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FURTHER STUDIES ON THE PERIPHERAL AUDITORY SYSTEM OF 'CF–FM' BATS SPECIALIZED FOR FINE FREQUENCY ANALYSIS OF DOPPLER-SHIFTED ECHOES

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SUMMARY

In the mustache bat (*Pteronotus parnellii rubiginosus*), the cochlear microphonic (CM) recorded from the round window is sharply tuned at 61 kHz and shows a prominent transient response to a tone burst at about 61 kHz (i.e. its amplitude increases exponentially at the onset of the stimulus and decreases at its cessation). In terms of the time constant ($1{\cdot}1 \pm 0{\cdot}3$ ms) and the resonance frequency ($61{\cdot}1 \pm 0{\cdot}43$ kHz) of this transient response, the Q of this system, assumed to correspond to a second-order filter, is 204 ± 57. Peripheral neurones sensitive to 61 kHz have a very sharp excitatory area (or tuning curve). The Q of a tuning curve markedly increases with the rise in best frequency up to 61 kHz and decreases beyond 61 kHz. The Q value of a single neurone with best frequencies between 60·76 and 61·75 kHz is 210 ± 89. If the assumption that the CM is directly related to the mechanical motion of the basilar membrane is correct, the very sharp tuning curves of single neurones at about 61 kHz could be simply due to the mechanical tuning of the basilar membrane. Since this animal predominantly uses a 61 kHz sound for echolocation and peripheral auditory neurones show a low threshold and extremely sharp tuning at about 61 kHz, its peripheral auditory system is specialized for the reception and fine-frequency analysis of the principle component of orientation sounds and echoes. Sharply tuned neurones can code a frequency modulation as small as 0·01%, so that the wing beat of an insect would be easily coded by them. Unlike the CM, N_1 is tuned at 64 kHz. This difference in best frequency is simply due to the properties of a sharply tuned resonator and N_1, and not due to a mechanism comparable to lateral inhibition.

INTRODUCTION

For echolocation, *Rhinolophus ferrumequinum* (greater horseshoe bat) and *Pteronotus parnellii rubiginosus* (mustache bat) use an orientation signal consisting of long constant-frequency (CF) and short frequency-modulated (FM) components. The CF component is an ideal signal for echo-detection and velocity measurement. The frequency of the CF component is about 83 kHz in *R. ferrumequinum* and about

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61 kHz in the second harmonic in *P. parnellii rubiginosus* (Schnitzler, 1968, 1970) The precise frequency employed may differ among individual bats, in particular among those belonging to different colonies. The cochlear microphonic of each of these bats is sharply tuned to the species-specific CF component (Pollak, Henson & Novick, 1972; Schnitzler, Suga & Simmons, 1976; Suga, Simmons & Shimozawa, 1974; Suga, Simmons & Jen, 1975), and peripheral auditory neurones with a best frequency of either about 61 or 83 kHz show extraordinarily sharp tuning curves, which are not due to a mechanism comparable to lateral inhibition but due to a mechanical specialization of the cochlea (Suga *et al.* 1975; Suga, Neuweiler & Möller, 1976).

After Békésy's series of experiments (1960), it is well established that sound analysis in the mammalian ear is performed by travelling wave, rather than by resonance as proposed by Helmholtz (1863). Our data from *P. parnellii rubiginosus* probably represent a unique case of sound analysis by both travelling wave and resonance (local resonator). The first aim of the present paper is to refine the previous work on the tuning properties of cochlear microphonic (CM) and single auditory neurones in *P. parnellii rubiginosus* (Suga *et al.* 1975) and to compare neural tuning curves with mechanical ones. The second aim is to explore more extensively the difference in best frequency between the cochlear microphonic and summated neural responses (N_1) described in the previous paper (1975). The third aim is to consolidate the single unit data obtained from three different species of bats: *P. parnellii rubiginosus*, *R. ferrumequinum* and *Myotis lucifugus* (little brown hat) and to discuss the specialization of the peripheral auditory system for the analysis of acoustic signