cell (hair cell) orientation patterns. The inner ears of lizards thus naturally lend themselves to the study of structure-function relationships (Manley, 1977). According to Miller (1966), "reptiles ... are remarkable in that they have experimented with the cochlear duct and modified almost all of its various parts." This is most true for lizards. The functional consequences of these modifications are accessible, at least in theory, if appropriate species are chosen for comparison. Even in a single papilla it may be tempting to look for morphological correlates to the variability in response properties of auditory neurons. This approach has been taken recently by studies on auditory nerve fibres in two lizards: the monitor (Manley, 1977) and the alligator lizard (Weiss et al., 1976). Both species have two fibre populations, a sharply tuned low frequency population and a broadly tuned one at higher frequencies. In each case, the fibres belonging to the two populations innervate different regions of the papilla. Weiss et al. and Manley have both suggested that a morphological dichotomy may underlie the

observed tuning differences.

The first paper in the thesis attempts to relate certain response properties of Tokay auditory nerve fibres to physical features of its inner ear, in addition to providing a general description of spontaneous and response activity. The second paper reports on the effects of temperature on the fibres' tuning properties.

An introductory outline of Tokay peripheral auditory anatomy follows.

Anatomy

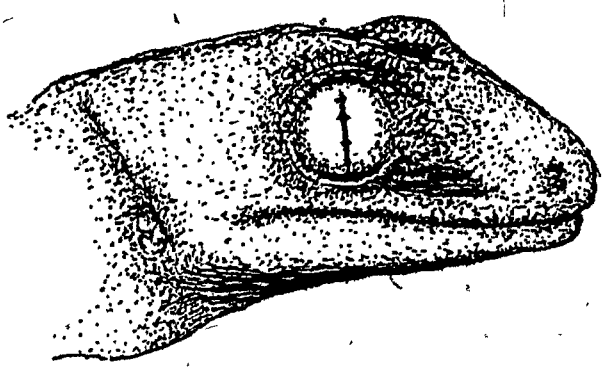
As in all lizards, the Tokay's eardrum is close to the surface of the head. The oval opening to the shallow (3 mm deep) external auditory meatus is slightly smaller than the meatus diameter, and can be closed by a constrictor muscle (Wever, 1963). The Tokay's middle ear is illustrated in Fig. 1b. The footplate of the columella (stapes) inserts on the oval window of the otic capsule. Lizards have a release window that is analogous to the mammalian round window (Henson, 1974).

Gekkonid lizards have a highly differentiated cochlear duct that has been described in detail by Miller (1966, 1973) and Wever (1974). The cochlear duct, saccule and utricle are the three major chambers of the labyrinth. The cochlear duct and lagenar macula lie together within the cochlear recess of the bony labyrinth (Fig. 2a). The periotic sac surrounding the cochlear duct consists of two compartments, the scala vestibuli and scala tympani, which communicate via a narrow channel, the helicotrema. This channel serves to eliminate slowly developing pressure differences between the scalae. It is ineffective with respect to the rapid pressure fluctuations created by sound; pressure is then exerted along the cochlear partition. The lagenar macula and vestibular membrane comprise the lateral wall of the cochlear duct. The vestibular membrane separates the periotic fluid of the scalae vestibuli and tympani from the endolymph of the cochlear duct. The endolymph has a different ionic composition than the perilymph. In mammals, this results in an 80 - 90 mV potential difference between the endolymphatic space (scala media) and the perilymphatic compartments. This potential is about 20 mV in birds and only a few

Figure 1. A) The Tokay gecko (Gekko gecko, family: Gekkonidae)

B) The middle ear of G. gecko, as viewed from an anterior, medial and ventral position. (Modified from Werner and Wever, 1972)

A. Gekko gecko



B.

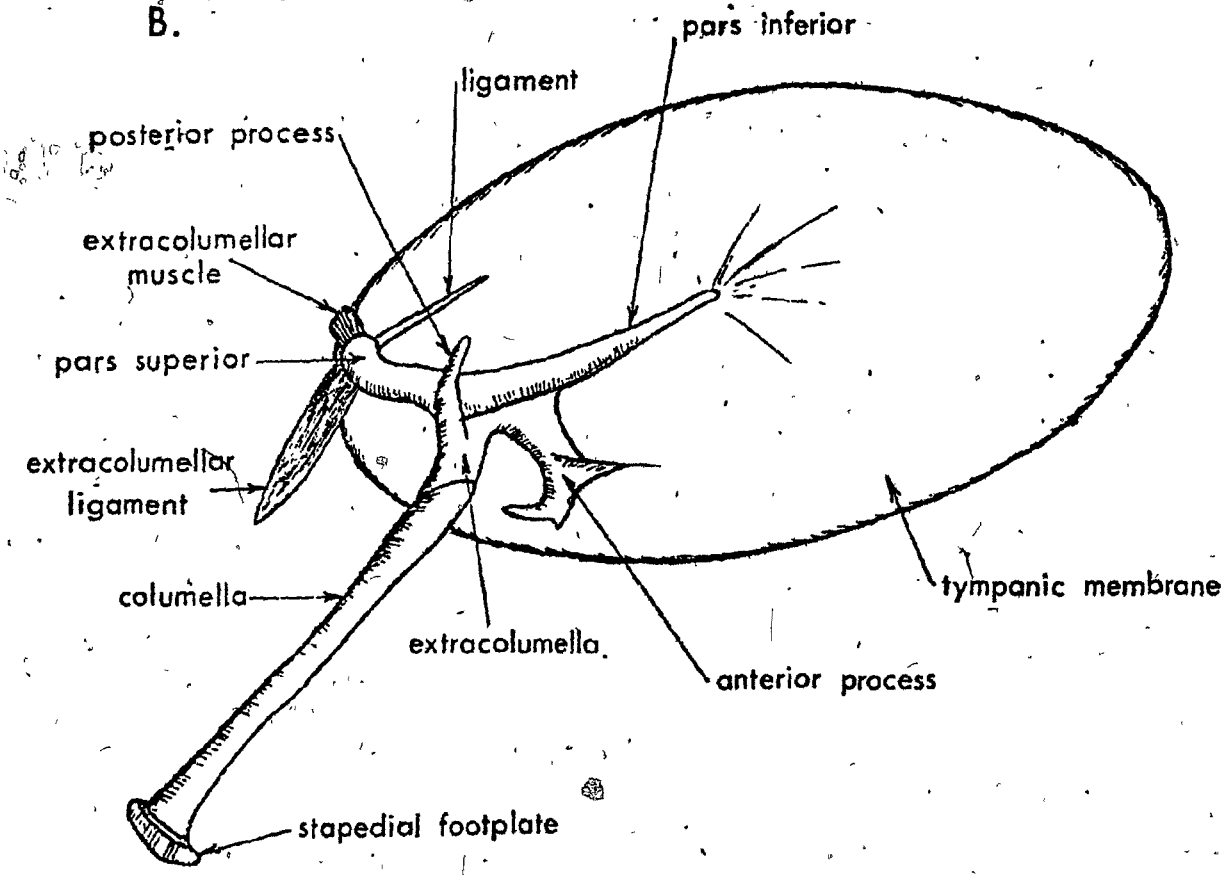


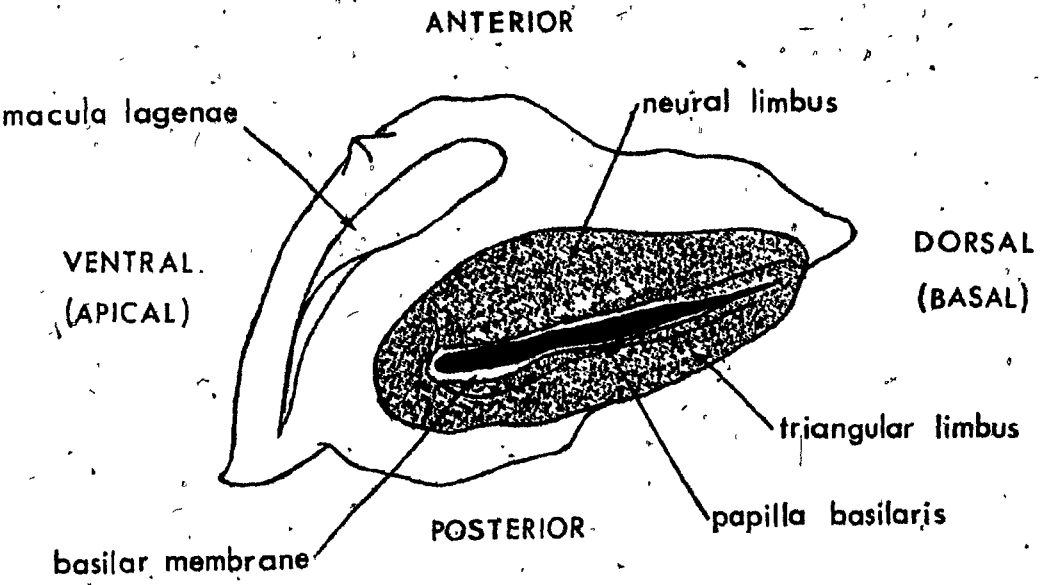
Figure 2. The cochlear duct of G. gecko.

A) lateral view of the left cochlear duct

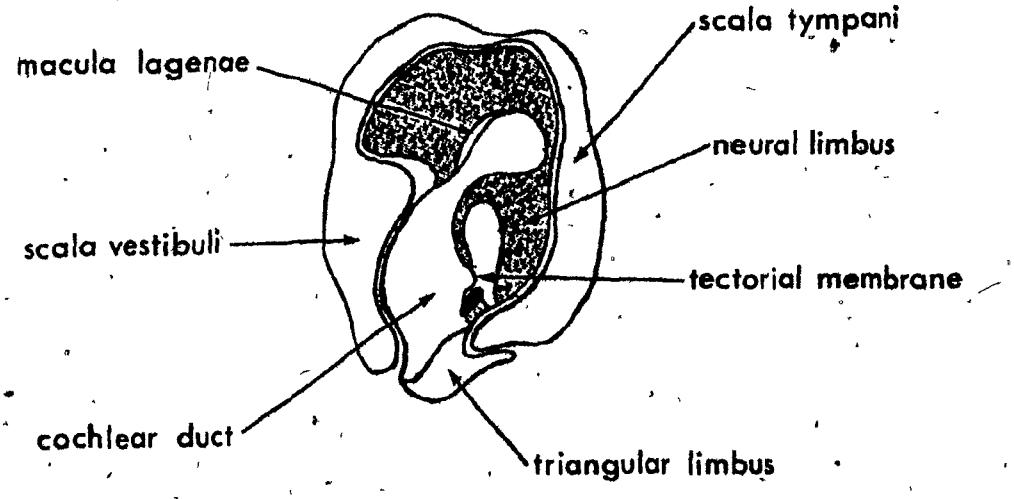
B) cross-section through the cochlear duct

(Modified from Miller, 1973)

A.


ANTERIOR
macula lagenae
neural limbus
VENTRAL
(APICAL)
DORSAL
(BASAL)
triangular limbus
papilla basilaris
POSTERIOR
basilar membrane

B.

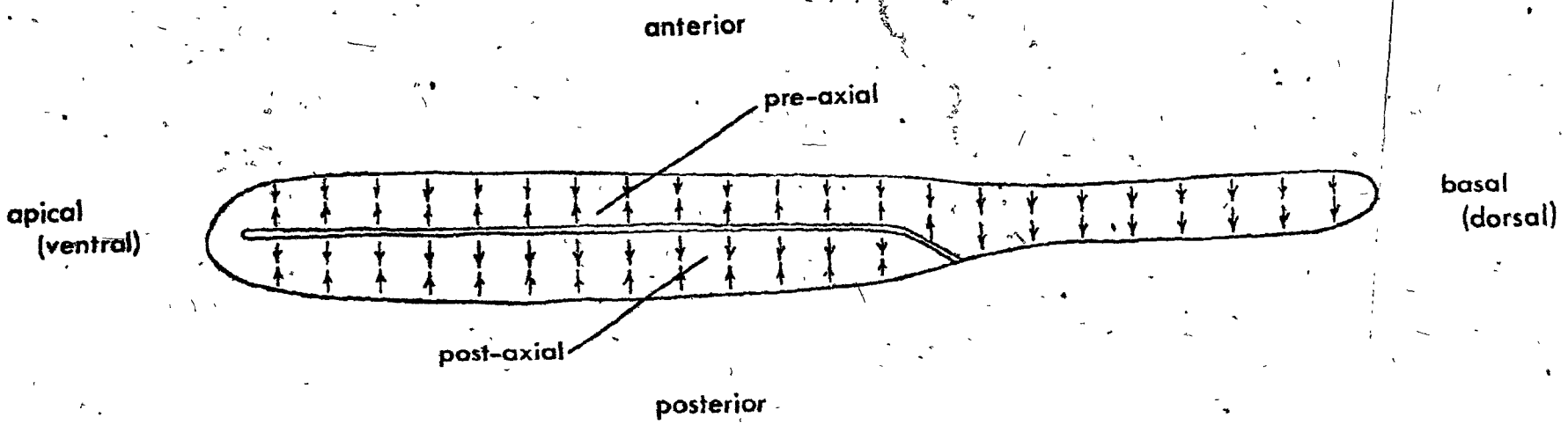

scala tympani
macula lagenae
neural limbus
scala vestibuli
tectorial membrane
cochlear duct
triangular limbus

millivolts in reptiles (Manley, 1973).

Along the medial wall of the cochlear duct are the limbus and the basilar membrane, which is itself modified limbic tissue. It bridges the gap between the dorsal neural limbus and the more ventral triangular limbus (Fig. 2b). The neural limbus is so named because it contains the cochlear ganglion. The bipolar primary cells of this ganglion each send a proximal process to the brain and a distal one to the basilar papilla. The latter consists of columnar hair cells and supporting cells. The fibres run between the supporting cells to terminate on the basal and basolateral surfaces of each hair cell. A relatively large projection of the neural limbus, called the limbic lip, overhangs the basilar papilla (Fig. 2). A tectorial membrane extends ventrally from the edge of the limbic lip to form attachments with the hair cell cilia.

The following description of the Tokay's basilar papilla is based on Miller's (1973) observations using scanning electron microscopy. The Tokay papilla is long (approximately 2 mm) by saurian standards, and has more hair cells (approximately 2100) than any other lizard papillae that have been examined. It tapers from an apical (ventral) width of about 130μ to 40-50μ basally (dorsally), becoming increasingly curved toward the basal end. In the apical two-thirds of the papilla, a prominent midline gap (Fig. 3) divides the apico-basal (longitudinal) rows of hair cells into an anterior or 'pre-axial' group that faces the neural limbus, and a posterior or 'post-axial' group that is directed more toward the triangular limbus. The midaxial hiatus is not present in the basal third of the papilla.

Figure 3. Schematic diagram of the basilar papilla of G. gecko. Small arrows indicate hair cell orientations. (Modified from Miller, 1974)


anterior
pre-axial
apical
(ventral)
basal
(dorsal)
post-axial
posterior

The tectorial membrane extends from nearly the entire length of the limbic lip. Tectorial fibres attach to all hair cells in the basal third of the papilla, and to the pre-axial cells of the apical two-thirds. The connections are filamentous on the basal end but thick apically. Each transverse (antero-posterior) row of post-axial hair cells on the apical part of the papilla is covered by a heavy tectorial mass, dubbed 'sallet' by Wever (1967). Sallets are connected to each other but are independent of the neural limbus. Each sallet is in contact with the kinocilia and possibly the tallest stereocilia of the underlying hair cells. The kinocilial head, or bulb, is embedded in the overlying salletal or tectorial process. According to Wever (1967), attachment of one end of the tectorial membrane to the limbus causes it to have rotational motion during sound stimulation, in contrast to the up-and-down vibration of the basilar membrane. Wever proposed that the rotation of the tips of the cilia relative to their bases stimulates the production of receptor potentials within the hair cells. The sallets were visualized as oscillating with some lag relative to basilar membrane motion, by virtue of their inertia. Again the critical stimulus would be provided by the difference in the motions of the tips and bases of the cilia.

The stereocilia on the surface of each hair cell are arranged in wedge-shaped rows that show a progressive decrease in height. The tallest row is in contact with the kinocilium. Hair cell orientation is defined by the location of the kinocilium on the cell surface. In the basal third of the Tokay papilla, all kinocilia are located posteriorly on the hair cell surfaces. Thus, this region of the papilla has a unidirectional hair cell orientation pattern. In the apical portion of the papilla, the kinocilia

in the posterior longitudinal rows of both pre- and post-axial divisions are located anteriorly on the cell surfaces (Fig. 3). The kinocilia in the anterior rows of both pre- and post-axial divisions are located near the posterior edges of the cell surfaces. Therefore, the pre-axial and post-axial regions each have a bidirectional hair cell orientation pattern, making the apical two-thirds of the papilla 'doubly bidirectional' (Miller, 1973).

The proximal processes of the cochlear ganglion cells join fibres from the other sensory epithelia of the otic capsule. Together they exit from the otic capsule as the eighth, vestibulocochlear nerve, which enters the rostral end of the medulla dorsolaterally. Basilar papilla fibres are distinguishable from the other, more variable fibre types in being rather small and uniform in diameter (Miller, 1975). They project to secondary auditory neurons in the cochlear nuclei, which extend caudally for about 1.5 mm from the junction between medulla and cerebellum (Miller, 1975). As in other animals, the cochlear nuclei are large enough to appear as visible elevations on the dorsal surface of the medulla.

Paper I:

Auditory Nerve Fibre Activity in *Gekko gecko*:

I. Spontaneous and response activity.

Introduction

Peripheral auditory processing in lizards is of special interest because of the extraordinary variability in cochlear duct morphology (Miller, 1966). Family-specific properties include: the shape of the basilar membrane; the shape and hair cell orientation patterns of the basilar papilla (homologous to the mammalian organ of Corti); the type and arrangement of tectorial and tectorial-like structures. Within a family, size differences and species-specific variations on the family plan occur. This great diversity of form invites comparison of inner-ear performance in different lizards. Numerous comparative studies of their cochlear microphonic responses have been done (eg. Wever and Peterson, 1963; Wever et al., 1963; Wever et al., 1964). A more recent approach has been to relate different types of response activity in a lizard's primary auditory fibres to structural features of its inner ear (Manley, 1977; Weiss et al., 1976).

This paper describes general features of spontaneous and response activity in auditory nerve fibres of the Tokay gecko, Gekko gecko. The Tokay was chosen for several reasons. Members of the family Gekkonidae are unusual lizards in that they use their hearing in communication. The Tokay has a variety of calls, some intraspecific. It is therefore possible in this lizard to study the activity of auditory neurons in response to its calls: natural, meaningful sound stimuli. The gekkonid cochlear duct is highly differentiated; the basilar papilla's hair cell orientation pattern and accessory tectorial structures are particularly complex (Miller, 1966, 1973, 1974; Wever, 1974). The Tokay's basilar membrane is large by lizards' standards (approx. 2 mm long) and has a

pronounced apico-basal (ventro-dorsal) taper (Miller, 1973, 1974; Wever, 1974). Because of its relatively advanced vocal behaviour and cochlear morphology, the Tokay's auditory system has received more attention than most lizards'. Studies have been done on its middle ear performance (Manley, 1972; Wever and Werner, 1970), its cochlear microphonics (Hepp-Reymond and Palin, 1968; Wever et al., 1963), the activity of its medullary (Manley, 1972, 1974) and midbrain (Sammaritano-Klein, 1976) auditory centres. The anatomy of its inner ear (Miller, 1966, 1973), cochlear nuclei (Miller, 1975) and torus semicircularis (Sammaritano-Klein, 1976) has been described. A primary fibre study seemed the obvious next step toward an understanding of processing at the Tokay's auditory periphery.

Methods

Gekko gecko weighing 40-200 g were obtained from Thailand. The data in this paper are from 410 units in 44 animals. 297 of these units are from 33 experiments whose main purpose was to observe temperature effects on tuning curves. These animals, therefore, contributed mostly tuning curve data.

The animals were anaesthetized with urethane (ethyl carbamate) at a dose of 10 ml. of 20% urethane per kg. Artificial respiration was unnecessary. In those experiments which were not part of the temperature effects study, the gecko was maintained at approximately 24°C with a heating pad draped around its body. In temperature effect experiments, oral temperature was varied between 20 and 30°C. A ventral surgical approach was used. An opening was made in the floor of the mouth to expose the palate. Muscle, bone and dura overlying left rostral medulla were removed. A hole was made in the arachnoid to reduce the cerebrospinal fluid pressure and create a space between the medulla and left otic capsule. Small pieces of tissue could then be inserted in this space, thus pushing the medulla slightly to the animal's right and exposing the left vestibulocochlear nerve (VIII). This was the proximal part of the eighth nerve, between its exit from the otic capsule and its entry on the dorsal side of the rostral medulla.

Glass microelectrodes were used to record from the posterior half of the nerve. Similar results were obtained whether the microelectrode was filled with 4M NaCl or 3M KCl. Using a remote hydraulic microdrive, the electrode was driven from outside the sound-attenuating semi-anechoic room containing the animal. Sound stimuli were delivered free-field

from a speaker 1 m from the gecko's head, facing its left ear. White noise pulses (50 ms, 2/s) were presented as search stimuli. Pure tone pulses (50 ms, 2/s, 5 ms rise-fall time) with trapezoidal rise-fall characteristics were used to obtain frequency-threshold tuning curves. Thresholds were determined audio-visually. Sound pressure levels were calibrated using a Brüel and Kjaer 0.5 in. condenser microphone, placed next to the left eardrum and a narrow-band wave analyser. Spontaneous and response activity were recorded on tape for off-line computer analysis using time interval histogram (TIH) and peristimulus time histogram (PSTH) programs, respectively.

Results

At the beginning of each electrode pass, the electrode was positioned over some point in the posterior half of the ventral surface of the eighth nerve. In vertical or near-vertical penetrations, all units encountered were auditory and easily identifiable as such by their time-locked responses to the search stimuli. When the electrode was angled caudo-rostrally, non-auditory units were obtained dorsal to auditory units, indicating that the electrode had passed out of the auditory part of the nerve. The non-auditory units often showed regular "spontaneous" discharge and were presumed to be vestibular. The transition from auditory to vestibular nerve was fairly abrupt.

Histological control indicated that the area of the nerve penetrated contained almost exclusively fibres and not cell bodies of the cochlear ganglion and nuclei. In a few electrode passes at the distal edge of the exposed nerve, units were occasionally encountered that differed from the others in spike waveform and which could be held over much longer distances (tens of microns rather than several). These few units were thought to be primary cell bodies.

(i) Tuning Curves

The tuning curve or frequency-threshold curve used in this study was obtained by exposing a unit to different frequencies of pure tone stimuli and determining the sound intensity required to evoke a threshold response at each frequency. A threshold response was defined as the smallest perceptible stimulus-locked increase in discharge rate over the spontaneous level. The frequency to which a unit is most sensitive is its

characteristic frequency (CF). Tokay tuning curves have a fairly simple V-shape (Fig. 1), in common with auditory nerve fibre data from mammals (eg. Evans, 1972; Kiang, 1965), birds (Manley and Leppelsack, 1977), reptiles (Klinke and Pause, 1977; Manley, 1977; Weiss et al., 1976), and amphibians (eg. Capranica, 1976). CFs in the Tokay auditory nerve ranged from 0.15 to 5 kHz. It is possible to derive a sensitivity curve from the cumulative tuning curve data (Fig. 2). Thresholds at various CFs are plotted and a curve is drawn through the lowest values. In Fig. 2, the sensitivity curve for auditory nerve fibre data is compared to that for cochlear nucleus neurones (from Manley, 1972) and to cochlear microphonic sensitivity functions (from Hepp-Reymond and Palin, 1968, and from Werner and Wever, 1972). The data from primary and secondary single units are in good agreement, and suggest two sensitivity maxima: one at 0.5 - 0.7 kHz and one at 2 - 2.5 kHz. These curves indicate that thresholds depend to some extent on the frequencies to which the units are responsive. That physiological condition is also a factor is indicated by the observation that variability among fibre thresholds was lower within an animal than over different animals. The high frequency maximum in the single unit curves is missing from both cochlear microphonic sensitivity functions. The possible significance of this discrepancy will be discussed later.

One measure of the sharpness of a unit's tuning is its Q_{10dB} value, obtained by dividing CF by the tuning curve bandwidth at 10 dB above CF threshold. In the Tokay, higher Q values appeared at higher frequencies, creating a greater spread of values than at lower frequencies (Fig. 3). Q values above 8 occurred almost exclusively above 2 kHz, while values of less than 4 occurred almost exclusively below 2 kHz. There are insufficient data per animal to determine to what extent the spread of Q values at a

Figure 1. Some tuning curves for Tokay gecko auditory nerve fibres, obtained from several animals. Sound pressure is expressed in this and subsequent graphs as dB SPL = dB re. 20 μNm^{-2}.

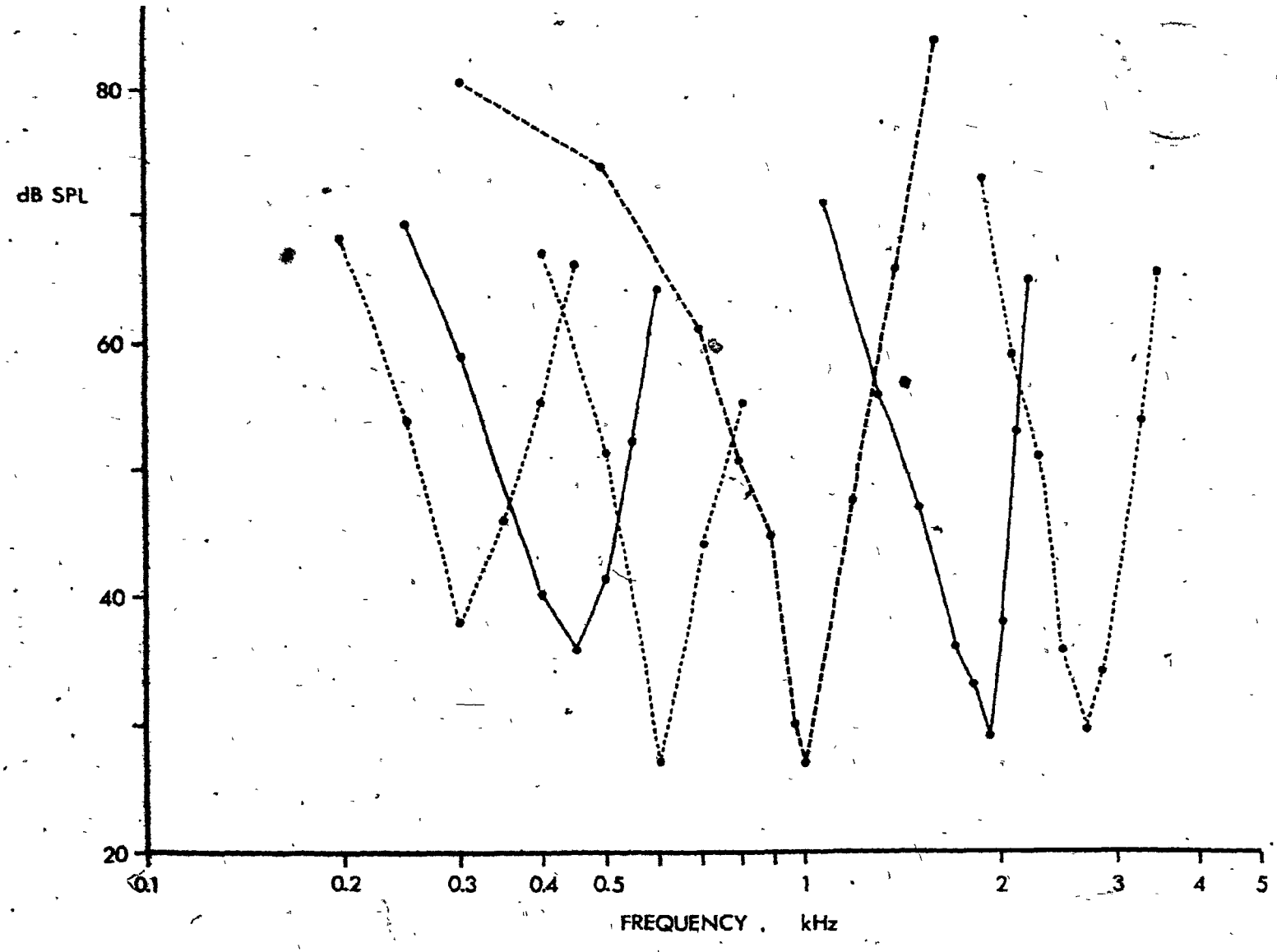

80
60
40
20
dB SPL
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
FREQUENCY , kHz

Figure 2. Auditory sensitivity curves for the Tokay gecko. The cochlear microphonic sensitivity functions are taken from Hepp-Reymond and Palin (1968) and Werner and Wever (1972). Both record the intensities required to produce a 'standard' microphonic response amplitude of 0.1 μV RMS at different frequencies of pure tone stimuli. The sensitivity curves derived from tuning curves of cochlear nucleus neurons (from Manley, 1972) and auditory nerve fibres (present data) were arrived at by drawing curves through the most sensitive CF thresholds across the Tokay's frequency range.

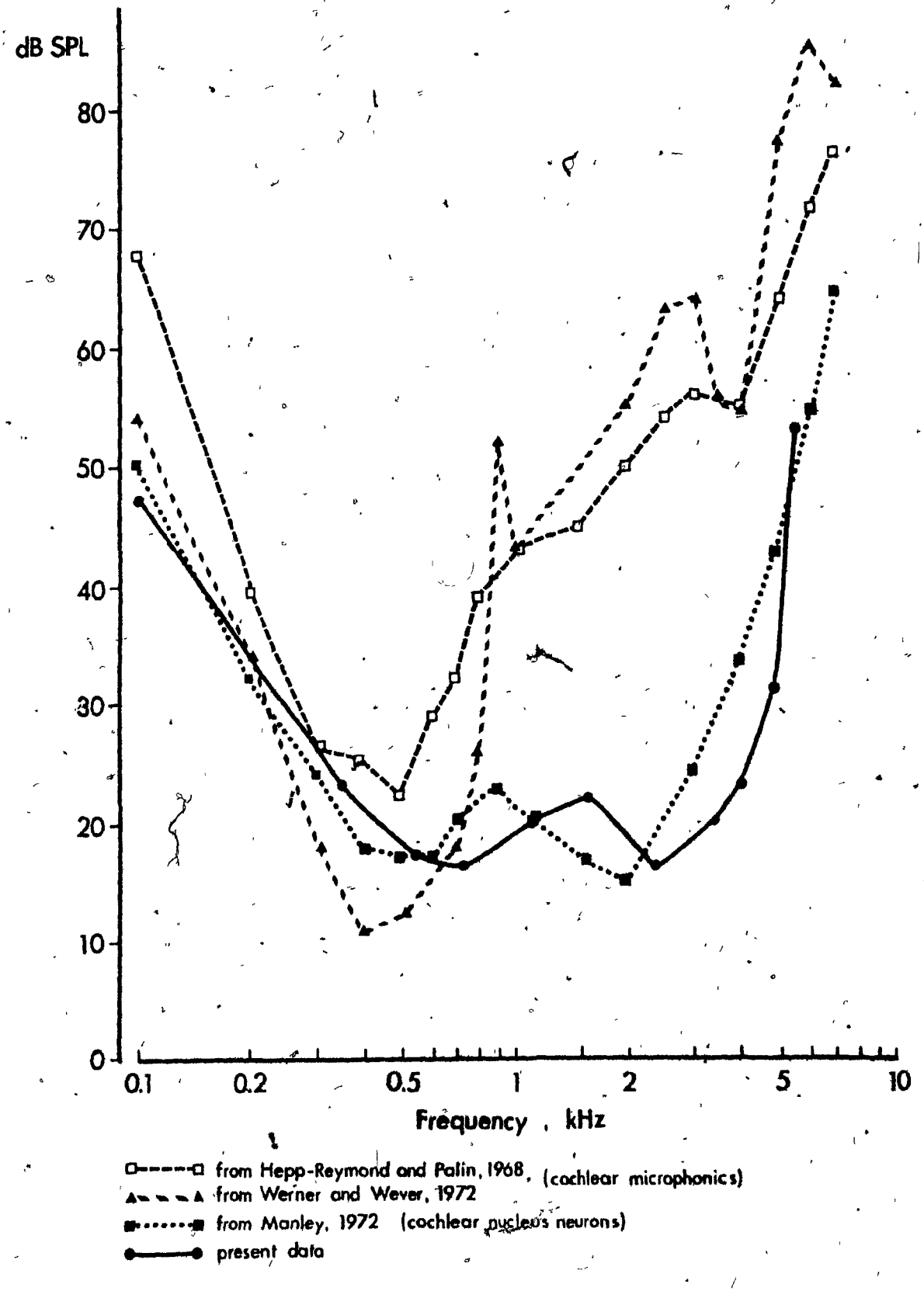
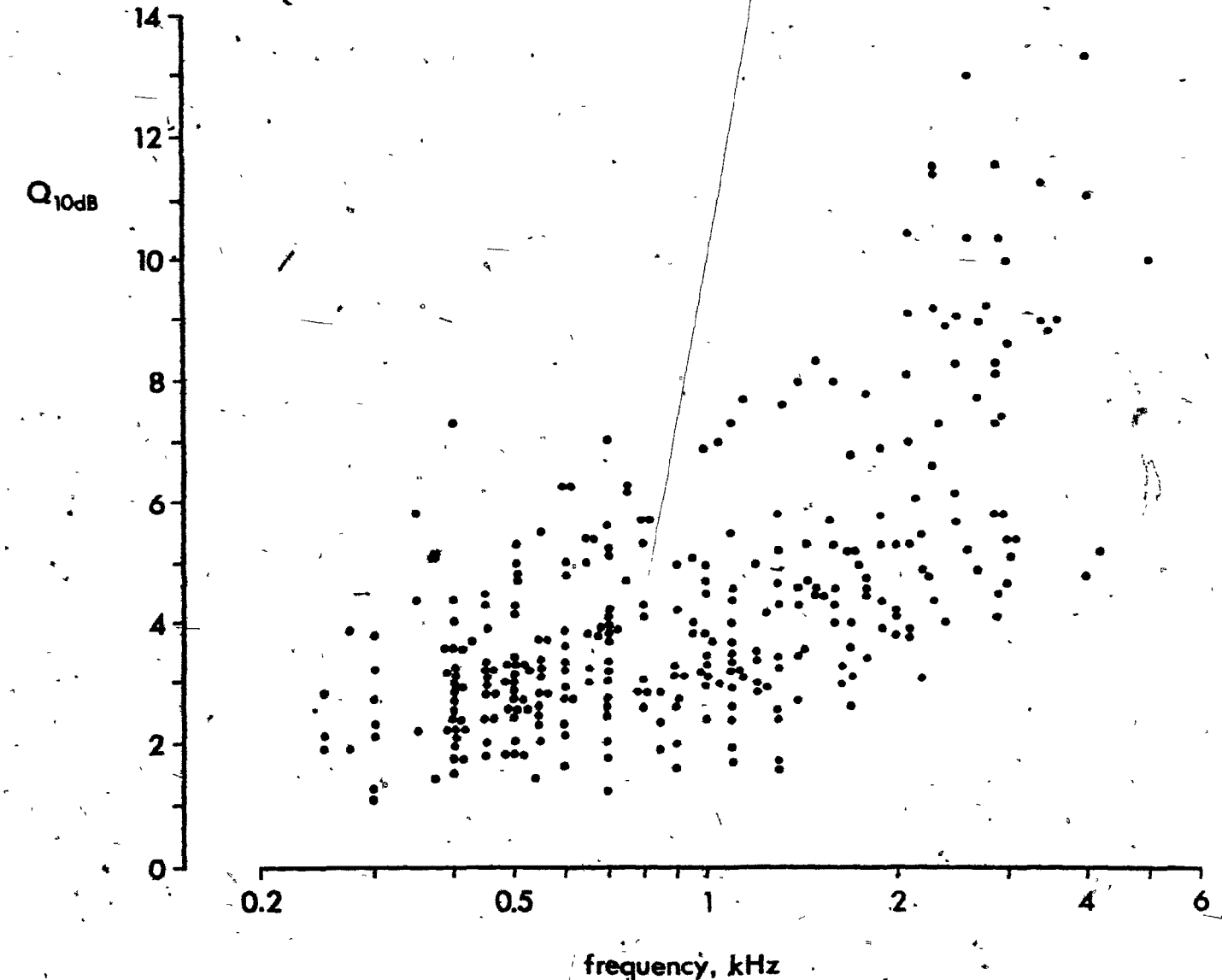

dB SPL
80
70
60
50
40
30
20
10
0
0.1
0.2
0.5
1
2
5
10
Frequency , kHz
from Hepp-Reymond and Palin, 1968, (cochlear microphonics)
from Werner and Wever, 1972
from Manley, 1972 (cochlear nucleus neurons)
present data

Figure 3. Q_{10dB} *v.* CF. The Q_{10dB} of a tuning curve is obtained by dividing its CF by its bandwidth at 10 dB above CF threshold.


14
12
Q_{10dB}
10
8
6
4
2
0
0.2
0.5
1
2
4
6
frequency, kHz

given frequency in Fig. 3 is the result of pooling the data. Q values do vary for fibres of similar CF in a given animal. Q_{10dB} was also plotted against CF threshold (Fig. 4) to determine whether there was any relationship between sharpness of tuning and sensitivity. Broad tuning has been shown to be associated with elevated thresholds in the event of cochlear trauma, hypoxia, etc. (Evans, 1974). In Tokay fibres there was a weak tendency for higher Q values to be associated with lower thresholds (Fig. 4). Thus in many instances low Q values were not associated with high thresholds and presumably did not reflect damage to the cochlea.

Sharpness of tuning can also be expressed in terms of the slopes of the two sides - low frequency and high frequency - of the tuning curve (Fig. 5). Low frequency slopes ranged from 20 to 240 dB/octave, and high frequency slopes from 20 to 300 dB/octave. As with Q_{10dB}, higher values and greater spread of both low and high frequency slope values occurred at higher frequencies. These trends are fairly continuous for high frequency slopes. In the graph of low frequency slope v. CF there is a discontinuity at 0.8 kHz, below which are higher values and a correspondingly greater spread of values than between 0.8 and 2 kHz. This has an effect on the graph of the ratio of high frequency: low frequency slope (HF:LF) v. CF (Fig. 6a) in that the ratios tend to be higher between 1 and 2 kHz than elsewhere. Below 0.6 kHz, low frequency slopes were on average as high as high frequency slopes.

To what extent do the changes with frequency of Q_{10dB} and low and high frequency slopes depend upon the frequency response of the middle ear? The velocity response of the Tokay's columella has been studied with the Mössbauer technique (Manley, unpublished data) (Fig. 7). The response falls off at rates of approximately 10 dB/octave below 0.4 kHz and 16

Figure 4. Q_{10dB} v. CF threshold

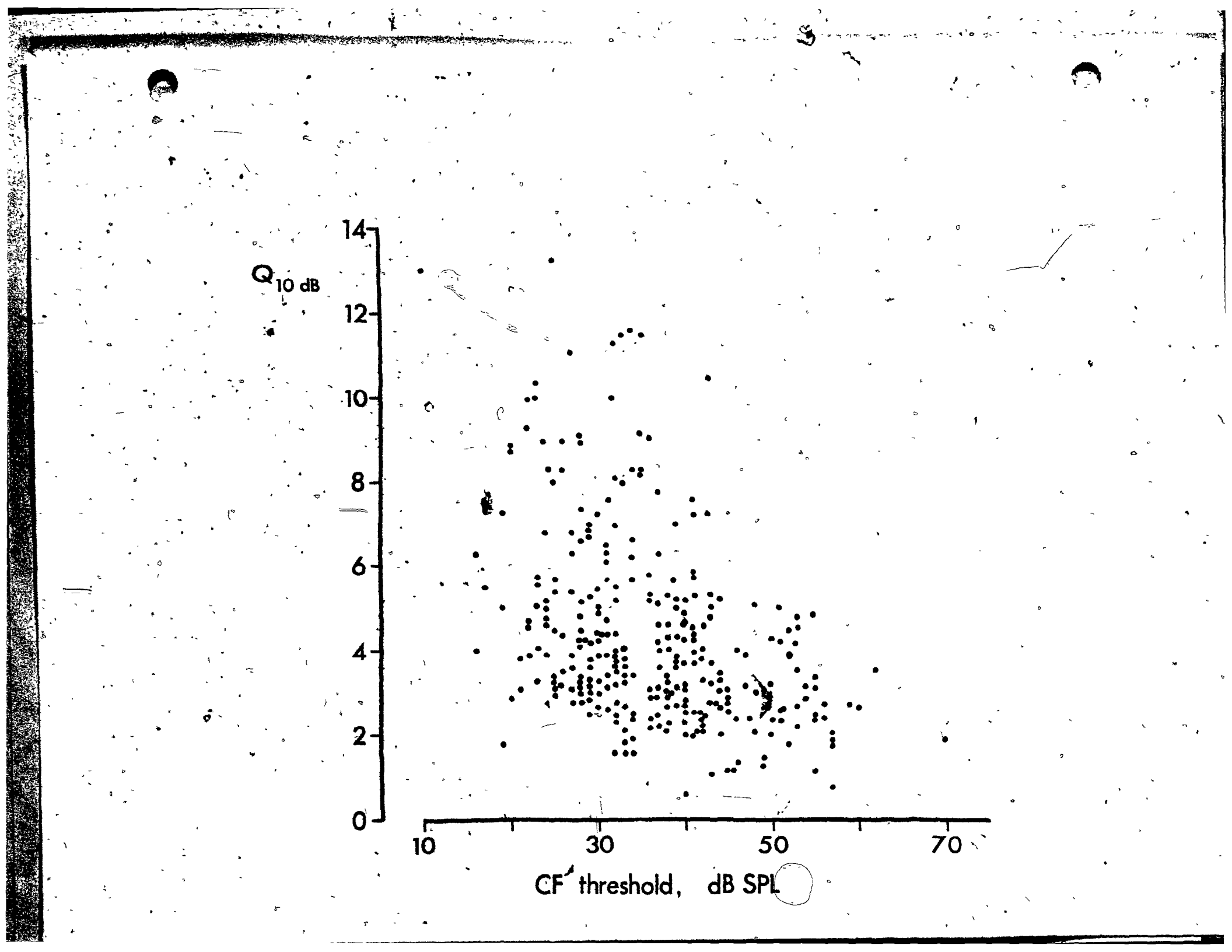
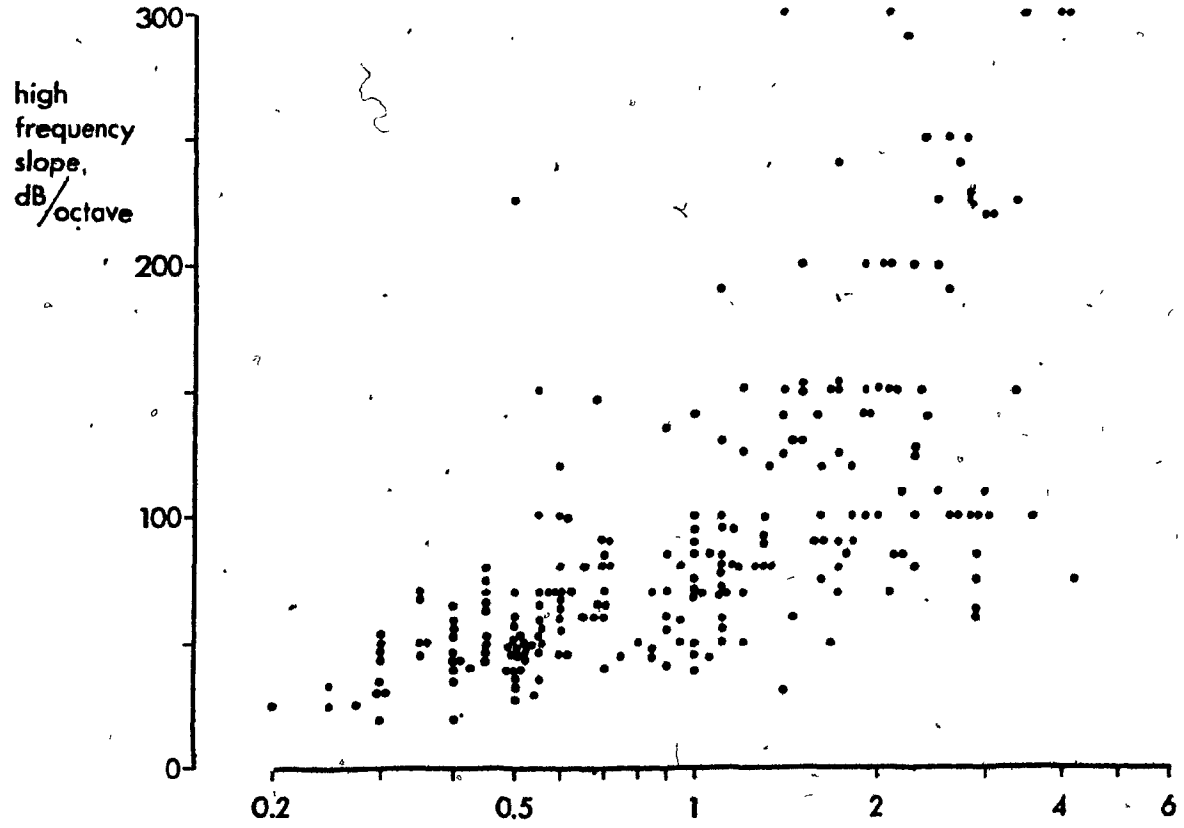

$Q_{10\ dB}$
14
12
10
8
6
4
2
0
10
30
50
70
CF threshold, dB SPL

Figure 5. Slopes of the A) high frequency and B) low frequency sides of the tuning curve v. CF. Slopes were measured between 3 and 23 dB above CF threshold.


high
frequency
slope,
dB/octave
300
200
100
0
0.2
0.5
1
2
4
6

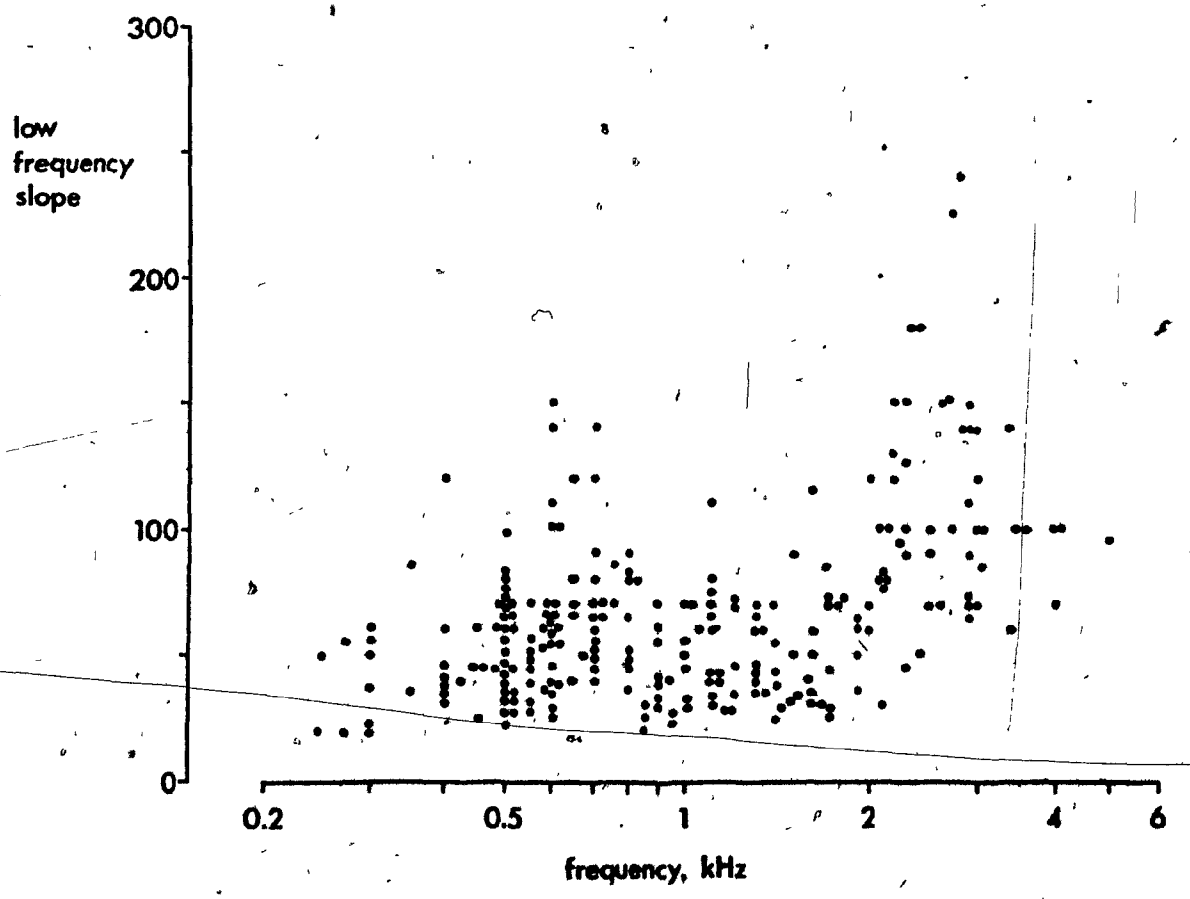
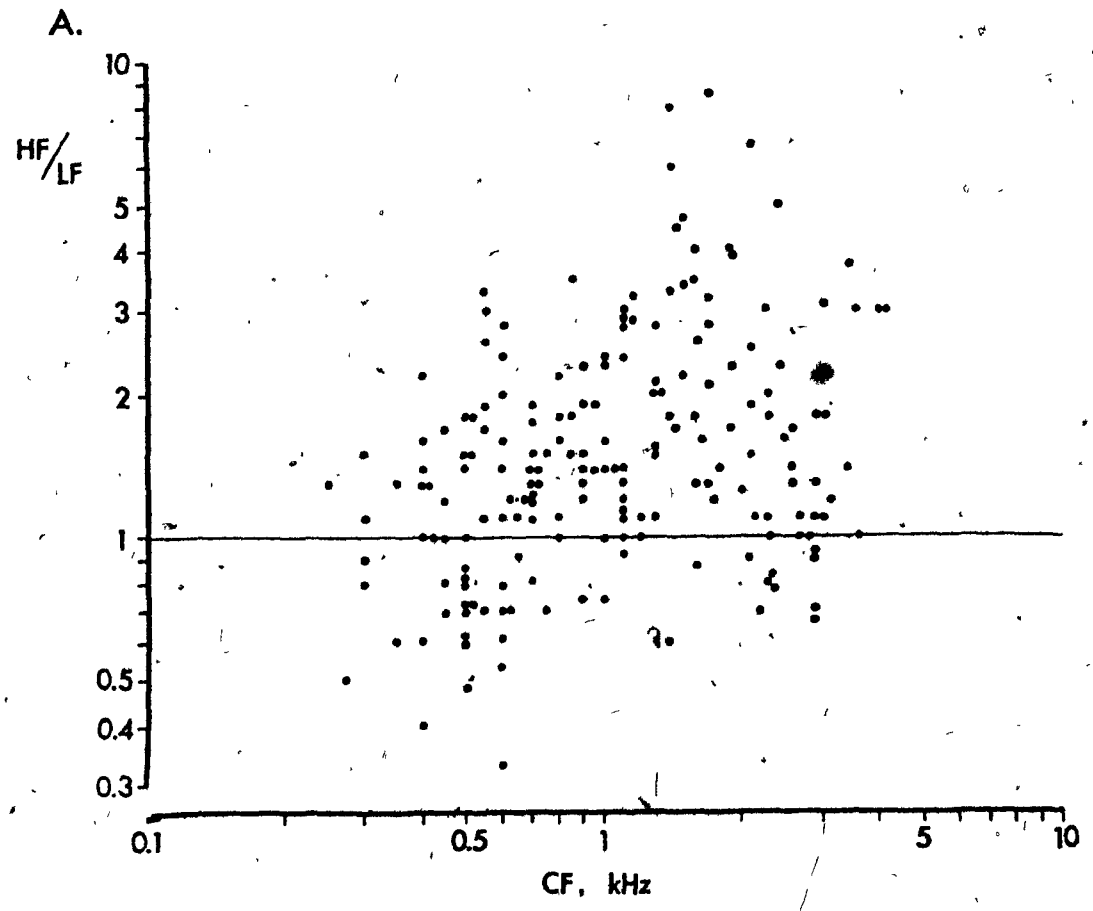
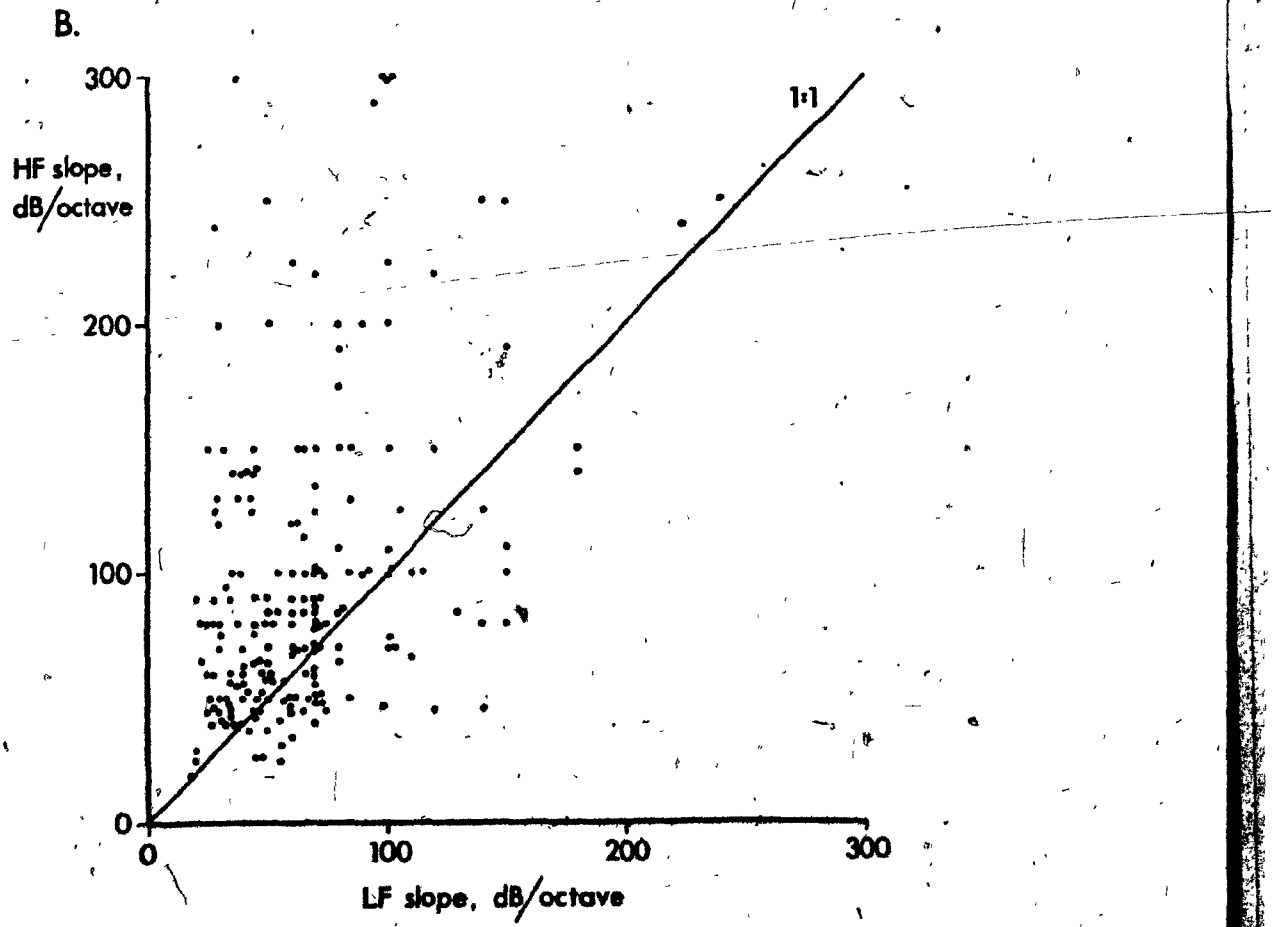

low
frequency
slope
300
200
100
0
0.2
0.5
1
2
4
6
frequency, kHz

Figure 6. A) The ratio of high frequency slope to low frequency slope for each tuning curve v. their CFs. Note that both axes are logarithmic.
B) High frequency slope v. low frequency slope.


A.
HF/LF
10
5
4
3
2
1
0.5
0.4
0.3
0.1
0.5
1
5
10
CF, kHz


B.
HF slope, dB/octave
300
200
100
0
1:1
0
100
200
300
LF slope, dB/octave

dB/octave above 2 kHz. For tuning curves above 2 kHz, the columella response acts to increase the high frequency slope and decrease the low frequency slope. The HF:LF ratio is thus somewhat inflated above 2 kHz. At frequencies below 0.4 kHz, the middle ear response contributes to the low frequency fall-off and detracts from the high frequency fall-off. This has the effect of making HF:LF ratios smaller than they would be if the middle ear response were completely flat. A more accurate representation of inner ear performance can be obtained by subtracting the middle ear response. Fig. 8 illustrates the effect this has on tuning curves and the overall sensitivity curve.

Though not studied systematically, some tonotopic organization of the nerve was evident. This was not unexpected, since the Tokay's cochlear nuclei have at least a rough tonotopic organization (Manley, 1972). Fibres along the posterior edge of the auditory nerve had CFs from 0.15 to 0.6 or 0.7 kHz. Vertical electrode passes through the middle of the auditory nerve contacted fibres with CFs from 0.2 to 2.5 kHz; there was some tendency for CF to increase with depth. Higher CFs, from 3 to 5 kHz, were only found deep in the anterior part of the auditory nerve - i.e. around the midline of the eighth nerve. Tonotopic organization of the nerve and cochlear nuclei implies a systematic distribution of frequencies along the basilar papilla (Konishi, 1970).

(ii) PSTHs

Peristimulus time histograms (PSTHs) were constructed off-line from the tape-recorded pure tone responses of 76 fibres from 17 animals. Each PSTH represents the summed activity of a fibre in response to 30 presentations of a tone burst, delivered at a constant intensity. The tone burst

Figure 7. Velocity response of the Tokay's columella (middle ear) v. stimulus frequency. The curve is an average from 3 Tokays.

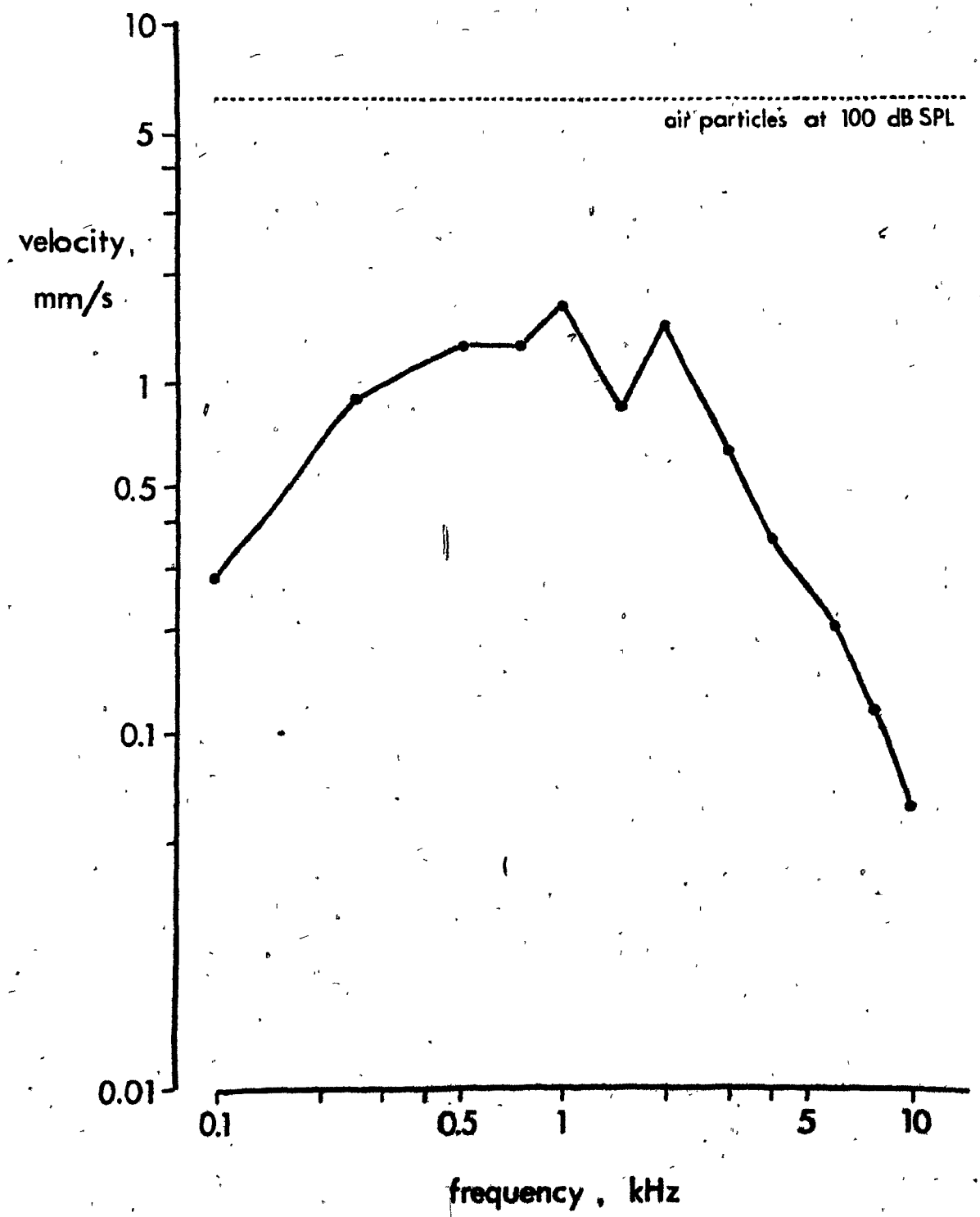
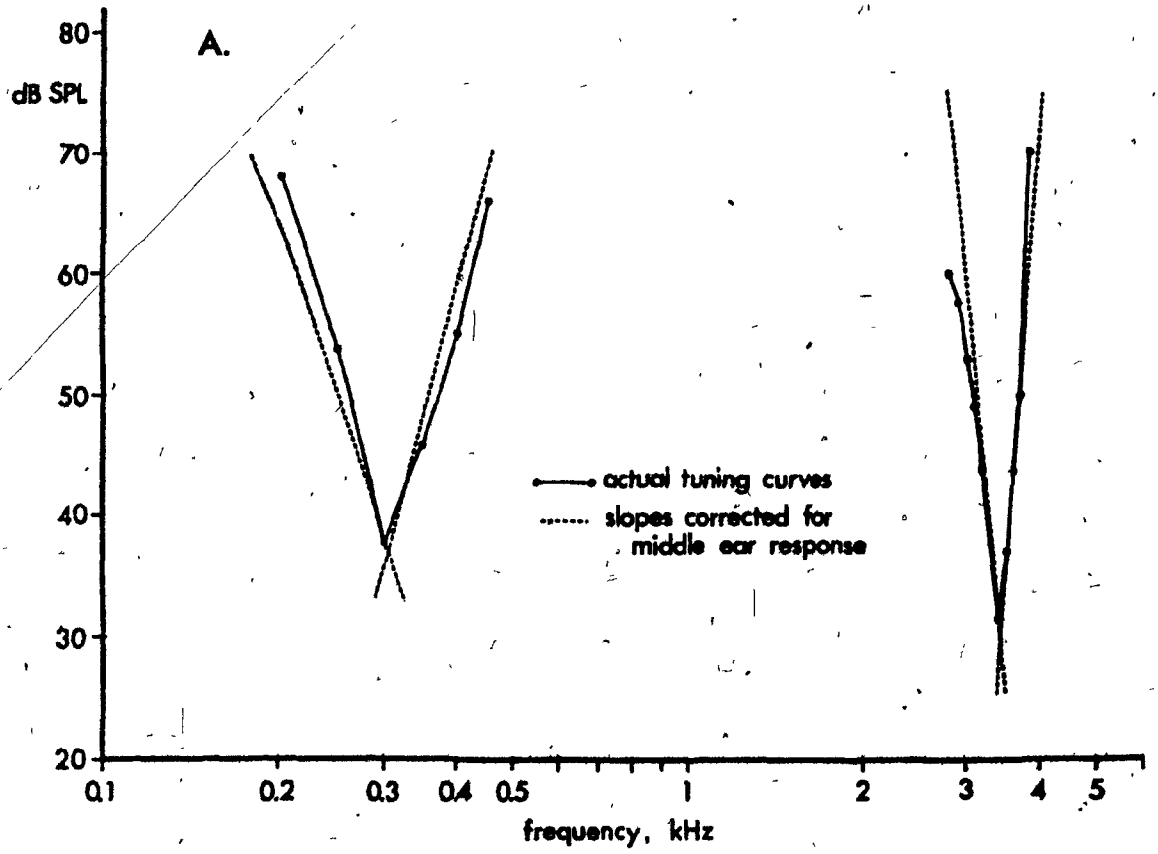
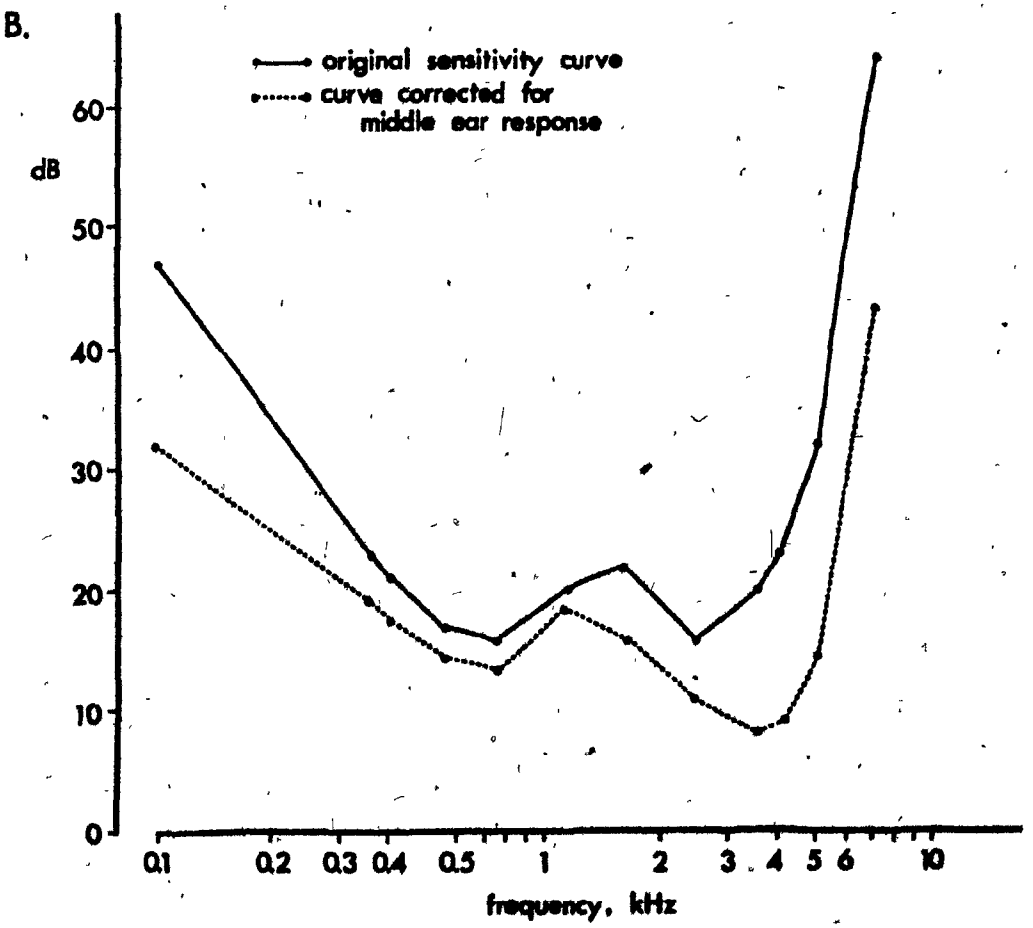

10
5
1
0.5
0.1
0.01
velocity, mm/s
air particles at 100 dB SPL
0.1
0.5
1
5
10
frequency , kHz

Figure 8. Correction of A) 2 sample tuning curves B) sensitivity curve derived from auditory nerve fibre data (as in Figure 2) for fall-off in the middle-ear response at low and high frequencies. The corrected curve in B) has been set at an arbitrary dB level.


A.
80
dB SPL
70
60
50
40
30
20
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
frequency, kHz
actual tuning curves
slopes corrected for middle ear response


B.
original sensitivity curve
curve corrected for middle ear response
dB
60
50
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
6
10
frequency, kHz

has a 5 ms rise-fall time unless otherwise stated. The beginning of fall-time was 50 ms after the beginning of rise-time, so that the tone burst was at full amplitude for 45 ms.

Three major categories of PSTH could be distinguished: 1) filled; 2) semi-peaky; 3) peaky with a) a single early peak or b) more than one peak (Fig. 9). PSTHs from 73 of the 76 fibres could be classified in this way. The filled PSTH indicates a sustained response to the tone-burst, with only gradual adaptation or none at all. In the third category, the impulses that comprise the initial peak are highly synchronized relative to stimulus onset. In the semi-peaky PSTH the phasic onset response is less synchronised and less pronounced than in the peaky type, while the subsequent discharge is at a proportionally lower level. PSTH type depended to some extent on CF and stimulus intensity. A unit could produce a semi-peaky PSTH at low intensity, a single-peak PSTH at moderate intensities, and one with two or three peaks at higher intensities (Fig. 10a). For uniformity's sake, a fibre was classified according to the PSTH it produced in response to a tone at or near CF and approximately 20 dB above threshold. The frequency distribution of each PSTH type is illustrated in Fig. 11. Filled PSTHs were only obtained from fibres with CFs of 0.7 kHz or less. Peaky PSTHs of both types were only produced by units with CFs of 0.7 kHz or more. Semi-peaky PSTHs occurred over the range of 0.4 to 2 kHz, which overlaps the distributions of both filled and peaky units.

To determine whether this classification scheme was to any extent an artifact of the arbitrarily chosen rise-time of 5 ms, the PSTHs of 7 fibres were also studied at rise-times of 2.5 and 1.0 ms. Stimulus frequency and intensity were kept constant. Three filled-type units

Figure 9. Examples of the major categories of peristimulus time histogram (PSTH) : A) filled B) semi-peaky C) peaky with single peak D) peaky with multiple peaks. The tone burst is depicted as beginning 16 ms later than the origin of the PSTH. This is the time delay (for a 0.5 ms rise-time) between the tape-recorded stimulus trigger and the calculated time of arrival of the tone burst at the lizard's ear.

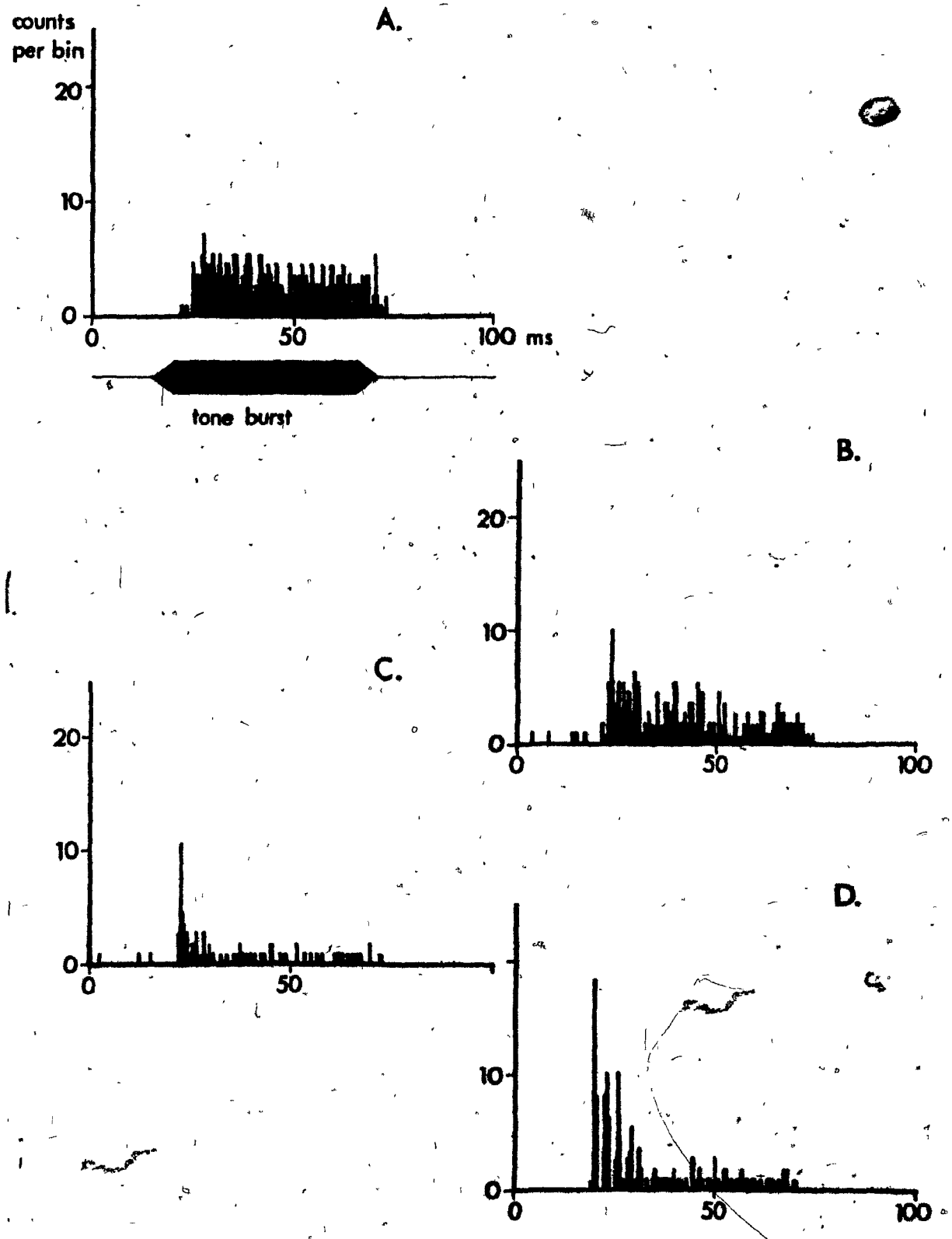

A.
counts
per bin
20
10
0
0
50
100 ms
tone burst
B.
20
10
0
0
50
100
C.
20
10
0
0
50
D.
10
0
0
50
100

Figure 10. Changes in PSTH with sound intensity

A) PSTHs from a unit with CF = 0.95 kHz, in response to 0.95 kHz tones at intensities of 8 dB, 20 dB and 40 dB above threshold intensity. The corresponding intensities in dB SPL were 50, 62, and 82 dB SPL.

B) PSTHs from a unit with CF = 2.9 kHz in response to 2.9 kHz tones at intensities 6 dB, 21 dB and 32 dB above threshold intensity. The corresponding dB SPL values were 49, 64, and 75 dBSPL.

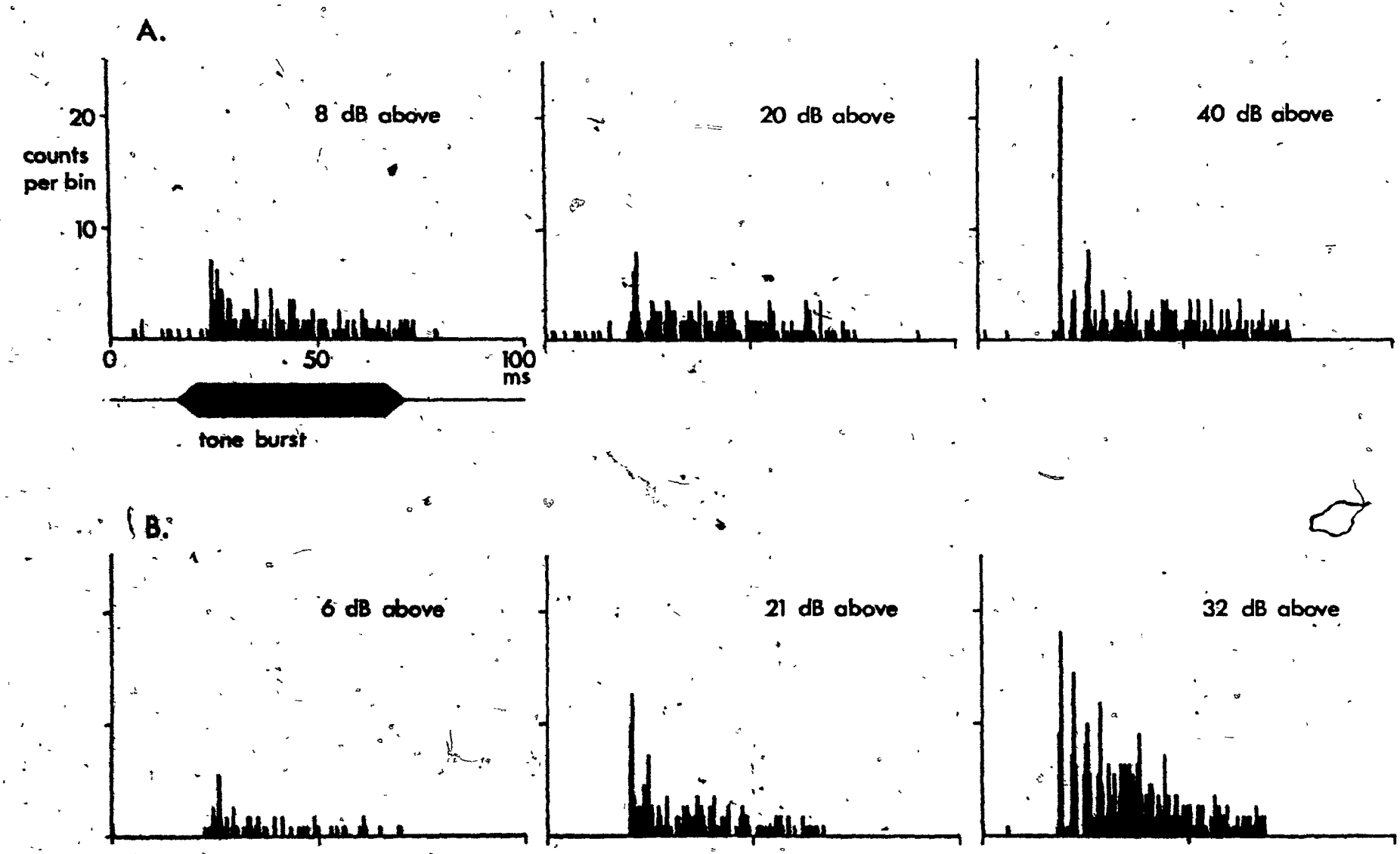
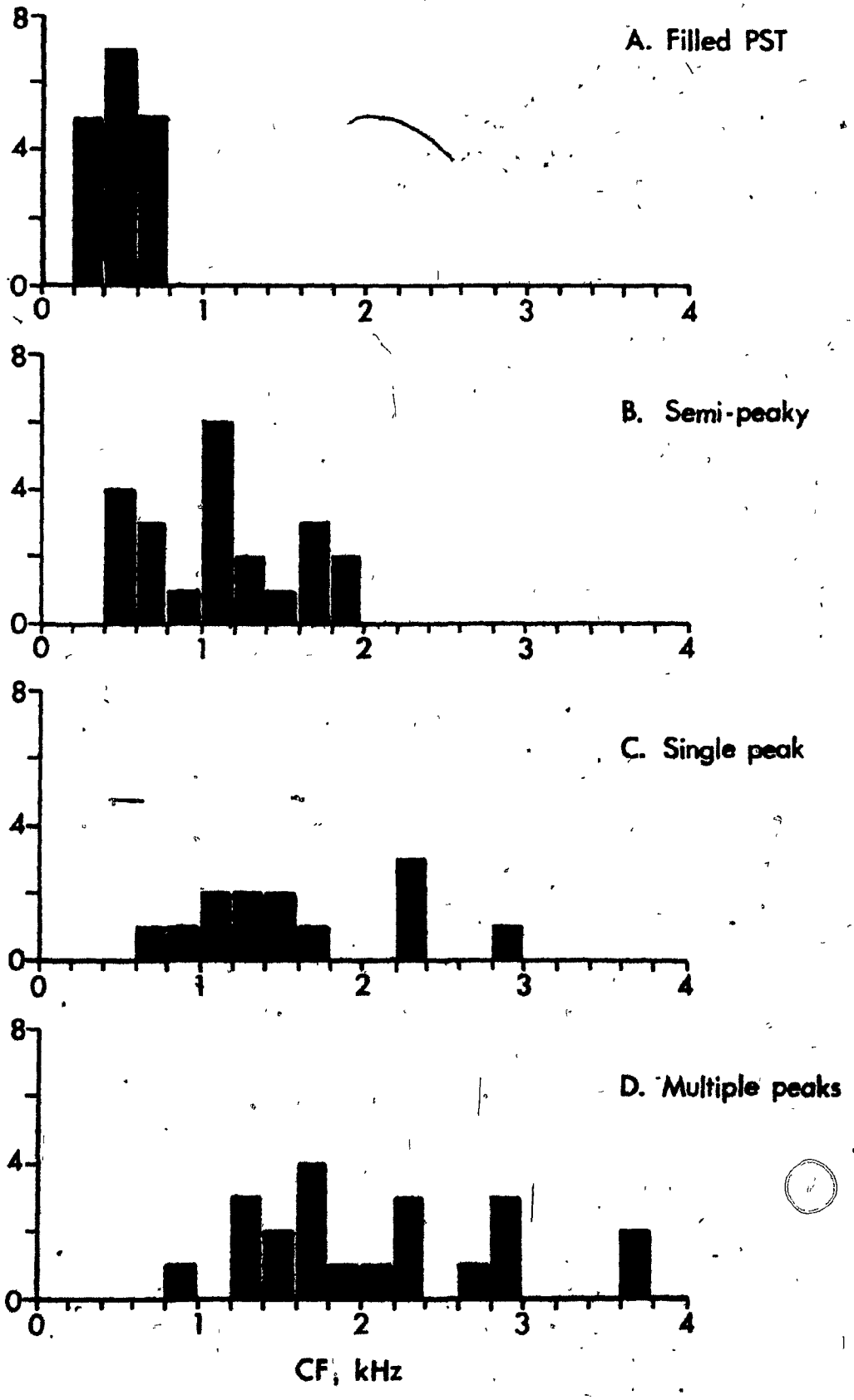

A.
20
counts
per bin
10
8 dB above
20 dB above
40 dB above
0
50
100
ms
tone burst
B.
6 dB above
21 dB above
32 dB above

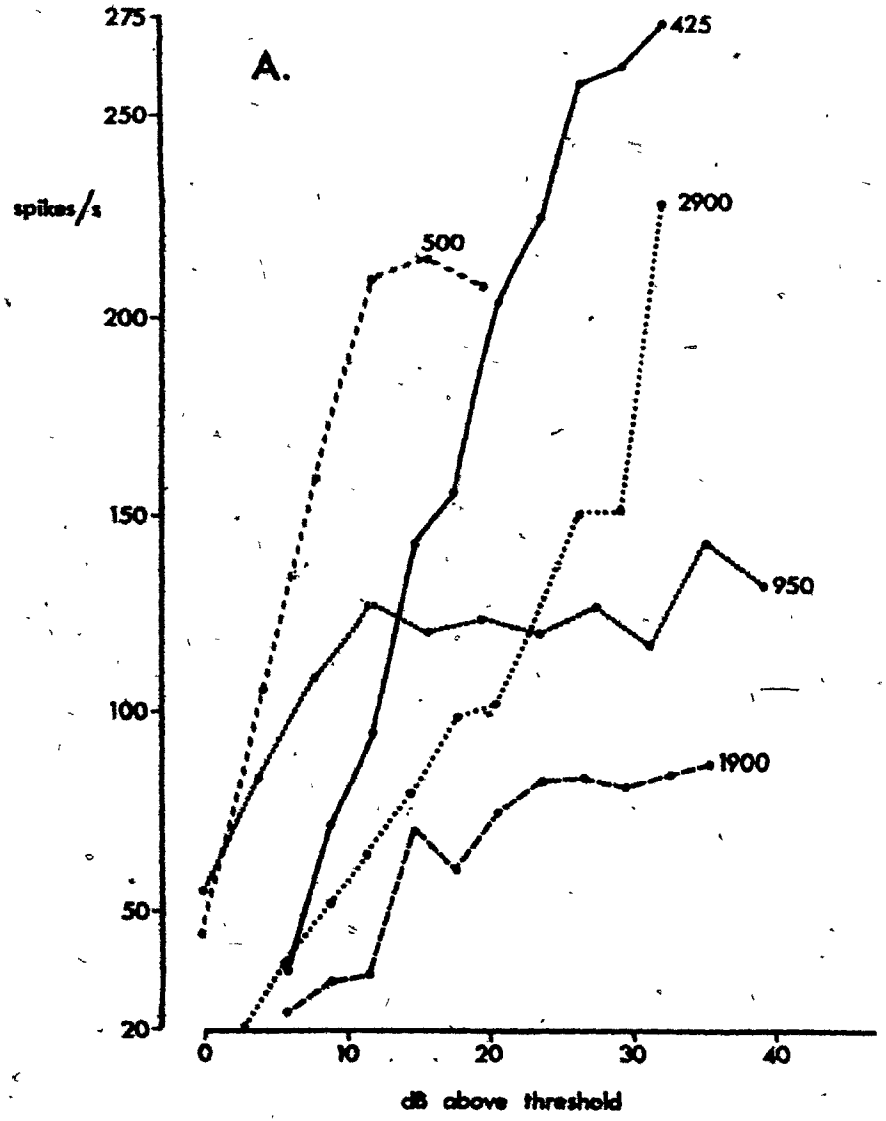
Figure 11. Distributions of PSTH type according to CF.

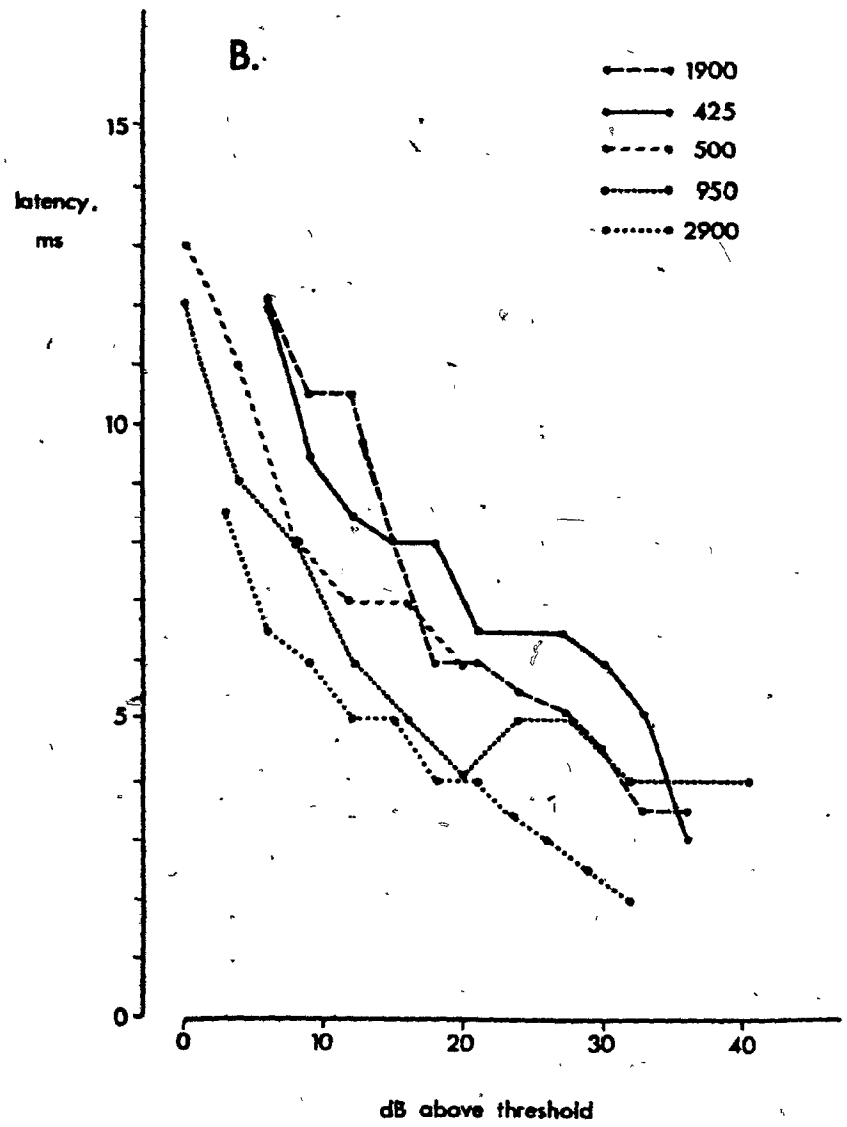

No. of fibres
A. Filled PST
B. Semi-peaky
C. Single peak
D. Multiple peaks
CF, kHz

remained true to form at the shorter rise-times. Peaky PSTHs tended to become peakier: individual peaks became more pronounced, and two single peak units gained a second peak. Although no semi-peaky units were tested, at least the filled and peaky categories remained stable over different rise-times. Several observations lead to the conclusion that single and multiple peak PSTHs have similar origins. (1) In one unit that produced two peaks at 5 ms rise-time, the second peak was greatly reduced at 2.5 ms and more pronounced at 1 ms. (2) The frequency distributions of the two PSTH types were nearly identical (Fig. 11c,d). (3) Although changes with intensity were not always examined, the overall impression was that peaky units produced single peaks at low intensities and multiple peaks at moderate to high intensities. Incipient second and third peaks were often visible at lower intensities. The rate at which such peaks developed with intensity determined whether a unit was a single or multiple peak unit at 20 dB above stimulus threshold.

Rate-intensity functions - graphs of average discharge rate v. stimulus intensity - were obtained for nine fibres with CFs ranging from 0.425 to 2.9 kHz. In each case a series of ten or more PSTHs was taken, using the same tone (at or near CF) at successively higher intensities. Increments of 3 or 4 dB were used. Examples of the different types of function obtained are shown in Fig. 12a. The discharge rates of the two low frequency filled-type units increased linearly with slopes of 12 and 14 spikes/s/dB, reaching maxima of over 200 spikes/s. Spikes were added uniformly over the PSTH, so that the filled form was retained. Peaky units increased their rates more slowly - with an average slope of approximately 6 spikes/s/dB - and often plateaued at much lower discharge rates. In these units, the initial peak became more prominent with

Figure 12. Intensity functions. In A), the discharge rates of 5 different units in response to pure tone stimuli at or near CF are plotted against stimulus intensity above threshold. The stimulus frequency used is indicated next to each curve. In B) the change in response latency with pure tone intensity is shown for the same 5 units. A) and B) are derived from the same PSTH series. Latency was measured from the beginning of stimulus rise-time.


A.
spikes/s
275
250
200
150
100
50
20
425
2900
500
950
1900
0
10
20
30
40
dB above threshold

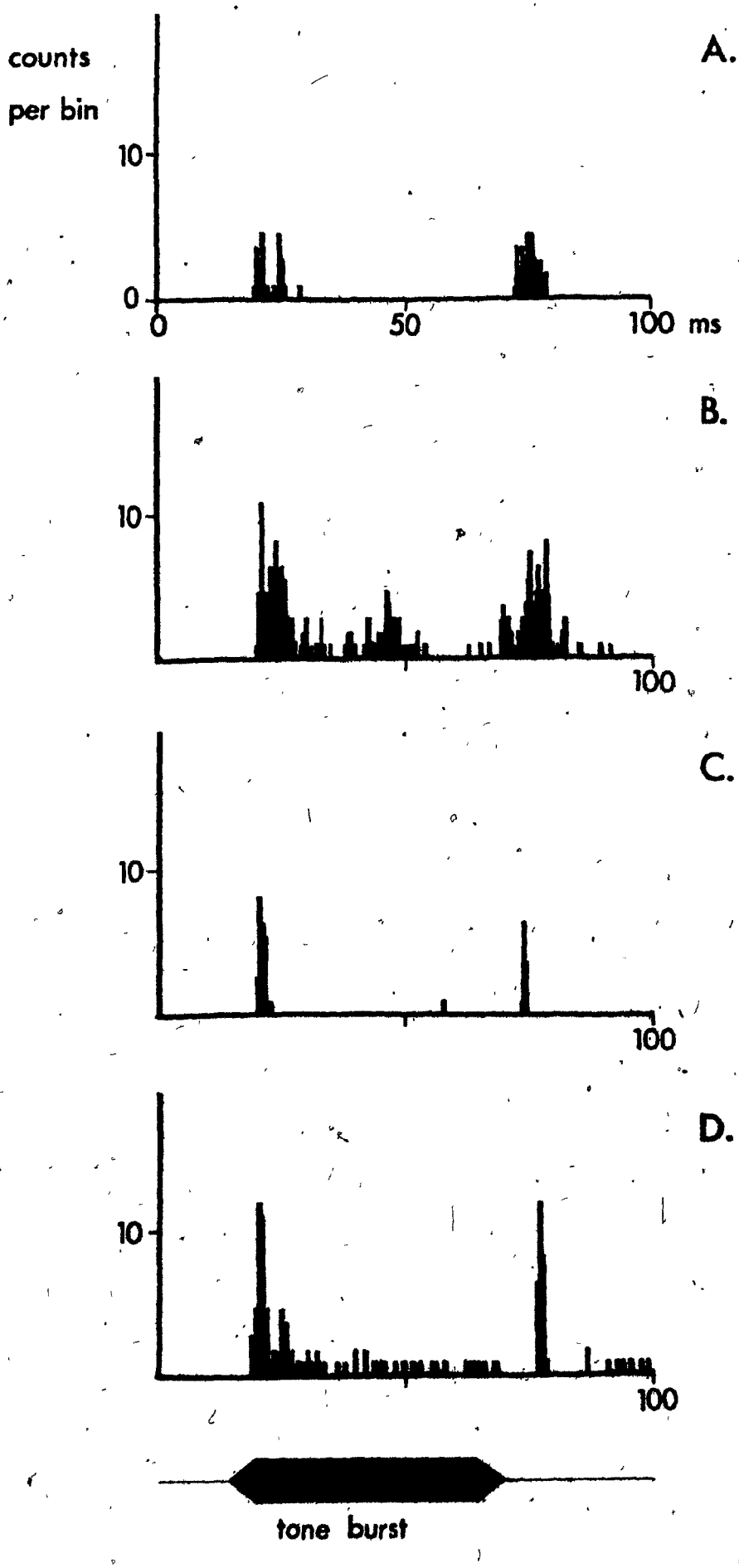

B.
latency,
ms
15
10
5
0
1900
425
500
950
2900
0
10
20
30
40
dB above threshold

increased intensity, while discharge rate over the rest of the histogram did not increase. In a couple of cases, however, spike rate began to rise again at intensities of 30 - 40 dB above threshold, after having plateaued. This second rise reflected the growth of second and third peaks within the histogram. The unit with CF of 2.9 kHz (Figs. 10b, 12), although peaky, never plateaued because the later peaks grew with the same time course as the initial peak.

From the nine PSTH series used to obtain rate-intensity functions, changes in response latency with intensity could also be determined. Latency always decreased with increased stimulus intensity (Fig. 12b). For eight of the nine fibres the slope of the change was greatest at lower intensities. The point at which the slope decreased occurred at different intensities above threshold for different units. At intensities above these transition points, the slope was between -0.14 ms per dB increase for seven of the nine units. For intensities below the transition points, slopes were 3 to 4 times as great.

A fourth, rare, category of PSTH was encountered: the on-off type (Fig. 13). Of the 410 fibres in this study, only about ten were observed to give on-off responses. It is probable that a number of examples were missed, especially in those cases where only tuning curve data were taken. Of the 77 fibres for which PSTH analysis was done, five gave on-off responses. Two of these were on-off at all frequencies on their tuning curves (Fig. 13a) while the other three were only on-off at certain frequencies removed from CF. In one case, a 0.3 kHz unit that produced a filled PSTH to CF stimulation was on-off at 0.25 kHz. Fig. 13c depicts an on-off response obtained at 0.5 kHz below a moderately high CF (2.1 kHz). The PSTH in Fig. 13d was obtained at 0.4 kHz above a low CF

Figure 13. On-off PSTHs. A) Response of a unit with CF = 0.15 kHz to stimulation with 0.15 kHz tones at 62 dB SPL. B) As in A) but with stimulus intensity raised to 72 dB SPL. C) A unit with CF = 2.1 kHz, CF threshold = 36 dB SPL, responding to 1.6 kHz tones at 70 dB SPL (1 dB above threshold at that frequency). The PSTH of this unit was peaky in type at higher frequencies. D) The response of a unit with CF = 0.3 kHz, CF threshold = 33 dB SPL, to 0.7 kHz tone bursts at 89 dB SPL (5 dB above threshold at that frequency).

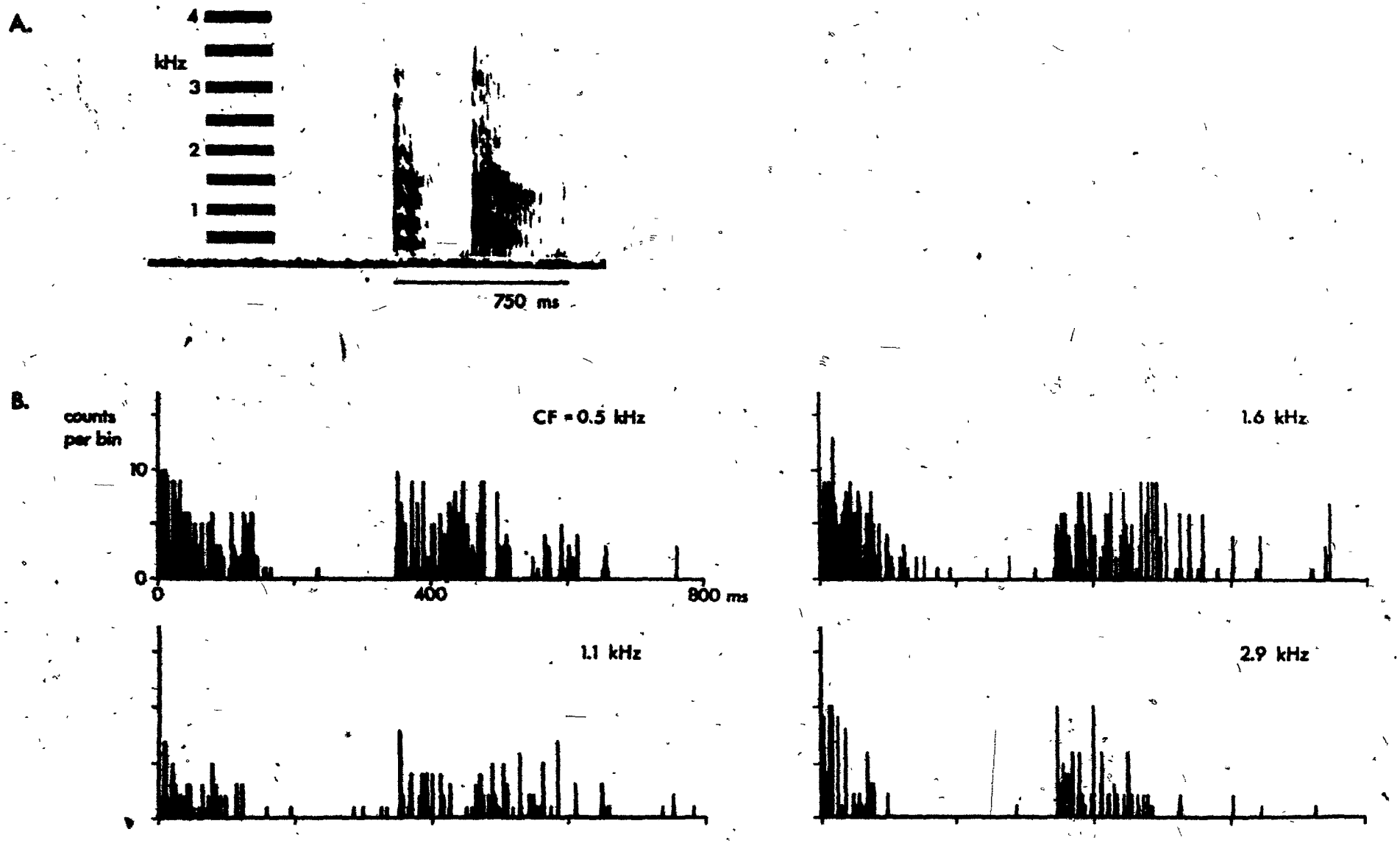

counts
per bin
A.
10
0
0
50
100 ms
B.
10
100
C.
10
100
D.
10
100
tone burst

(0.3 kHz). In all five cases, threshold was above 60 dB SPL at the frequencies that evoked the on-off response. Thus the units that were on-off at CF were relatively insensitive.

PSTH analysis was also done on the responses of 20 fibres to a tape-recorded Tokay call. The entire call was 750 ms long, and consisted of a 150 ms early component followed by a 190 ms pause and a 400 ms late component. A wide-band sonagram of the call showed it to be broad-band with a pronounced time structure (Fig. 14a). Its energy was concentrated below 1.6 - 1.7 kHz. Higher frequencies (up to 3.5 kHz) occurred, but were restricted to the early part of each of the two main components. The 20 fibres had CFs ranging from 0.3 to 3.6 kHz. As expected with a broad-band call, responses were obtained from all 20. The PSTHs consisted of successive peaks (Fig. 14b), reflecting the pulsed nature of the call. PSTHs from different units with similar CF had superimposable peaks, indicating that each peak was a response to a specific pulse within the call. A unit responded maximally, of course, to those parts of the call with most energy around its CF. For instance, 0.5 kHz units (Fig. 14b) responded throughout both components of the call, since energy was always present at 0.5 kHz. The response of the 2.9 kHz unit was much shorter, and corresponded to the occurrence of higher frequencies early in the call.

It is interesting to note that a temporally structured call such as the one used provides an optimum stimulus for units in the peaky PSTH category. Within the call, each pulse containing appropriate frequencies evokes an onset response. The discontinuities prevent the sort of adaptation obtained from peaky-type fibres when pure tone stimuli are used. Such units respond to the call as if it were a series of onsets, and so produce a series of peaks.

Figure 14. A) Wide-band sonagram of 2-part Tokay call used as a sound stimulus. B) PSTHs of vocalization responses of 4 different units to the call in A). The CFs of the units are indicated. The maximum intensity within each component was similar. For the PSTHs shown, the maximum intensity of the call was 35-40 dB above the units' CF thresholds. This, of course, says nothing about the intensities of the frequency components relevant to each unit.


A.
4
kHz
3
2
1
750 ms
B.
counts
per bin
CF = 0.5 kHz
10
0
0
400
800 ms
1.6 kHz
1.1 kHz
2.9 kHz

(iii) Spontaneous Activity

The spontaneous activity of 64 units from 19 Tokays was recorded on tape and analyzed in the form of time interval histograms (TIHs). As noted by Kiang (1965) the term "spontaneous" can only refer to activity in the absence of experimentally controlled sound stimulation. Within the 64 fibres, spontaneous rates varied from 1.6 to 40 spikes/s. Units were occasionally encountered that were never seen to discharge spontaneously. Apart from the omission of such units, the data probably represent a fairly random sample of spontaneous activity. The distribution of rates for the 64 fibres (Fig. 15a) indicates a much lower average rate and smaller range than are found in cochlear nerve fibres in mammals (Kiang, 1965; Manley and Robertson, 1976) and birds (Manley and Leppelsack, 1977). The range of spontaneous activity recorded in auditory nerve fibres in the monitor lizard, Varanus bengalensis (Manley, 1977) was not very different from the Tokay's. In caiman (Klinke and Pause, 1977) and alligator lizard (Weiss et al., 1976), the upper limits of the range were higher (70 and 80 spikes/s, respectively).

A TIH indicates interspike interval along the abscissa and number of spikes per given interval along the ordinate. The zero-order histogram of a TIH divides the period during which activity was recorded into small units of time, and indicates the discharge rate per unit of time. Spontaneous data were usually collected for 1-6 min, and were only considered acceptable when the zero-order histogram indicated a relatively constant discharge rate throughout the recording period.

Six representative TIHs are illustrated in Fig. 16. (A) and (B) were from units with spontaneous rates of 29 and 11 spikes/s, and CFs of 1 and

Figure 15. The distributions of spontaneous A) discharge rates, B) modes, and C) dead times, within the present sample.

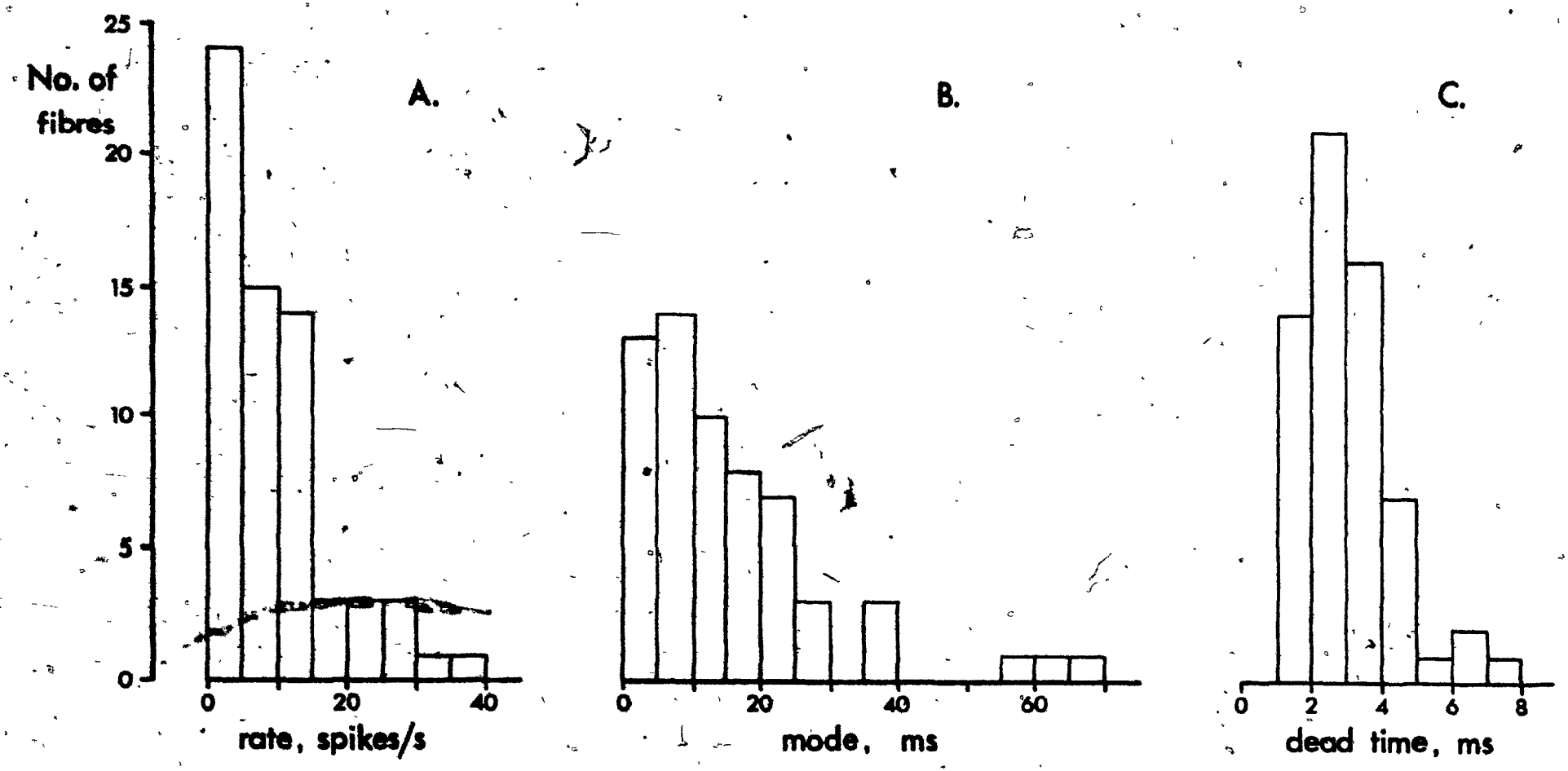
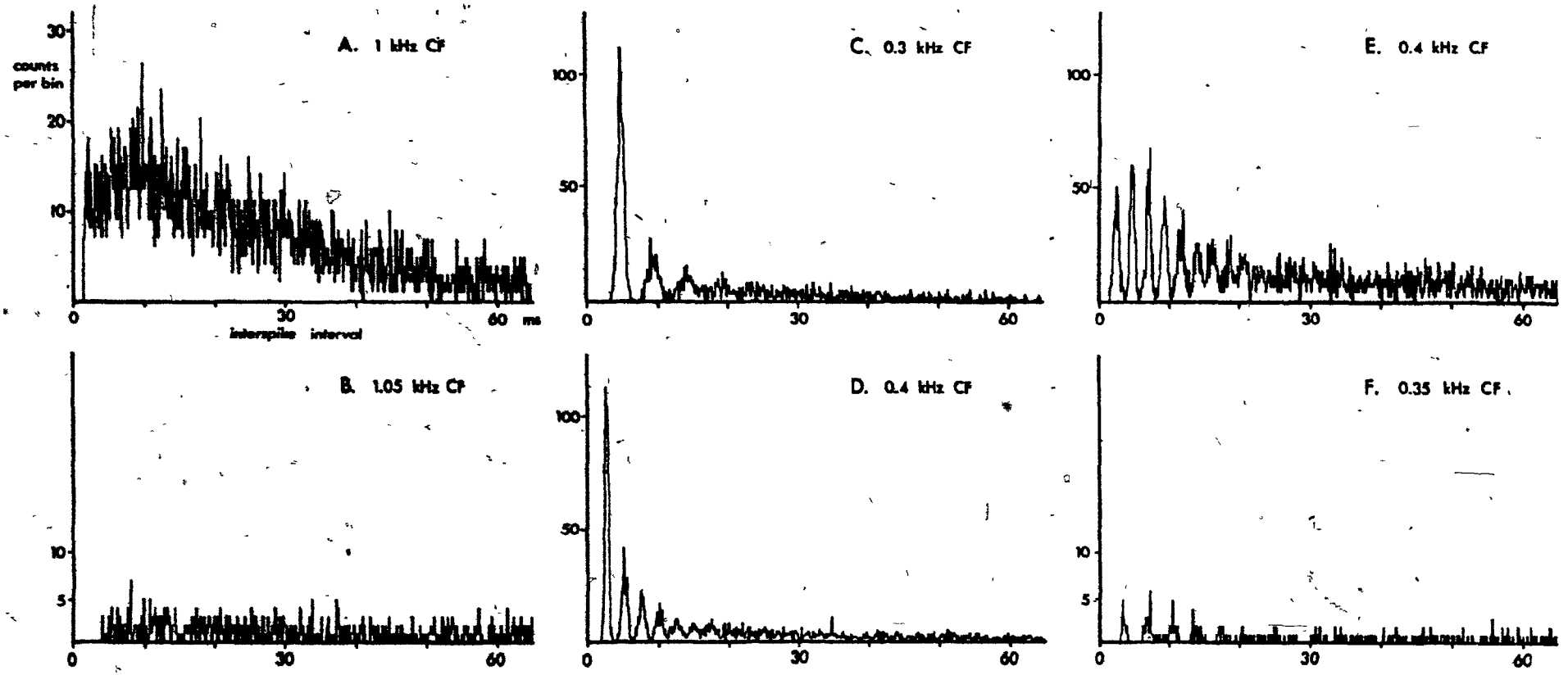

No. of fibres
25
20
15
10
5
0
A.
0
20
40
rate, spikes/s
B.
0
20
40
60
mode, ms
C.
0
2
4
6
8
dead time, ms

Figure 16. Representative time interval histograms (TIHs) of spontaneous activity, from 6 fibres. Abscissa: interspike interval, divided into 0.1 ms bins. Ordinate: number of spikes that occur at a given interval after the previous one. Note the different ordinate scales.


A. 1 kHz CF
counts per bin
30
20
10
0
30
60 ms
interspike interval
B. 1.05 kHz CF
10
5
0
30
60
C. 0.3 kHz CF
100
50
0
30
60
D. 0.4 kHz CF
100
50
0
30
60
E. 0.4 kHz CF
100
50
0
30
60
F. 0.35 kHz CF
10
5
0
30
60

1.05 kHz, respectively. TIHs from units with CFs above 0.5 kHz were generally comparable to TIHs from mammals (Kiang, 1965; Manley and Robertson, 1976) and birds (Manley and Leppelsack, 1977) despite the lower spontaneous rates. Units with CFs below 0.5 kHz, on the other hand, produced TIHs with unusual early peaks (Fig. 16c,d,e,f). It was disconcerting to discover that these peaks occurred at regular intervals that corresponded at least roughly, and sometimes closely, to the reciprocal of the unit's CF. It was at first assumed that the units were being inadvertently stimulated. Since the trigger normally used for tones and white noise pulses was continuously recorded on tape even during spontaneous data collection, it was possible to check for stimulation through the sound system by doing PSTHs on the spontaneous activity. PSTHs for all spontaneous data from low frequency units were flat, indicating that the units had not been responding to sounds coming from the speaker. In subsequent experiments, the amplifier input to the speaker was removed. Care was taken to keep noise outside the semi-anechoic sound-attenuating chamber to a minimum. In several instances, spontaneous activity was recorded both with and without background noises in the room outside the recording chamber. No differences were discernible in the zero-order histogram or when the TIHs were compared. In summary, if these units were responding to low-level sounds, then they were doing so with a sensitivity far greater than would be expected from their tuning curves. One 0.25 kHz fibre whose TIH had several prominent peaks separated by 4 - 4.5 ms intervals had a CF threshold of 65 dB SPL. A number of other fibres with CF-related oscillations in their TIHs had CF thresholds of 40-50 dB SPL. The sound level (dBA) within the sound-attenuating chamber has been measured at 18 dB SPL, with someone in the chamber. In addition, the ambient sound level as measured by a 1/2 inch Brüel and Kjaer condenser microphone

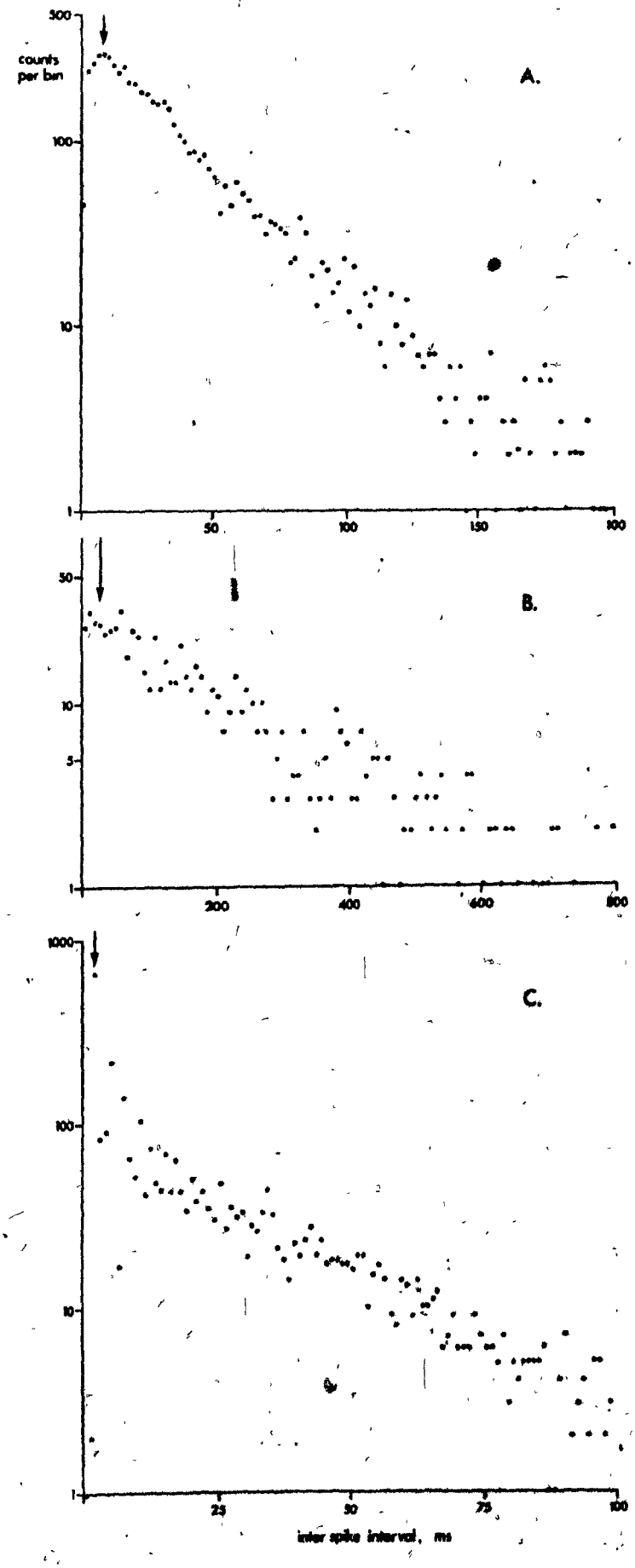
falls within the noise level of the instrument (30 dB SPL).

Peaks at intervals corresponding to the reciprocal of the CF were always obtained from units with a CF of 0.4 kHz or less, occasionally from 0.5 kHz units, and never from units with higher CFs. Inter-peak intervals were consistent within a TIH, and were in some cases almost exactly the reciprocal of the CF (Fig. 16d). The intervals could, however, be somewhat shorter or longer than the reciprocal of the CF. The discrepancy between inter-peak interval and CF reciprocal in Fig. 16c was the largest seen. The unit had a CF of 0.3 kHz and inter-peak interval of 5 ms. The initial peak was not always the most prominent (Fig. 16e). Peaks were apparent even in low-rate units with low counts in their TIHs (Fig. 16f).

The mode or preferred interval of a TIH is generally taken to be the highest point in a semilogarithmic plot of the number of intervals v. the interspike interval (Fig. 17). In mammals, the mode is followed by a simple exponential decay in number of interspike intervals (Kiang, 1965; Manley and Robertson, 1976). This exponential decay was also observed for Tokay units with CFs greater than 0.4 kHz. It was especially clear in the units with relatively high discharge rates (Fig. 17a). There was more jitter in the semilogarithmic plots for low-rate units (Fig. 17b) partly because of the lower numbers of spikes recorded from such units. The presence of peaks in the TIHs of low frequency units complicated the appearance of the corresponding semilogarithmic plots (Fig. 17c). The decay in these cases did not become exponential until some time after the mode, when the oscillations in the histogram became weak.

The exponential decay in occurrence of interspike intervals is thought to indicate an underlying process that is roughly Poissonian, except for intervals smaller than the mode. The reduced number of such intervals

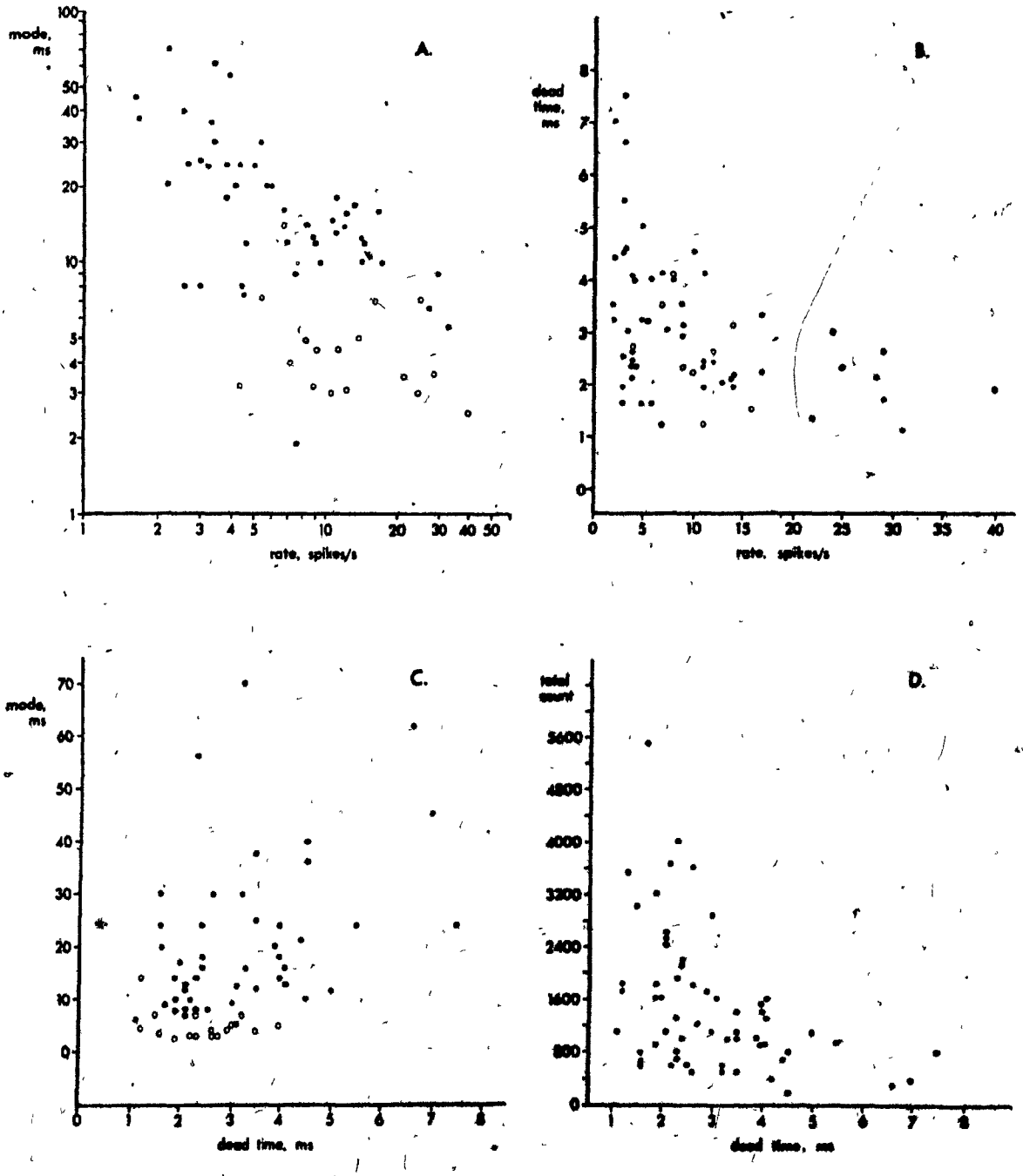
Figure 17. Semilogarithmic plots of TIH data from 3 fibres: A) A relatively high-rate unit (29 spikes/s) with a CF of 1 kHz. This is the same unit as in Fig. 16a. The abscissa is divided into 2 ms bins. The estimated mode (arrow) is 9 ms. B) A fairly low-rate (5 spikes/s) unit with a CF of 0.7 kHz. Bin width is 8 ms. Mode is more difficult to estimate in such units, partly because the total number of counts tends to be lower. The mode of this unit was estimated at 28 ms. C) A high-rate (40 spikes/s) unit with a CF of 0.4 kHz and oscillations in its TIH. This unit is the same as in Fig. 16d. Bin width is 1 ms. Mode is taken to be the point at which the highest peak occurs; here it is 2.5 ms.


counts per bin
A.
500
100
10
1
50
100
150
200
B.
50
10
5
1
200
400
600
800
C.
1000
100
10
1
25
50
75
100
inter spike interval, ms

is presumed to be related to unit refractoriness (Kiang, 1965). This reasoning cannot hold true for the long modes that have been recorded from auditory nerve fibres in the monitor lizard (Manley, 1977) and the Tokay (Fig. 15b). Unlike mammalian modes, which are all within several ms of one another (Manley and Robertson, 1976), modes in the Tokay varied from 2.5 to 70 ms. There was a tendency for lower rate units to have longer modes (Fig. 18a). The wide range of modal values and relationship between mode and rate were also found in auditory nerve fibres of the monitor lizard (Manley, 1977). It should be noted that in the Tokay, all modes of 5 ms or less were contributed by the low frequency units with peaky TIHs. In such units, mode was taken to be the interval corresponding to the highest peak (Fig. 16). Modes greater than 5 ms were obtained from several peaky TIHs in which the initial peak was not the largest.

The smallest interspike interval, or dead time, of a TIH indicates the interval below which the unit is refractory to spontaneous discharge, for whatever reason. The distribution of dead times in the present sample is shown in Fig. 15c. Although mode and dead time were not consistently related to each other (Fig. 18c), both showed a tendency toward larger values at low spontaneous rates (Fig. 18a,b). Dead times of 4.5 ms or more only occurred at rates of 5/s or less. Some bias may have been introduced by the tendency to record fewer spikes at the low discharge rates. The accuracy of a dead time estimation is probably more dependent than that of a mode on the total number of spikes recorded. More data are needed because the chance of a unit firing at its true dead time is almost by definition quite low. This might be expected to be especially true for units with long modes, for which the preferred inter-

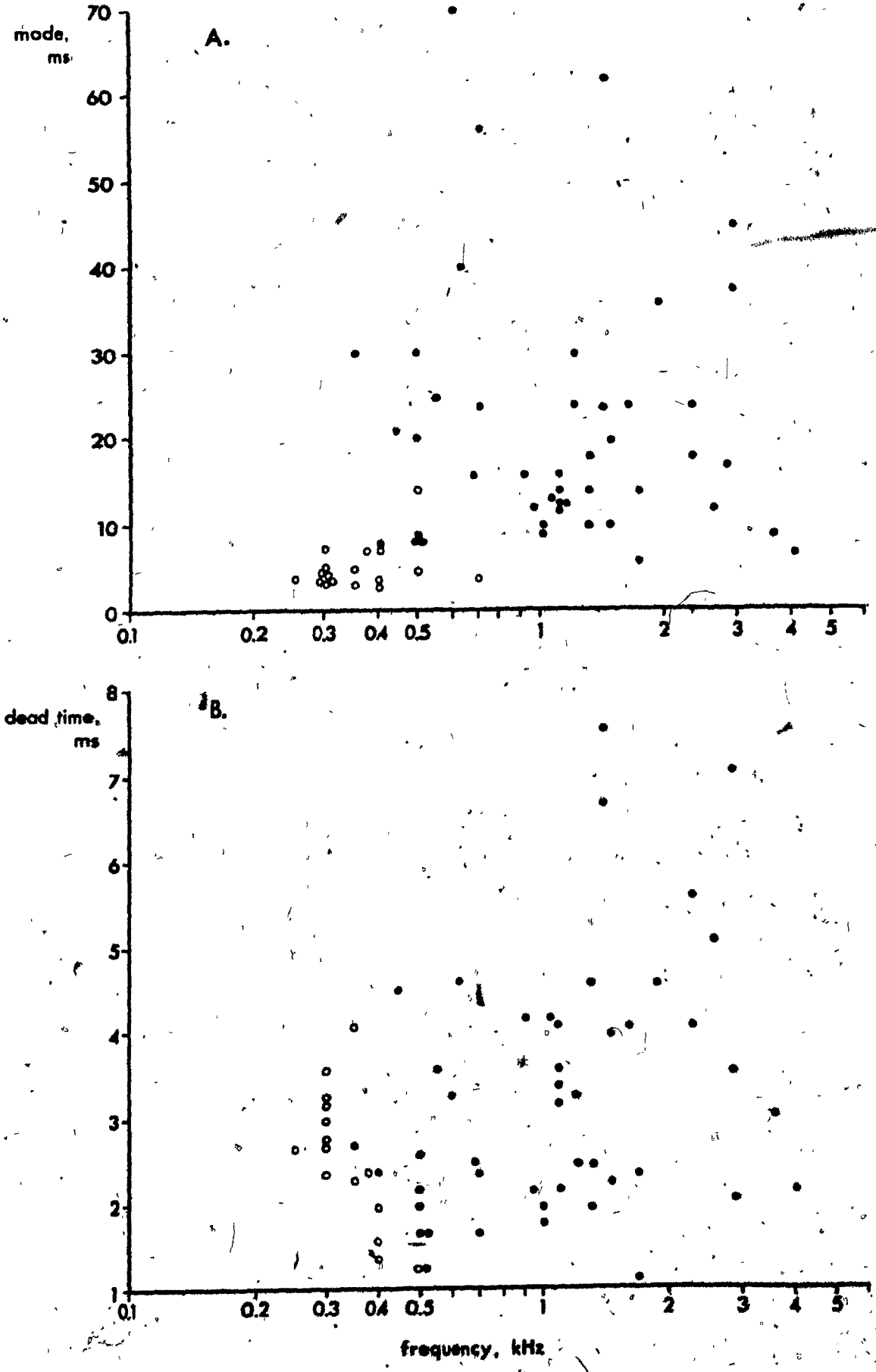
Figure 18. A) Mode (preferred interspike interval) v. spontaneous rate. In A), B) and C), the open circles represent the low frequency fibres with CF-related peaks in their TIHs. B) Dead time (smallest interspike interval) v. spontaneous rate. C) Mode v. dead time. D) The total number of spikes recorded for each unit v. dead time. Dead times longer than 4 ms correspond to counts of less than 1200.


A.
mode, ms
rate, spikes/s
B.
dead time, ms
rate, spikes/s
C.
mode, ms
dead time, ms
D.
total count
dead time, ms

spike interval is far removed from the intervals at which refractoriness could play a rôle. A graph of dead time v. total number of spikes recorded (Fig. 18d) does show that all units with dead times of 4.5 ms or more also had low total spike counts in their TIHs. It is impossible to say whether the long dead time values were a function of the low discharge rates or of the low numbers of spikes recorded, or both. The obvious solution is to record spontaneous activity from low-rate units over longer periods of time.

No relationship between dead time and CF could be discerned (Fig. 19b). In a graph of mode v. CF (Fig. 19a) the modes from low-frequency units with oscillations in their TIHs form a cluster of low values. In contrast, the dead times of these units covered a wide range.

Figure 19. A) Mode v. CF. B) Dead time v. CF. In A) and B), open circles represent the low frequency units with CF-related peaks in their TIHs.


A.
mode, ms
70
60
50
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
B.
dead time, ms
8
7
6
5
4
3
2
1
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
frequency, kHz

Discussion

(i) Tuning curves

Comparison with data from other species indicates that auditory nerve fibres in the Tokay are quite sensitive and relatively sharply tuned. Q_{10dB} values are on average higher than have been reported for auditory nerve fibres in the alligator lizard (Weiss et al., 1976) and monitor lizard (Manley, 1977). The frequency distribution of Q_{10dB} values (Fig. 3) is not unlike that found in starling auditory nerve fibres (Manley and Leppelsack, 1977). Graphs of Q_{10dB}, low and high frequency slopes v. CF have a very different appearance in the monitor and alligator lizards in that two distinct fibre populations are apparent. Both species have a high frequency fibre population that is more broadly tuned than fibres at lower frequencies. In the Tokay, changes with CF of Q_{10dB} and tuning curve slopes tend to be gradual, so that separate fibre populations are not evident. There was a discontinuity in the relationship between low frequency slope and CF at 0.7 kHz. Higher slope values occurred at and below this frequency than over the range 0.75 to 2 kHz. This is not completely attributable to changes in middle ear transmission efficiency. Although below 0.5 kHz fall-off in the columella velocity response augments low frequency slopes to some extent, the response is relatively flat from 0.5 to 2 kHz (Fig. 7). It appears that there is an inner ear contribution to the increased slope values below 0.75 kHz. (It is interesting to note that this is the range over which filled PSTHs were observed). The middle and inner ear contributions together have the effect of making tuning curves more symmetrical at low CFs (Fig. 6a). At higher CFs tuning curves tend to have higher high frequency slopes. A graph of high v. low frequency slopes (Fig. 6b)

gives an overall impression of higher high frequency slopes. This is reportedly the case in caiman (Klinke and Pause, 1977) but contrasts with the apparent symmetry of tuning curves in monitor lizard (Manley, 1977) and starling (Manley and Leppelsack, 1977).

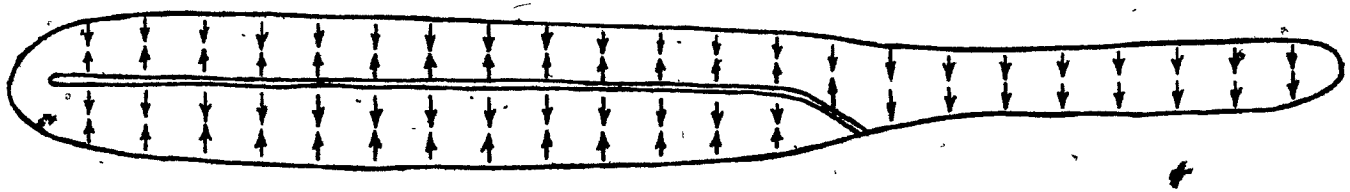
Comparison of the present results with guinea pig cochlear nerve fibre data over the same frequency range (0.1 - 6 kHz) reveals a relatively poor performance by a mammalian ear at these low frequencies (Evans, 1972; Harrison, personal communication). Tuning curve slopes and Q_{10dB} values are on average significantly lower than in the Tokay. The marked asymmetry of mammalian tuning curves at higher CFs, however, is retained down to 0.1 kHz.

(ii) PSTHs

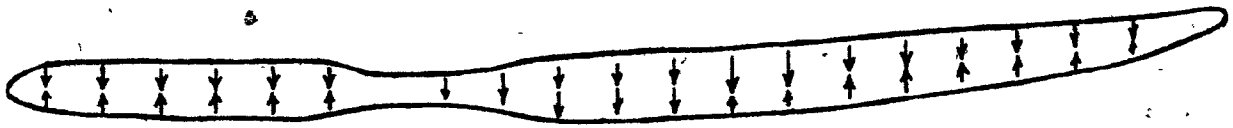
The variety of PSTH types obtained from Tokay auditory nerve fibres indicates a greater degree of peripheral filtering than occurs in mammals. With the exception of some bats, PSTHs from mammalian cochlear nerve fibres have been relatively uniform in shape (Kiang, 1965). The key to peripheral filtering in the Tokay is presumably in the morphological specializations of its inner ear. All lizard basilar papillae have discrete areas of unidirectional and bidirectional hair cell orientation (Miller, 1974). In the Tokay the unidirectionally oriented hair cells are confined to the narrow basal (dorsal) third of the papilla (Fig. 20). The wider apical (ventral) two-thirds of the papilla is unusual in being doubly bidirectional; i.e. there are two bidirectional areas separated by a longitudinal hiatus (Miller, 1973). In the monitor lizard, Varanus bengalensis, mapping of the distribution of CFs along the papilla has revealed a correlation between hair cell orientation pattern and PSTH type (Manley, 1977). Filled, semi-peaky and peaky PSTHs were also obtained

Figure 20. Schematic drawings of the basilar papillae of A) the Tokay: Gekko gecko (from Miller, 1974) B) the monitor lizard: Varanus bengalensis (from Miller, 1974, and personal communication). Areas of unidirectional and bidirectional hair cell orientation are represented by arrows in the same and opposing orientations, respectively.

(A) Gekko gecko



apical (ventral) basal (dorsal)



(B) Varanus bengalensis

from low, intermediate and higher CF primary fibres, respectively, in this species. The varanid papilla has a constriction approximately one-third of the papilla's length from its apical end (Fig. 20b). (Miller, 1974). Low frequency fibres were found to emanate from a unidirectional region basal to the constriction. Intermediate frequencies were located more basally in a bidirectional area. High frequency fibres arose from a bidirectional area occupying the third of the papilla apical to the constriction. Thus in Varanus, filled and peaky PSTHs were recorded from fibres innervating unidirectional and bidirectional areas of the papilla, respectively. It was suggested that hair cell orientation may be the factor determining PSTH type in Varanus.

It is tempting to apply this hypothesis to the Tokay, where remarkably similar PSTH types are produced by a different papilla. If peaky PSTHs are related to the presence of oppositely oriented hair cells, then higher frequencies would be located apically on the Tokay's papilla. Filled low frequency PSTHs would originate in the basal unidirectional third of the papilla. Such an arrangement would be somewhat surprising since familiarity with the mammalian cochlea has conditioned us to think of low frequencies as apical and high frequencies as basal. However, it is not known whether or how mechanical waves occur along the Tokay's basilar membrane. Although it has an apicobasal taper, it is only 2 mm in length. von Békesy (1960) observed travelling waves along the chicken's basilar membrane, which is 4 mm long. Using the Mossbauer technique, Weiss (1977) found no evidence for mechanical tuning in the small (0.4 mm) papilla of the alligator lizard. As in Varanus, mapping of the alligator lizard's auditory nerve showed that low and high frequencies are

represented on unidirectional and bidirectional areas of the papilla, respectively (Weiss et al., 1976).

That this may also be the case in the Tokay receives further indirect support from the discrepancy between cochlear microphonic and single-unit sensitivity curves (Fig. 2). The fact that the cochlear microphonic functions are based only on the fundamental component of the response may account at least in part, for the discrepancy. In round-window recordings in the Tokay, Hepp-Reymond and Palin (1968) found that the second harmonic was often as large as or larger than the fundamental. Lateral line receptor cells of opposing orientations are known to produce microphonic potentials that are out of phase (Flock and Wersäll, 1962). The presence of significant second harmonic in the cochlear microphonic is therefore thought to reflect the activity of oppositely oriented hair cells (Capranica, 1976). This reasoning provides a fairly simple explanation for the difference between cochlear microphonic and single-unit sensitivity curves in the Tokay. The fall-off of the fundamental above 0.6 - 0.8 kHz would be expected if high frequencies stimulate a bidirectional area of the papilla. The much greater sensitivity of the fundamental at low frequencies would be explained if low frequencies are represented on a unidirectional region.

Assuming that hair cells of opposing orientations do produce microphonic potentials that are out of phase, recordings based on the fundamental are clearly inappropriate when bidirectional areas are involved. The second harmonic cannot be used as a measure of sensitivity either, since its amplitude will vary with the relative phase of the microphonics produced by the opposing hair cell populations. Since amphibian and lizard

papillae have bidirectional areas, cochlear microphonic data from these animals are of questionable value.

The multiple peaks present in many of the PSTHs from units with CFs above 0.8 kHz have not been reported in other species. The inter-peak interval varied from 1.5 to 4 ms for different units, remaining constant at different stimulus intensities for the same unit. Considering that the dead times for fibres with CFs greater than 0.8 kHz were generally 2 ms or more (Fig. 19b), it is conceivable that multiple peaks reflect a tendency of these units to fire as rapidly as possible in response to the early part of the stimulus. If a unit were driven to fire several times in succession as rapidly as possible, spikes following the highly synchronized initial spike would also be synchronized.

PSTHs with one or two initial peaks have been recorded from Tokay cochlear nucleus neurons (Manley, 1974). In the torus semicircularis in the Tokay midbrain, only single peak PSTHs have been reported (Sammaritano-Klein, 1976). Discharge following the initial peak is more reduced in the secondary neurons than in the primary fibres, and even more reduced in the higher-order neurons of the midbrain. Contralateral input could contribute to this reduction. Although it has not been studied in the Tokay, binaural interaction has been shown to occur in bullfrog secondary auditory neurons (Feng and Capranica, 1976). It is conceivable that the peaks, which are highly synchronized with respect to stimulus onset, are important for sound localization (Manley, 1977).

On-off responses have also been reported in auditory nerve fibres of the green frog (Sachs, 1964) and a CF-FM bat (Suga et al., 1975). They are apparently as rare in the green frog as in the Tokay. The on-off responses in the bat are obtained from units with best frequencies around

the constant frequency component of the orientation pulse. Specialization of the area of basilar membrane on which these frequencies are represented provides a simple mechanical explanation for the off response. The on-off phenomena in CF-FM bats and Tokays are not likely to have much in common.

The use of a tone burst with trapezoidal rise-fall characteristics rather than a gaussian envelope raises the possibility that the off response is caused by bandspread during stimulus fall-time. The fall of a tone burst introduces new frequencies which could provide an effective stimulus. However, if bandspread were the source of the off responses reported here, they would be expected to occur much more commonly. In addition, relative to peaky PSTHs, on-off PSTHs showed suppression of discharge following the onset peak. This difference in on-off responses cannot be related to bandspread.

(iii) Spontaneous Activity

If dead time is assumed to be an indicator of refractoriness, then the preferred spontaneous interspike interval (mode) is clearly independent of refractoriness in these animals (Fig. 18c). The range of dead times in the Tokay (1.1 - 7.5 ms) was very different from the range for guinea pig spiral ganglion cells: 0.4 - 3.6 ms (Manley and Robertson, 1976). Comparison of spike waveforms recorded with similar microelectrodes from Tokay primary fibres and guinea pig spiral ganglion cells (Robertson, 1976) reveals that the time-course of the spikes is significantly longer in the Tokay. This would influence refractory period and so contribute to the difference between Tokay and guinea pig dead times.

It is impossible to rule out low intensity sound as the source of the oscillations observed in the interval histograms of low frequency fibres. However, it is difficult to understand how the units with elevated CF thresholds - 65 dB SPL in one case - could respond to such low-level stimulation. The absence of CF-related peaks in TIHs from fibres with CFs above 0.5 kHz is also not easily explained with this interpretation.

It is interesting to speculate on other possible sources of this phenomenon. It is conceivable that it originates at the level of individual hair cells. In weakly electric fish (mormyrids and gymnotids) certain phasic electroreceptor cells exhibit large membrane potential oscillations when the electrical loading of the cells is reduced - eg. by removing the overlying water (Bennett, 1967). In gymnotids, where afferents to phasic electroreceptors are tuned to the frequency of the electric organ discharge (EOD) (Hopkins, 1976), the receptor cell oscillations occur at the EOD frequency. The receptor cells which exhibit this behaviour are of a type that responds regeneratively to electrical stimuli. Hair cells have nonregenerative membranes (Flock, 1971), but then it is not necessary to postulate that they are capable of the large-amplitude oscillations recorded from the electroreceptor cells. Instead, passive filter properties in hair cell membranes might be sufficient to select low frequency oscillations from membrane noise. The implication is that these filter properties could form a basis for tuning.

If one chooses to speculate that the peaks in the TIHs originate in the hair cells, it becomes necessary to explain how such oscillations could be followed by fibres that presumably innervate more than one hair cell. Although papillar innervation has not been examined in the Tokay,

auditory afférents are known to each contact several hair cells in all non-mammalian systems that have been studied: anuran (Frishkopf and Flock, 1974; Lewis and Li, 1975), bird (Takasaki and Smith, 1971), caiman (von During et al., 1974). A fibre will only reproduce oscillations in the hair cells it innervates if they are in phase. This would be the case if neighbouring hair cells were electrically coupled. Weiss et al. (1974) have suggested that lateral electrical interaction may occur between hair cells in the alligator lizard. Their proposal was based on two findings in this animal: (1) that receptor potentials can be recorded from supporting cells as well as hair cells, implying electrical coupling between the two types of cell; (2) that specialized junctions occur between supporting cells and between supporting and hair cells, providing a possible pathway for electrical interaction. The observation that inter-peak interval in the Tokay TIHs was sometimes less or greater than the reciprocal of the CF could be explained as the result of coupling between hair cells of slightly different CFs. Coupling junctions could conceivably be low-pass filters that prevent coupling at frequencies greater than 0.5 kHz. Of the TIHs recorded from 0.5 kHz fibres, about half did not show peaks. In those that did, the oscillations were weak relative to those seen in lower-frequency units. It is interesting that at 0.4 kHz or less, all fibres produce peaky TIHs and filled PSTHs (Fig. 11).

Of major concern is the fact that similar TIHs have not been reported for other animals. Since they only occur in very low frequency units, it is possible that they have been overlooked. In mammals especially, most data are collected from higher-frequency units, since such low frequency fibres constitute only a small proportion of the population. On the other hand, it would not be surprising if this phenomenon, apparently

related to low frequency tuning in the Tokay, did not occur in mammals. Considering the differences between mammalian cochlear and Tokay inner ear morphology, it may be that very different tuning mechanisms have developed. This possibility would be confirmed if it were shown that, as has been suggested, low frequencies are represented basally on the Tokay's papilla.

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Paper II:

Auditory Nerve Fibre Activity in *Gekko gecko*:

II. Temperature effects on tuning.

Introduction

The influence of temperature on peripherally recorded auditory potentials has been studied in a variety of animals. It was established early that in mammals the cochlear microphonic (CM) is more resistant to change with temperature than are the neural components (N_1,N_2) of the round-window recording (Coats, 1965; Fernandez et al., 1958; Kahana et al., 1950). With hypothermia, N_1 and N_2 decrease in amplitude and increase in latency. CM amplitude and latency both remain constant down to 5 or 10°C below normal body temperature; further cooling reduces CM amplitude, but to a lesser and more variable extent than the amplitude of the neural components. More recently, the mammalian summating potential has been shown to decrease linearly with temperature (Manley and Johnstone, 1974). In birds, Necker (1970) found that both CM and SP changed little with cooling down to 30°C, below which they decreased in amplitude.

Campbell (1969) recorded CM and evoked potentials from the cochlear nuclei in eight lizard species, and found them to be generally most sensitive at temperatures within the animals' preferred thermal ranges. Werner (1972, 1976) arrived at the same conclusion after studying changes in CM with temperature in iguanids and geckos. Intriguingly, Werner also found that the frequencies to which the microphonic was most sensitive increased somewhat with temperature. In a single experiment, Manley and Werner looked at the effect of temperature on the tuning of cochlear nucleus neurons in the Tokay gecko (Gekko gecko) (unpublished data). The results they obtained in several cells recorded from at different temperatures supported the cochlear microphonic data and suggested to us that closer study might prove worthwhile. The possibility of an effect

of temperature on auditory tuning was interesting both from a behavioural-ecological point of view and as a potential source of information about vertebrate tuning mechanisms.

It was decided to investigate the influence of temperature on the frequency selectivity of Tokay auditory nerve fibres, these being more peripheral than cochlear nucleus neurons and much more accessible than hair cells. It was felt than an effect on primary fibre activity would be easier to interpret in terms of cochlear mechanisms than an effect observed in a higher-order auditory centre. The Tokay seemed an appropriate choice of experimental animal because its auditory neurons are sensitive and sharply tuned (Manley, 1972; Paper 1 of this thesis) and because it is an ectotherm active over a relatively broad thermal range (20 - 40^{o}C) (Werner, 1976). The results obtained with temperature changes within this range would presumably not be complicated by any adverse effects on the animal's general condition.

Methods

Gekko gecko weighing 40-200 g were imported from Thailand. The data reported in this paper are from 75 auditory nerve fibres in 22 animals. These were the fibres for which it was possible to obtain partial or complete tuning curves at more than one temperature.

The surgical approach to the animal's left proximal eighth nerve and the stimulation and recording techniques have been described (Paper 1 of this thesis). The animal's temperature was controlled by means of a heating pad wrapped loosely around its head and body. This arrangement permitted more rapid warming of the head, when desired, than was possible with the heating pad around the body alone. In most experiments, temperature was measured orally with a thermistor probe (0.25" diam., 0.063" thick) placed against the right cochlear duct - i.e. contralateral to the exposed auditory nerve. In the last 3 of the 22 experiments, the temperature inside the right cochlear duct was measured with a 24-gauge hypodermic probe. The footplate and part of the columella were removed to allow insertion of the probe through the oval window. Temperature was monitored and controlled with a thermistor probe monitor accurate to $\pm$ 0.1°C and located outside the sound-attenuating semi-anechoic chamber containing the animal.

Glass microelectrodes filled with 3M KCl or 4M NaCl were used to record from the posterior, auditory, half of the proximal portion of the vestibulocochlear nerve. The procedure upon encountering an auditory fibre (identified as such by its time-locked responses to white noise pulses) was to: 1) obtain a frequency-threshold or tuning curve at one temperature; 2) change the temperature, usually only by 0.5 - 2°C;

3) redo the tuning curve. In a number of cases it was possible to repeat these steps several times before contact was lost. For the 75 fibres that contributed data, two-thirds of the temperature changes were decreases and the remaining third were increases. Three of the fibres were both warmed and cooled. The range of temperatures covered by this study was from 19 to 31°C.

Cooling was usually accomplished by simply switching off the heating circuit. The rate of cooling then depended on the animal's temperature as the heat was switched off, and the ambient temperature. In a couple of experiments more rapid, localized cooling was achieved with a stream of cool damp air from a metal tube connected to an aquarium pump. The tube was positioned over the exposed braincase and left otic capsule; its end was flattened so that the opening was 8 mm by 1 mm.

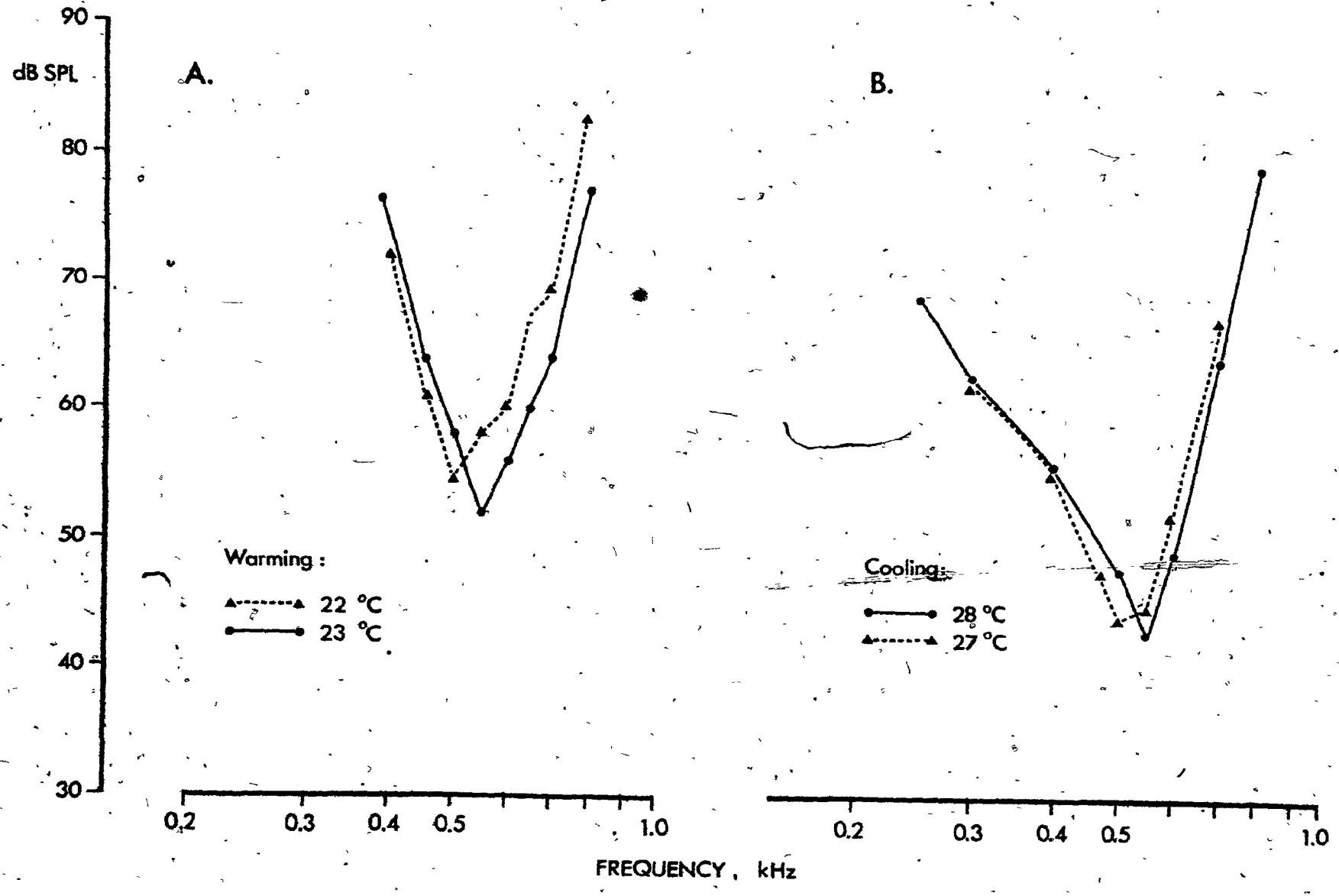
Results

It proved difficult to maintain contact with or 'hold' a fibre while increasing temperature; thus, only one-third of the results reported here were obtained while warming the animal. For 25 of the 75 fibres it was possible to obtain tuning curves (complete or partial) at more than two temperatures. Temperature changes between the curves obtained from an individual fibre varied from 0.3 to 4 or 5°C, but were usually between 0.5 and 1.5°C. Often a given tuning curve was obtained over a temperature range of 0.5 to 1°C, because temperature had not stabilized when tuning was begun.

The temperature effects shown in Fig. 1 are fairly representative of the results. A 1°C increase in temperature shifted the tuning curve of the unit in Fig. 1a toward higher frequencies. At the new temperature, thresholds on the high frequency side of the tuning curve were consistently lower while those on the low frequency side were higher. The frequency to which the fibre was most sensitive (its characteristic frequency or CF) changed from 0.5 kHz at 22°C to 0.55 kHz at 23°C. The reverse shift is shown for a different unit of similar CF (Fig. 1b), cooled from 28 to 27°C. After cooling, thresholds on the low frequency side were lower while those on the high frequency side were higher.

A unit's threshold at CF provides a measure of its overall sensitivity. Although changes in CF threshold (threshold at new CF minus threshold at original CF) with temperature were almost always seen, they were not consistent. In other words, it was not possible to predict a change in sensitivity from the magnitude (within the limits studied) or sign of the temperature change. Cooling and warming were both seen on different

Figure 1. Temperature effects on the tuning of 2 auditory nerve fibres with CFs around 0.5 kHz. A) Tuning curves from the same fibre before and after warming the gecko from 22 to 23°C (oral temperature). B) Tuning curves from a fibre while the animal's temperature was decreasing. The curves were taken at oral temperatures of 28 and 27°C.

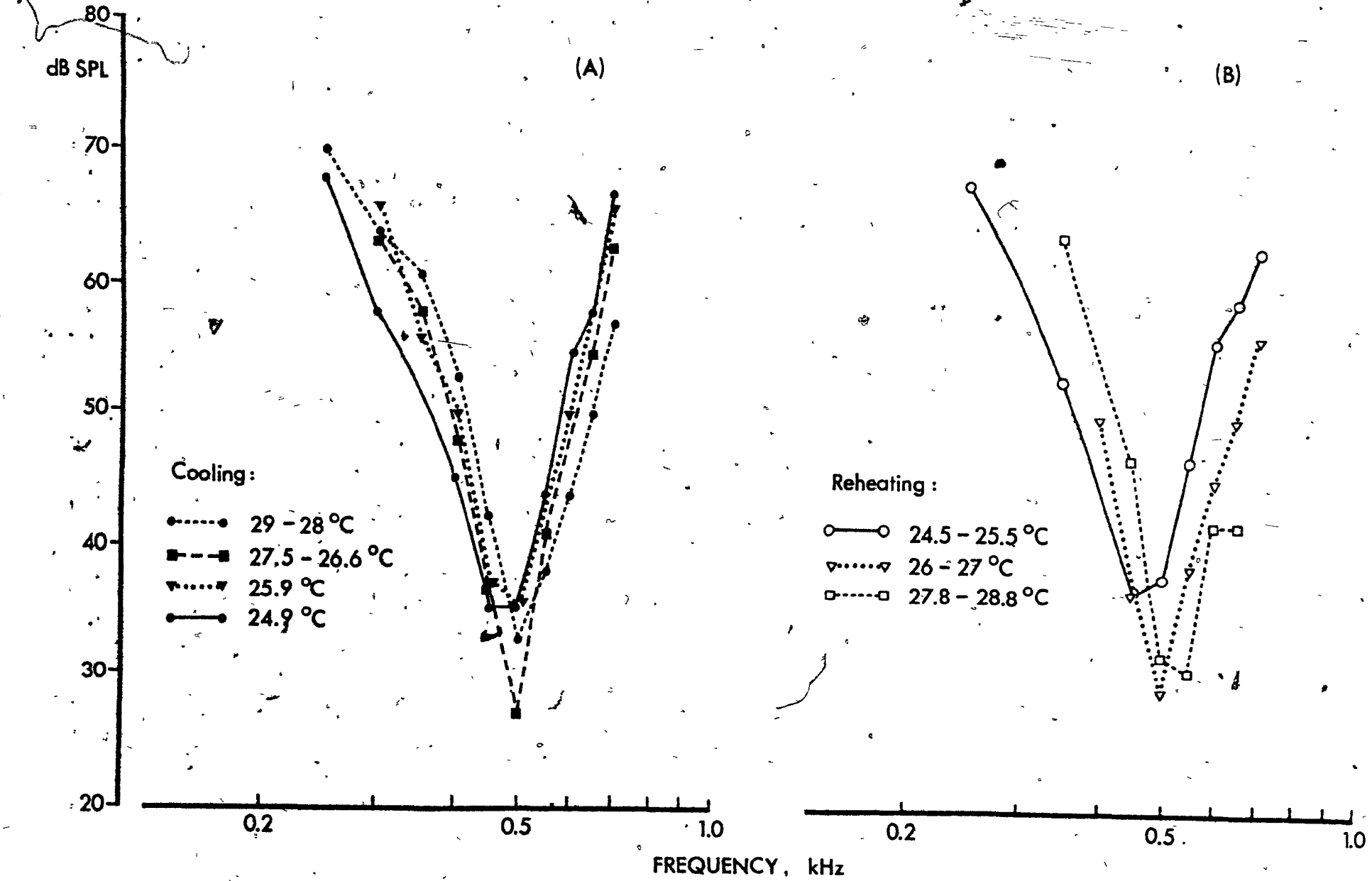

90
dB SPL
80
70
60
50
40
30
A.
B.
Warming :
22 °C
23 °C
Cooling:
28 °C
27 °C
0.2
0.3
0.4
0.5
1.0
0.2
0.3
0.4
0.5
1.0
FREQUENCY , kHz

occasions to reduce, increase, or not affect the fibre's lowest threshold value. There was no discernible relationship between the sign of the threshold change and the actual temperatures involved. Thus, there was no tendency for fibres to be more sensitive at any temperature or range of temperatures between 20 and 30°C. Threshold changes with temperature were also unrelated to CF. Perhaps larger and more rapid temperature changes than were used in this study, and/or changes below 20°C and above 30°C would have had a definite effect. The picture may also have been somewhat obscured by those occasions in which it could not be said with confidence that threshold at CF had actually been determined - that is, those cases in which the true CF may have been missed.

The shape of the tuning curves also showed no consistent changes with temperature. The shape or sharpness of tuning can be expressed in terms of Q_{10dB} (CF divided by tuning curve bandwidth at 10 dB above threshold) or the slopes of the low and high frequency sides of the tuning curve. Plotting the changes in Q_{10dB} and low and high frequency slopes per °C against CF revealed that although changes often occurred, they were highly variable and in no preferred direction.

The frequency shifts were, on the other hand, completely consistent in direction. Warming and cooling always shifted tuning curves to higher and lower frequencies, respectively. The reversibility of the effect is illustrated in Fig. 2. This fibre was held long enough to give four tuning curves while temperature was decreasing and three more during reheating. The shifts obtained with cooling and reheating were opposite in direction and comparable in magnitude. There was a pause for several minutes between cooling and reheating during which response activity was recorded. It is worth noting that the tuning curve taken at 24.9°C,

Figure 2. The reversibility of the temperature effect. In A), 4 tuning curves were obtained from a fibre while the animal's oral temperature was dropping from 29 to 24.9°C. After a pause, the same fibre as in A) was held while the animal was warmed back up to 28.8°C. The 3 tuning curves obtained from it during this reheating are shown in B). The unit's CF varied from 0.45 to 0.55 kHz during these procedures.

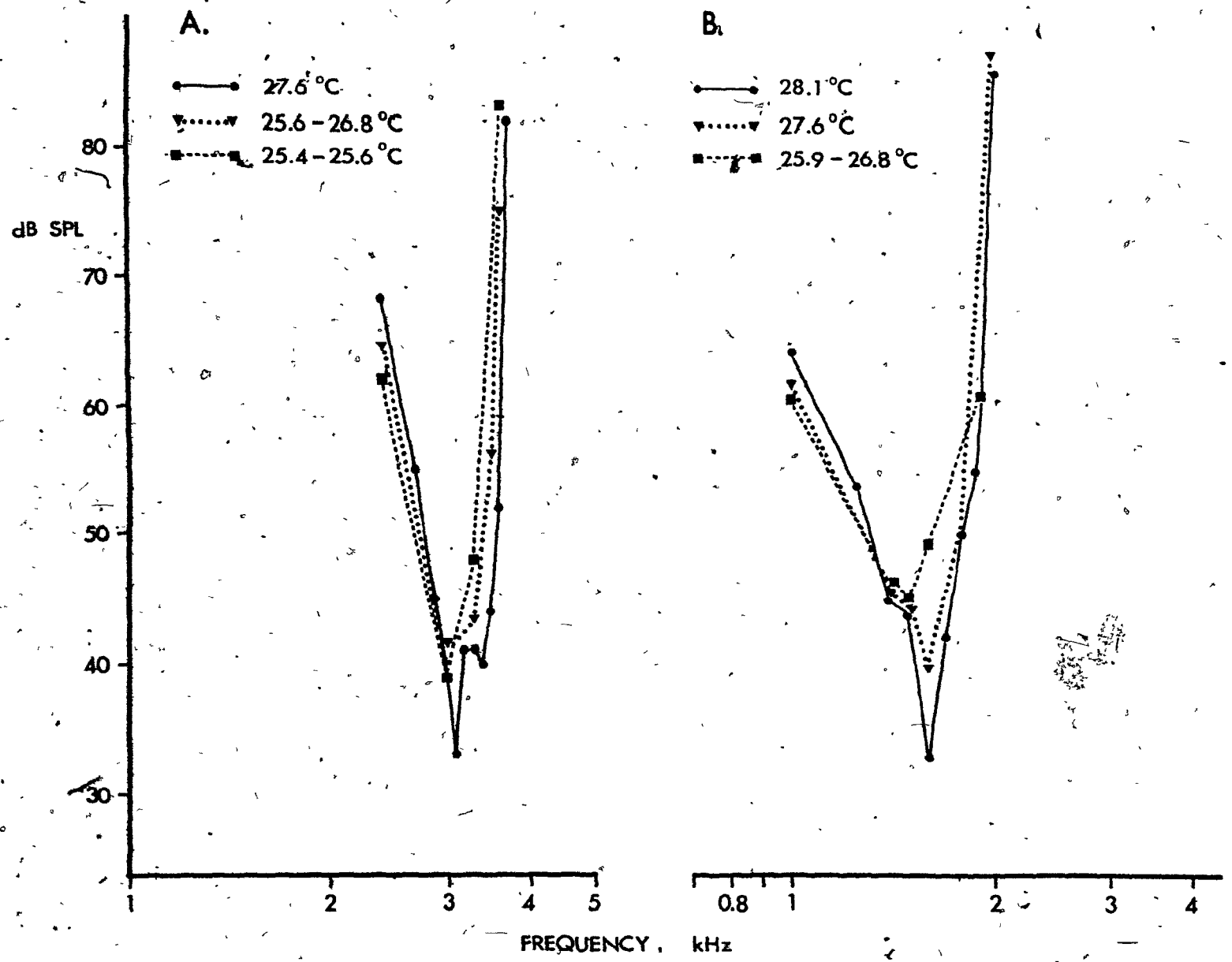

dB SPL
80
70
60
50
40
30
20
(A)
(B)
Cooling :
29 – 28 °C
27.5 – 26.6 °C
25.9 °C
24.9 °C
Reheating :
24.5 – 25.5 °C
26 – 27 °C
27.8 – 28.8 °C
0.2
0.5
1.0
FREQUENCY , kHz

before the break, is almost identical to the curve taken between 24.5 and 25.5°C, immediately after the break and at the start of reheating.

Although more data were obtained at lower frequencies, the effects were similar for higher-frequency units (eg. Fig. 3b). The results in Fig. 3 were obtained by cooling with a stream of air, as described in Methods. The noise of the airstream (on continuously for 30 s) may have been at least partly responsible for the increases in CF threshold in these units with cooling. To test for this possibility, a couple of units were subjected to 30 s of continuous white noise while temperature was held constant. CF sensitivity was reduced by about 5 dB; no frequency shift occurred. Thus, in Fig. 3, the frequency shifts are probably reliable while the threshold changes are probably not. It should be noted that although the rate of cooling was much faster with this method, there were no obvious differences in the magnitude of the observed frequency shift; that is, the rate of change of temperature may not be important. The results obtained with the intracochlear thermistor probe were indistinguishable from those obtained with the oral probe. As might be expected there was some lag between intracochlear and oral temperature - eg. intracochlear temperature was slower to increase when the heating pad was on and would continue to increase after it was switched off.

There are several ways of expressing the cumulative frequency shift data in graphic form. One is to simply look at changes in CF with temperature (Fig. 4a). The problem with this approach is that to be very informative it requires accurate determination of CF. The fineness of tuning required to determine small CF shifts was often not possible in the time available between contacting the fibre and losing it. In Fig. 1b, for instance, visual inspection of the tuning curve slopes at

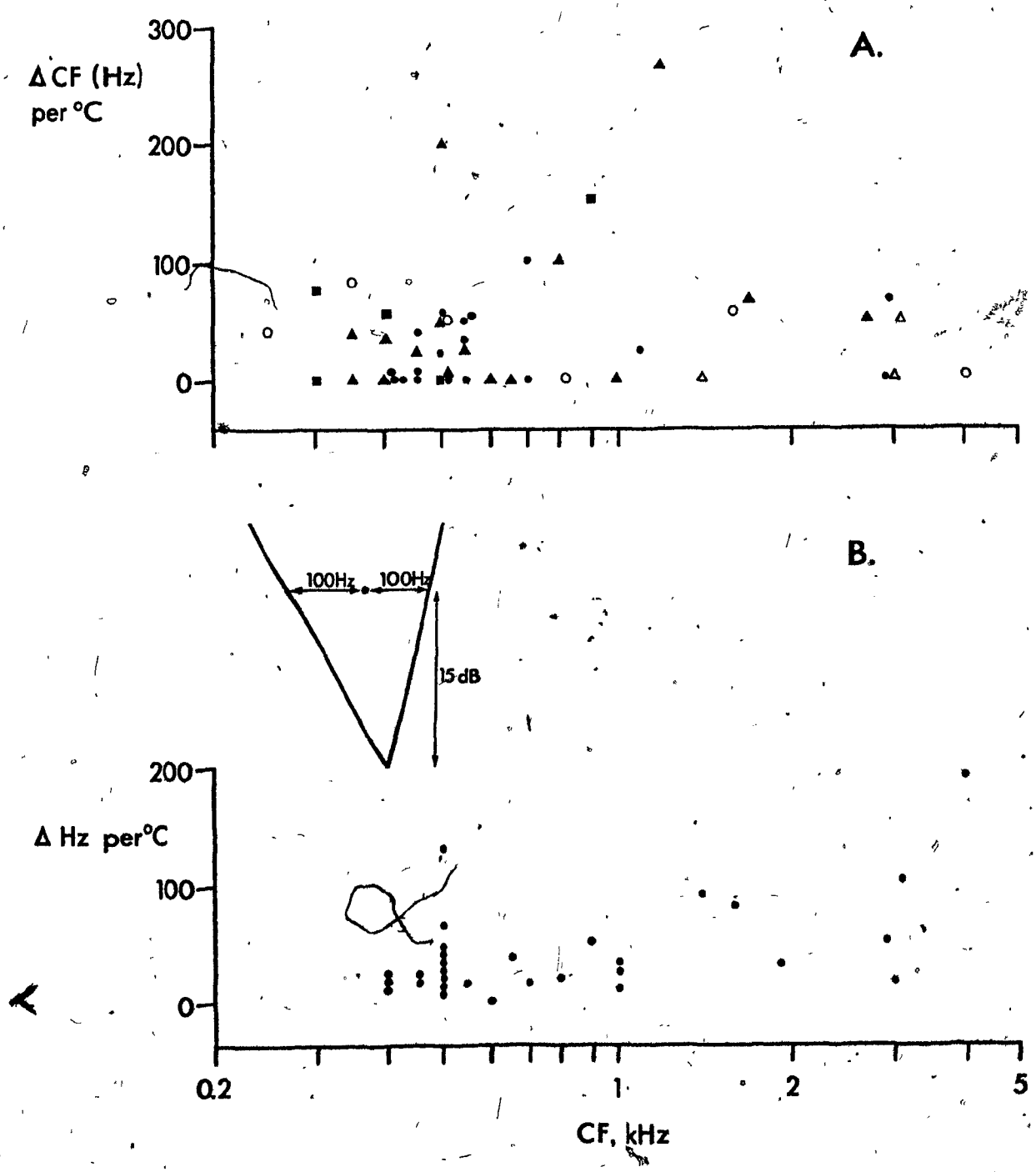
Figure 3. The effect of cooling with a stream of air on the tuning of A) a unit with a CF of 3.1 kHz at 27.6°C. B) a unit with a CF of 1.7 kHz at 28.1°C and 27.6°C.


A.
27.6 °C
25.6 – 26.8 °C
25.4 – 25.6 °C
dB SPL
80
70
60
50
40
30
1
2
3
4
5
B.
28.1 °C
27.6 °C
25.9 – 26.8 °C
0.8
1
2
3
4
FREQUENCY, kHz

the lower temperature would predict CF to be approximately 0.525 kHz, since Tokay tuning curves typically have a fairly simple V-shape (Paper 1 of this thesis). Unfortunately no threshold values were taken between 0.5 and 0.55 kHz, so that the prediction cannot be confirmed. If the CF of each of the two tuning curves is simply taken to be the frequency corresponding to the lowest threshold value recorded, then CFs of 0.55 and 0.5 kHz and a CF shift of 50 Hz are obtained. It is possible, however, that the CF shift was less than 50 Hz in this case. For other fibres CF shift appeared to be underestimated because of the same problem: insufficient threshold data around the original CF value. Threshold differences at each frequency contributing a point to the tuning curve were a more reliable indicator of the temperature-mediated changes. An effort was made to take thresholds at the same frequencies when doing tuning curves at different temperatures for a given fibre.

What can be observed in the graph of change in CF with temperature (Fig. 4a), despite its limitations, is that CF never decreased with warming or increased with cooling. CF was seen to shift to higher frequencies with warming, to lower frequencies with cooling, or not at all. The largest shifts were obtained from fibres with CFs below 1.3 kHz. The few data points above 1.3 kHz suggest that CF shift may not be proportional to CF. Although the data are insufficient to warrant serious conclusions, they are more in keeping with a CF shift that is independent of original CF. It should be noted that error has been introduced by standardizing all shifts to a $1^{o}C$ temperature change. This makes the unwarranted and probably false assumption that temperature change and CF shift are linearly related, but is the only way of coping with changes of different magnitudes.

Figure 4. A) Changes in CF per ^{o}C, v. the original CF. ΔCF = final CF - original CF. In this and subsequent graphs: (1) the temperature change used to obtain ΔCF per ^{o}C was also given a sign: negative for cooling (ie. final minus original temperature), and positive for warming. Thus, both a decrease in CF with cooling and an increase with warming have positive values ; (2) only 2 tuning curves per unit were compared, so that each unit made an equivalent contribution to the data. Symbols are used to break down the total temperature range covered by this study into 3 smaller ranges, and to differentiate between data points obtained with oral and intracochlear temperature measurements. Filled squares: 19-22^{o}C (oral temperature); filled triangles: 22-26^{o}C (oral); open triangles: 22-26^{o}C (intracochlear); filled circles: 26-31^{o}C (oral); open circles: 26-31^{o}C (intracochlear). B) Changes in 'central frequency' with temperature v. original CF. The 'central frequency' was determined as shown above the graph. The number of data points is reduced relative to A), because in some cases, one side of a tuning curve did not extend to 15 dB over CF threshold (i.e. only a partial curve was obtained).


A.
Δ CF (Hz)
per °C
300
200
100
0
B.
100Hz
100Hz
15 dB
200
Δ Hz per°C
100
0
0.2
1
2
5
CF, kHz

One way of avoiding the problem of inaccurate CF determination is to look instead at the frequency midway between the frequencies intersected by the low and high frequency sides of the tuning curve at an arbitrary intensity (eg. 15 dB SPL in Fig. 4b) above CF threshold. In Fig. 4b, changes with temperature of "central frequencies" determined in this way are plotted against CF. By comparing Figs. 4a and 4b it can be seen that the "central frequency" shifts more with temperature than the CF. This is particularly obvious at frequencies above 2 kHz.

More information can be obtained from each tuning curve shift if one examines threshold changes with temperature at various frequencies along the tuning curve. In Fig. 5 the tuning curves have been normalized so that each frequency is expressed as a fraction of the original CF. The ordinate is change in threshold (final threshold minus original threshold) per ^{o}C. To arrive at this value, the temperature shift was given a sign: positive for an increase in temperature and negative for a decrease in temperature. Thus, a decrease in threshold (increase in sensitivity) with temperature increase will have a negative value on this graph, as will an increase in threshold with temperature decrease. A positive value on the graph indicates that threshold either increased (sensitivity decreased) with warming or decreased with cooling. In this way equivalent effects are given the same sign and the direction of the temperature change is not indicated. The validity of this design is demonstrated by the reversibility of the temperature effect (Fig. 2). Threshold difference values were only calculated for those frequencies at which thresholds had been estimated both before and after the temperature change - i.e. threshold was never estimated from the tuning curve. In

Figure 5. Temperature-induced changes in threshold at various frequencies along the tuning curve, expressed as fractions of CF. Refer to text for explanation.

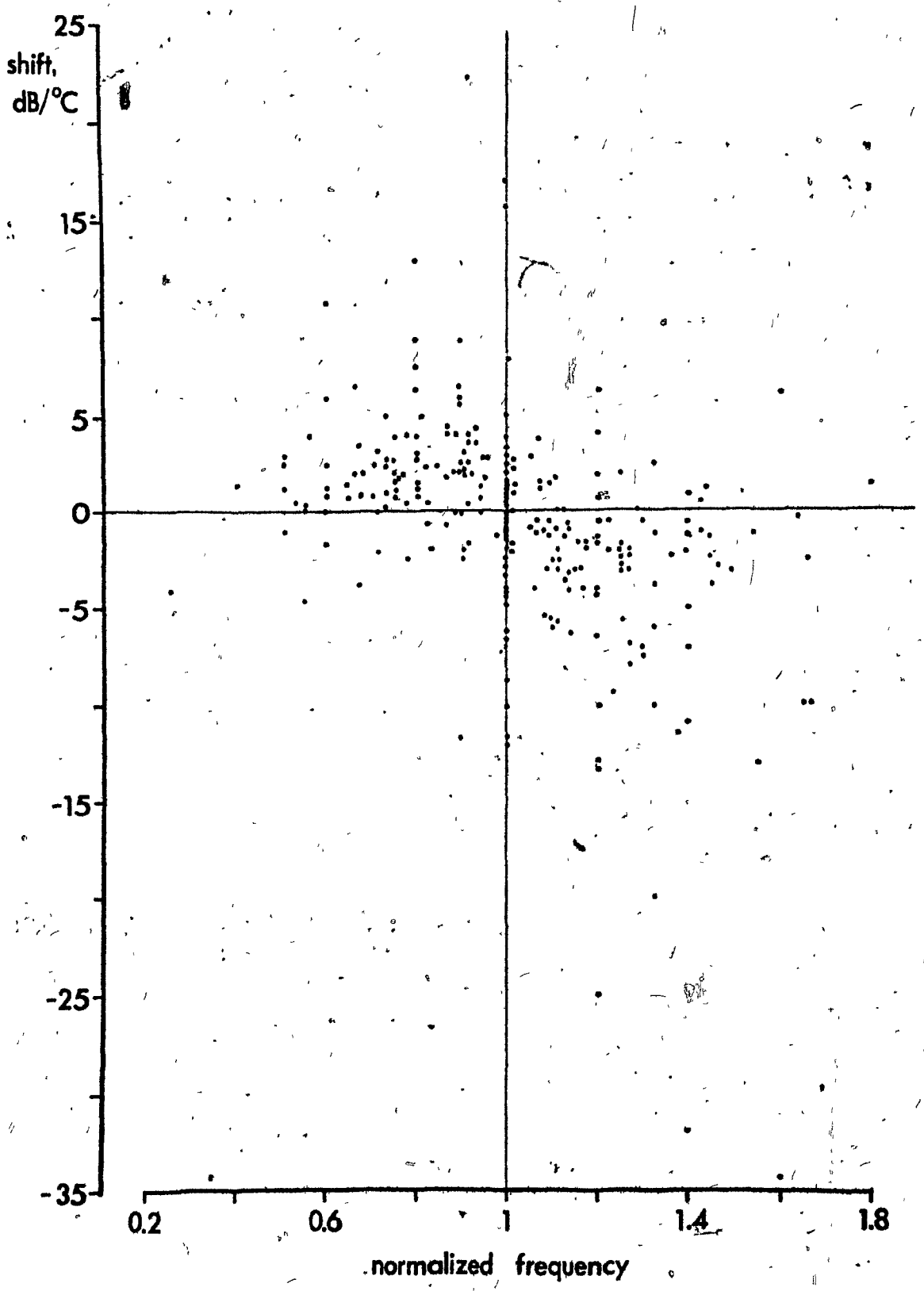
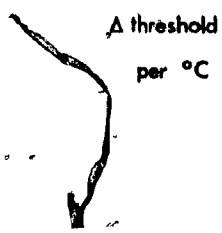

25
shift,
dB/°C
15
5
0
-5
-15
-25
-35
0.2
0.6
1
1.4
1.8
normalized frequency

Fig. 1b, for instance, threshold differences were calculated at 0.3, 0.4, 0.5, 0.55, 0.6 and 0.7 kHz. The threshold shift at 0.5 kHz was 44-48 dB = -4 dB, while the temperature change was 27-28 = -1^{o}C. Thus the threshold shift per ^{o}C at 0.5 kHz had a value of: -4/-1 = +4 on Fig. 5.

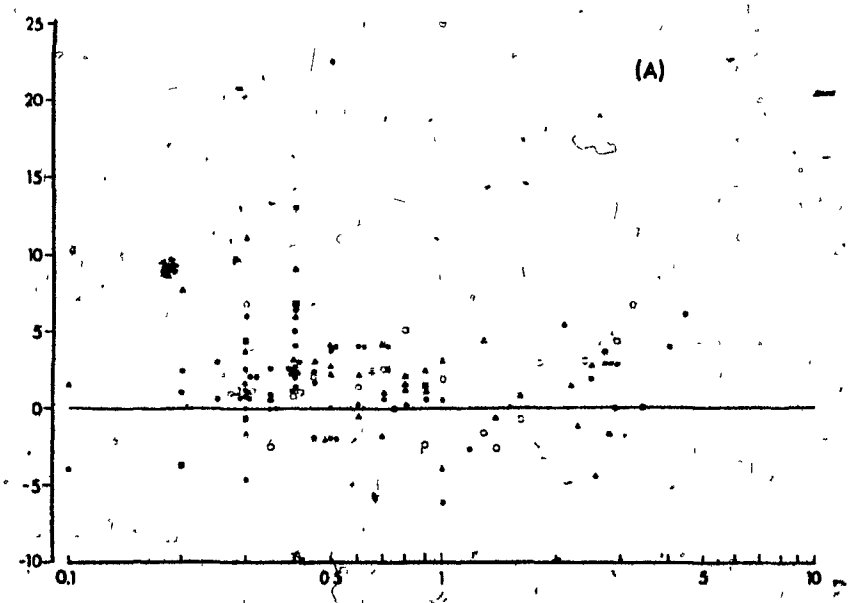
From Fig. 5, it can be seen that threshold changes at CF show no significant trend toward either positive or negative values. Thus threshold changes above and below CF can be considered relatively independent of overall changes in sensitivity. Below CF, threshold shifts tend to be positive in value. This means that the low frequency side of the tuning curve tends to become less sensitive with warming and more sensitive with cooling. Above CF, the opposite tends to occur, so that most threshold shifts have a negative value. The negative values at frequencies below CF and the positive values above CF do not indicate that whole tuning curves occasionally shifted in a direction opposite to the usual shift. This was never the case. Instead, the scatter reflects the tendency for there to be one or more frequencies on a tuning curve (other than CF) at which the threshold shift deviated from the shift seen at most frequencies. For instance, in Fig. 2, although the trend toward lower frequencies with cooling was clear, there was some cross-over on the low-frequency side of the first three tuning curves taken. The large negative threshold shifts in Fig. 5 were obtained from units that showed large shifts for very small temperature changes (eg. 10 dB per 0.3^{o}C). This illustrates the problem associated with standardizing all temperature changes to 1^{o}C. The evidence from the other fibres indicates that it is highly improbable that such large shifts would have actually occurred over a 1^{o}C change.

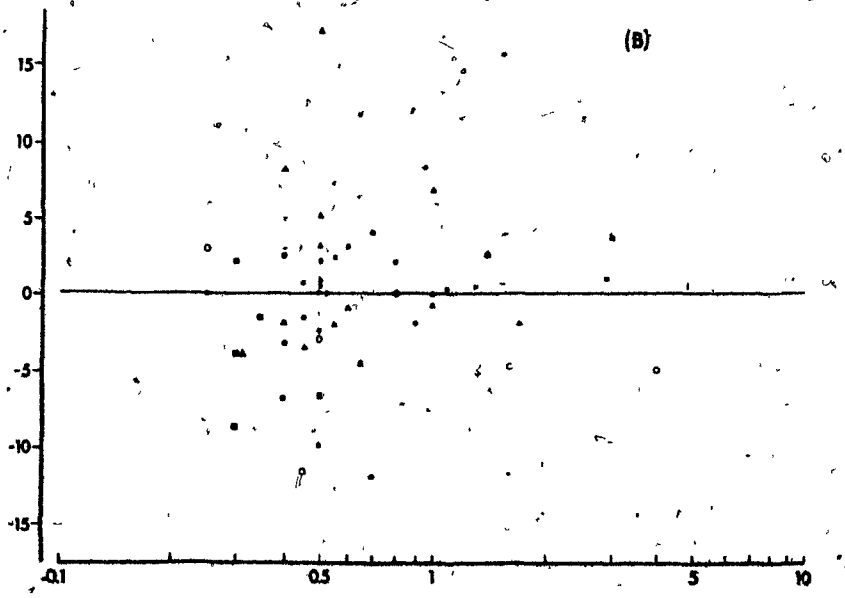
In Fig. 6, the same threshold shift data are presented somewhat differently. Frequency is not normalized to CF. The data are divided into 3 main categories: threshold shifts obtained A) at frequencies less than CF - i.e. on the low frequency arm of the tuning curve; B) at CF; C) at frequencies greater than CF. Thus actual frequency is shown, not frequency relative to CF. These graphs also do not indicate how far removed from CF a given point is along the tuning curve. In Fig. 6, different symbols are used to represent three different temperature ranges, and to distinguish intracochlear temperatures from those measured orally. As in Fig. 5, it is clear that threshold shifts on the low frequency side of the tuning curve tend to have positive values; that is, thresholds tend to increase with warming and decrease with cooling. Again, the opposite tends to occur on the high frequency side, and CF thresholds show no consistent trend to either positive or negative values. In addition, Fig. 6 indicates that threshold shifts show similar trends over different temperature ranges and different frequencies. Data points from animals whose intracochlear temperature was measured seem to fit well with those from animals in which oral temperature was measured.

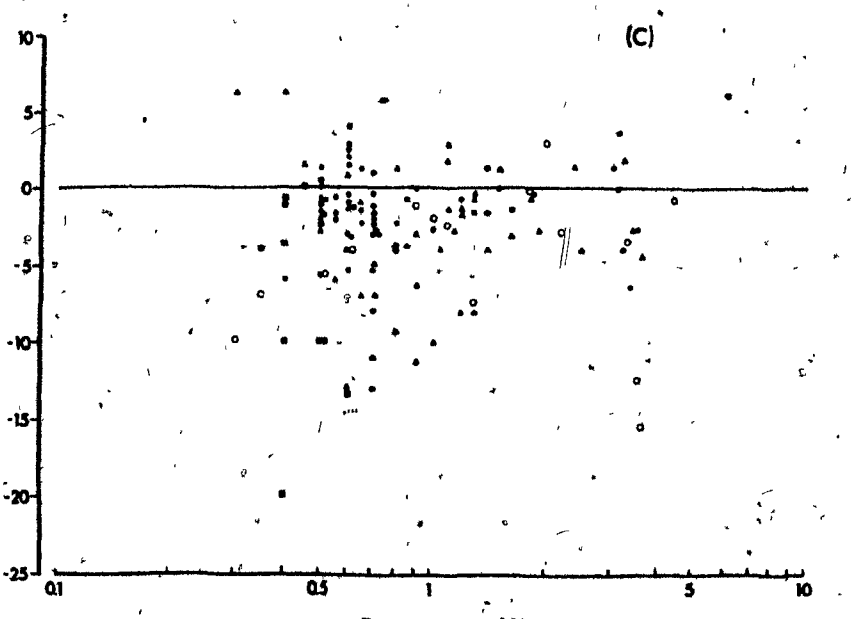
Figure 6. Temperature-induced changes in threshold at given frequencies along the tuning curve. Refer to text for explanation. As in Fig 4: different symbols represent different temperature ranges within which the data points fall, and also differentiate between points obtained with oral temperature measurements and those obtained with intracochlear temperature measurements. Filled squares: 19-22°C (oral temperature); filled triangles: 22-26°C (oral); open triangles: 22-26°C (intracochlear); filled circles: 26-31°C (oral); open circles: 26-31°C (intracochlear).



Δ threshold per °C







Discussion

Werner (1972, 1976) observed that in iguanids and geckos the frequencies to which the microphonic is most sensitive increase with temperature up to a point, beyond which CM sensitivity deteriorates. In the Tokay, he found that the overall sensitivity of the CM response varied little between 19 and 32°C. For temperature changes over the same range, tuning curves of Tokay auditory nerve fibres show frequency shifts in the same direction as well as no consistent changes in best threshold. In a single experiment on Tokay cochlear nucleus neurons, temperature change was found to shift tuning curves in a manner indistinguishable from the primary fibre results (Manley and Werner, unpublished data). The influence of temperature on frequency selectivity is thus clearly established at the Tokay's auditory periphery, although the relationship between the CM effect and the tuning curve effect may not be simple.

Werner (1972, 1976) has also demonstrated that the middle ear is not implicated in the effect of temperature on lizard CM. CM sensitivity functions obtained by direct stimulation of the footplate following columella (middle ear) removal were affected by temperature in the same way as functions recorded with the middle ear intact. Further evidence for the insensitivity of middle ear transmission to temperature comes from mammalian studies (Fernandez et al., 1958; Kahana et al., 1950) in which CM latency was found to remain constant over large temperature reductions. For the Tokay, an ectotherm with a broad active thermal range, it is perhaps not remarkable that temperature changes within this range have little or no effect on overall auditory sensitivity. That cooling

from 40 to 30°C or less does not influence CM amplitude in mammals (Drescher, 1974, 1976) and birds (Necker, 1970) is less easily understood. In contrast, the latency and amplitude of the whole-nerve action potential (AP) change systematically with temperature (Coats, 1965; Kahana et al., 1950). The poor correlation between CM and AP amplitude changes with temperature led Coats (1965) to suggest that temperature acts primarily on an excitatory process intermediate to the generation of microphonic and excitation of the afferent nerve terminals. By this reasoning, the summating potential, though concurrent with CM, would be a candidate for Coats' excitatory process by virtue of its linear decrease with cooling. Correlation between changes in intracochlear potentials and changes in AP may, however, not be very meaningful. Kahana et al. (1950) suggested that the decrease in AP amplitude that is concurrent with constant CM output may simply reflect the progressive desynchronization of fibre activity with cooling. Changes in CM amplitude are small even with drastic cooling (Coats, 1965) and may be obscured between 40 and 30°C by variable phase relationships within the output.

Tuning curves from certain auditory nerve fibres in the toad, Bufo americanus, show frequency shifts with temperature similar to those reported here (Moffat and Capranica, 1976). Curiously, fibres from only one of the toad's two auditory papillae (the amphibian and basilar papillae) exhibit this behaviour. Neither frequency selectivity nor thresholds of fibres from the toad's basilar papilla - considered by some to be homologous to the reptilian basilar papilla - are significantly altered by temperature changes of 5 to 10°C. Tuning curves of fibres from

the amphibian papilla not only shift to lower frequencies with cooling, but also become less sensitive. The reduced sensitivity may have resulted from the use of larger temperature shifts than in the Tokay experiments.

Although it may seem surprising that tuning should ever be consistently affected by temperature, it is even more remarkable when the effect is restricted to one of two auditory papillae in the same animal. What are the relevant differences between the anuran basilar and amphibian papillae? One obvious possibility is the mechanical basis for tuning. The anuran basilar papilla, which is responsive to a relatively narrow band of frequencies, is thought to be a "simply tuned resonant structure" (Capranica and Moffat, 1977). For the amphibian papilla, with its wider distribution of frequencies, Capranica and Moffat have proposed that place-determined frequency analysis is operating. Mechanical tuning of the mammalian type is not possible since both anuran papillae are supported by the wall of the otic capsule rather than a suspended basilar membrane. The amphibian papilla may be mechanically tuned through its tectorium. Temperature could conceivably affect its tuning by altering the physical properties - eg. elasticity, density - of structures contributing to its mechanical tuning.

On the other hand, the temperature sensitivity of tuning in certain electroreceptors raises the possibility that temperature acts directly on receptor cells. Hopkins (1976) studied the effect of small, localized temperature changes on the activity of the afferents to phasic electroreceptors in gymnotids. A $2^{\circ}C$ increment at the receptor site was found to more or less reversibly increase a fibre's best frequency by 10-30 Hz - a significant amount relative to CFs around 130 Hz. A slight reduction in

sensitivity at the higher temperature (22°C) may have been related to the fish's prior acclimation at 20°C. Unlike Tokay tuning curves, which did not consistently alter shape, tuning curve bandwidth increased with warming in the gymnotids. Referring to large membrane potential oscillations that occur when the electrical loading of phasic electroreceptor cells is reduced, Hopkins discussed the possibility that electroreceptor tuning is dependent upon the membrane filter properties responsible for these oscillations (Paper 1 of this thesis). In gymnotids the oscillations occur at the frequency of the electric organ discharge (EOD) (Bennett, 1967). Hopkins suggested that temperature might affect the frequency of such oscillations and hence tuning. It is tempting to consider a mechanism for the temperature effect that would apply generally to lateral-line derivatives and so encompass both hair cells and electroreceptors. Membrane conductance changes with temperature are well-established in a number of systems (eg. Lieberman and Lane, 1976). It is interesting to note that in hair cells of the sea slug's statocyst, the slow generator potential evoked by a mechanical stimulus is both secondary to conductance changes and temperature-sensitive in its time course (Detwiler and Fuortes, 1975). The rate of rise of the generator potential increases with temperature.

An obvious and serious objection to the suggestion that temperature may act primarily on the receptor cell membrane is raised by the insensitivity of the toad's basilar papilla to temperature change. It is intriguing to note in this regard that the tuning of anuran basilar papilla fibres is also resistant to anoxia (Capranica, personal communication), unlike the tuning of amphibian papilla fibres and mammalian cochlear fibres (Robertson and Manley, 1974). Clearly, tuning in the

toad basilar papilla has a different basis than it does in the amphibian papilla and presumably auditory organs in other animals. Its insensitivity to anoxia and temperature shift implies an independence from normal aerobic metabolism.

It has recently been reported that tuning in cat cochlear fibres is also unaffected by temperature change (Klinke and Smolders, 1977b; Smolders and Klinke, 1977). Local warming of the basal cochlea by 2 - 4 ^{o}C was reported to increase fibre sensitivity but not significantly affect CF. Apparently, however, a CF shift of "not more than 0.04 octave per 4^{o}C" toward higher frequencies did occur. Several comments can be made in this regard. (1) The stimuli used were 8 s tone sweeps fed to 200 ms tone bursts. The summed activity in response to 5 tone sweeps was used to evaluate CF. This method has the advantage of speed but lacks the precision of a tuning curve. (2) Almost all of the small sample of fibres studied had CFs in the vicinity of 20 kHz. A 0.04 octave per 4^{o}C shift for such units is in the order of 800 Hz. Standardized to 1^{o}C, as was done for the Tokay data, this becomes a CF shift of 200 Hz per ^{o}C. The decision as to whether such a shift is significant or not appears to be rather arbitrary. If, as may be the case in the Tokay, frequency shifts are not proportional to CF, then similar shifts might occur at lower frequencies where they would be unquestionably significant relative to CF. (3) CF changes with temperature were found to give rather inferior information about the frequency shifts seen in Tokay fibres. In other words, the observed CF shift was a much less reliable indicator of the overall frequency shift of a tuning curve than were threshold changes along its sides. With these points in mind it

may be prudent to withold judgement on the question of a temperature effect on mammalian tuning until tuning curve data is obtained from a large sample of mammalian fibres, including some low-frequency ones. This is not to say that one should expect a temperature effect in mammals similar to that found in toad and Tokay, or that with more data the picture in the cat will necessarily change from that already presented. Differences in the magnitude of the effect in various animals should provide clues to differences in tuning mechanisms.

Klinke and Smolders have also investigated the influence of head temperature on auditory nerve fibre tuning in the caiman (Klinke and Smolders 1977a; Smolders and Klinke, 1977). With cooling over a temperature range similar to that used in the present study, CF was found to decrease by approximately 0.3 octave per 6°C (personal communication). Although the CF shifts are apparently much more regular than those in the Tokay, values of 0.05 octave per °C (standardized from 0.3 per 6°C) fit well within the scatter of points in Fig. 4a, for CFs below 2 kHz. It is not known whether the Tokay data are representative above 2 kHz, or whether caiman data have been obtained above 2 kHz. The caiman's frequency range only extends to 3 kHz (Klinke and Pause, 1977; Manley, 1970).

Spontaneous and evoked activity were found to decrease with cooling in caiman (Klinke and Smolders, 1977a; Smolders and Klinke, 1977) and in fibres from both the amphibian and basilar papillae in the toad (Moffat and Capranica, 1976). This was not studied systematically in the Tokay. The presence of an effect on discharge rates from the toad's basilar papilla suggests that it is unrelated to the effect on tuning. Temperature-mediated changes at the afferent synapse could provide a simple explanation for the reduction in discharge rates. Cooling depresses

the rate of transmitter release at the squid giant synapse (Weight and Erulkar, 1976) and the neuromuscular junction in frog sartorius muscle (Katz and Miledi, 1965). In the latter preparation, ACh release following the arrival of an impulse at the terminal is both delayed (Q_{10} approximately 3) and dispersed in time during hypothermia.

It is impossible at this point to suggest a behavioural significance for the sensitivity of the Tokay's peripheral tuning to temperature. The Tokay's behaviour has not been studied to any extent, and the durability of the temperature effects reported here is not known. Fibres were usually held for only short periods of time. Examples such as Fig. 3 impress one with the consistency of the effect over perhaps half an hour of recording. However, temperature was not held constant for very long so that the stability with time of a given frequency shift has not been assessed. There are, however, interesting behavioural correlates to the temperature sensitivity of gymnotid and toad amphibian papilla afferents. Let us assume that the temperature-mediated frequency shift seen in the toad probably also occurs in fibres from the amphibian papilla in other anurans. It has been shown that both the pulse repetition rate of the male treefrog's mating call and the pulse rate preferred by the female treefrog increase, in step, with temperature (Gerhardt, 1977). Although this indicates that anuran calls can change with temperature, it is not known whether the frequency spectra of the calls vary. In gymnotids, the EOD frequency - to which the phasic electroreceptors are tuned - increases with temperature (Hopkins, 1976). Frequency shifts with temperature in communicatory signals could be secondary to the physiological effect on auditory tuning. Selection pressure for behavioural adaptations to the sensory phenomenon would presumably be high in animals

that rely heavily on their perception of communicatory and orientation signals.

Temperature must be expected to influence the inner ear in a number of ways. More comparative data are needed to decide which of these is/are critical to frequency selectivity. For instance, it would be interesting to look for a temperature effect on the tuning of fibres from the alligator lizard's papilla which, like the anuran basilar papilla, is thought to be a simply tuned resonator (Weiss, 1977). It is not at the moment necessary to postulate a single mechanism for temperature effects on tuning; it is conceivable that temperature exerts its influence in different ways in different systems. Admittedly the similarity of the frequency shifts in caiman, gymnotid, toad amphibian papilla and Tokay suggest a common mode of action. Since the mechanics of these systems differ greatly, an effect at the receptor cell level would seem more plausible were it not for the results from the toad's basilar papilla. Further information about this unusual papilla would seem important to understanding the source of temperature's effects on tuning.

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Concluding Remarks

While the details of auditory processing in a lizard can be fascinating in their own right, clearly most of the interest in such a system depends upon its relevance to other systems. This is particularly true for animals whose behaviour related to audition is either uninteresting or unknown. The Tokay is known to vocalize, but in-depth behavioural studies to determine what sounds have most significance for the animal are missing. Such information is important to understanding the more specialized forms of peripheral filtering - i.e., the dynamic properties of fibre responses rather than basic tuning. The various PSTH categories (Fig. 9, Paper 1) indicate that the Tokay's inner ear performs more than just frequency analysis. Even without good behavioural data on the Tokay, it is interesting to speculate on the selection pressure(s) that produced these PSTH patterns. The need to localize sound has been suggested (Manley, 1977). Lizards are poorly equipped to handle sound localization cues available to other animals. They lack pinnae and have small heads that create little sound shadow over the relatively low frequencies to which they are sensitive. The highly synchronized onset peaks in response to tone bursts at intermediate and high frequencies (within the Tokay's range) may be a device to emphasize the very small differences in time of arrival of sounds at the two ears. The increasing emphasis of the onset peak, relative to the rest of the histogram, in higher-order centres indicates its importance to the animal's perception. The absence of onset peaks in PSTHs from units sensitive to low frequencies may simply reflect the impossibility of binaural temporal discrimination at such long stimulus wavelengths.

The origin of onset peaks in the inner ear is as interesting to consider as their significance to the animal. In Paper 1, a relationship between onset peaks and hair cell bidirectionality was suggested. It should be pointed out that in the Tokay, the bidirectional area also differs from the unidirectional region in its accessory tectorial structures (refer to p. 8, this thesis) (Miller, 1973). The Tokay papilla is actually divisible into three regions on the basis of tectorial attachments: apical pre-axial, apical post-axial, and basal (Fig. 3, Introduction). This might seem to relate less well to the data than division of the papilla into two areas (apical and basal) according to hair cell directionality, because of the basic dichotomy in PSTH pattern: peaky v. filled. However, innervation patterns help determine functional divisions of the papilla as 'seen' by the primary fibres. For instance, if each afferent innervates all cells in a transverse row (pre-axial and post-axial), then the apical two-thirds is, in terms of fibre responses, a single division of the papilla. If cells underlying the post-axial sallets are separately innervated, then the division between pre-axial and post-axial apical papilla is reproduced in the auditory nerve. Unfortunately, the innervation of the Tokay's papilla has not been examined. In any case, it is conceivable that the sallets contribute to or cause the onset peak. The growth of multiple peaks with intensity (Fig. 10, Paper 1) can be visualized as the result of the sallets' tendency to oscillate at a preferred frequency that is precisely determined by their inertial properties. Such oscillations would damp out more slowly at high intensities. The weak point in this suggestion is that monitor lizards, whose primary fibres also produce peaky and filled PSTHs, have a continuous plate-like tectorial membrane over all hair cells on the papilla (Miller, 1974).

Evidently in the monitor the tectorial membrane cannot be responsible for the differences in PSTH pattern. It is possible, however, that different mechanisms produce onset peaks in the two species.

Although not studied systematically, the phenomenon of two-tone rate suppression[1] was observed in the present study. Two-tone rate suppression is the reduction of a unit's response to a tone around its CF that is caused by simultaneous presentation of a second, louder tone of different frequency. It has recently been found to occur in Tokay primary fibres with CFs between 0.4 and 2 kHz (Manley, personal communication). It was not investigated in units with higher CFs. As in mammals, the tones which proved effective in reducing the response of a Tokay fibre to a CF stimulus were often frequencies to which the fibre was relatively insensitive when they were presented alone. It is worth noting that of the two types of primary fibre described by Weiss et al. (1976) in the alligator lizard, only the low frequency, sharply tuned fibres demonstrate two-tone rate suppression (Holton and Weiss, 1977). The absence of the phenomenon in the higher-frequency fibres may be related to their broad tuning. It has been shown that under certain conditions that produce broad tuning in mammalian primary auditory neurons (eg. removal of perilymph from, or mechanical damage to, the cochlea), two-tone suppression can no longer be observed (Robertson, 1975).

The purpose of this thesis has been to extract from the recorded activity of Tokay primary auditory fibres information relevant to current problems in peripheral auditory physiology. Since the major advantage to recording from primary fibres is their proximity to the sense organ, an effort has been made to

[1] Terminology of Holton and Weiss (1977).

relate patterns of activity to inner ear morphology. The elaborate cochlear duct of the Tokay provides fascinating material for speculation of this nature. Such speculation is not idle in that it is often testable and at the same time suggestive of appropriate follow-up experiments. For instance, the postulated frequency distribution along the Tokay papilla (Paper 1) could be tested by recording from primary fibres as they emanate from the papilla. If this revealed that, as suggested, low frequencies are represented on the narrow basal end, it would be interesting to study the mechanical response of the Tokay's elongate basilar membrane using the Mössbauer technique. If, on the other hand, mapping of the papilla revealed that low frequencies are apical and high frequencies basal, then the correlation between hair cell orientation pattern and PSTH type in the monitor lizard (Manley, 1977) would be reversed in the Tokay.

The question of how tuning is achieved is the most basic problem in peripheral auditory physiology and hence the one that has attracted the most attention. The temperature effects on tuning curves described in this thesis and by others as yet make no clear contribution to an understanding of tuning. Since these studies are all very recent, and since the published reports are based on very few units, it can be hoped that the picture will be clarified by detailed studies on more species. From the consistency and specificity of the described temperature effect, it appears to be a creditable new source of information on tuning.

Summary

Spontaneous and response activity were recorded from single fibres in the auditory nerve of the Tokay gecko. Standard techniques of recording and sound stimulation were used. Spontaneous discharge rates were found to vary from 0 - 40 spikes/s. Interspike interval histograms from units with CFs greater than 0.5 kHz were comparable to those of other animals. Interval histograms from fibres with CFs less than 0.5 kHz were unusual in that they had oscillations at intervals approximating the reciprocal of the CF. For several reasons it was concluded that these oscillations are probably real and not the result of unintentional sound stimulation. A possible mechanism for the production of these oscillations at the hair cell level is discussed.

The range of characteristic frequencies (CFs) observed was from 0.15 to 5.2 kHz. Tuning curves had fairly simple V-shapes, with a tendency toward steeper high frequency slopes than low frequency slopes. The curves were on average quite sharp relative to those from other animals over the same frequency range.

Peristimulus time histograms (PSTHs) of response activity can be divided into three main types: filled, semipeaky, and peaky. These correspond to low, intermediate, and higher CFs. Similar PSTH classes are observed in the monitor lizard (Manley, 1977), in which the presence of the onset peak is correlated with hair cell bidirectionality. It is suggested that this correlation may also exist in the Tokay, in which case low frequencies would be represented basally and high frequencies apically on the papilla - the reverse of the mammalian frequency distribution.

Several on-off responses were observed. Fibres with CFs from 0.3 to 3.6 kHz responded to a broad-band Tokay call. Series of peaks in the PSTHs

of the responses reflect the pulsed nature of the call. It is suggested that onset peaks may play a rôle in sound localization.

The effect of temperature change on tuning curves was investigated. Temperature was measured orally or in the cochlear duct contralateral to the exposed auditory nerve. Temperature changes were effected with a heating pad, and were usually small (0.5 - 2°C). The range of temperatures covered by the study was from 19 to 31°C. Temperature change did not consistently affect either sharpness or overall sensitivity of the tuning curves, but did produce a consistent frequency shift. Tuning curves shifted toward higher frequencies with warming and lower frequencies with cooling. These temperature effects are discussed in relation to similar results obtained recently in other animals.

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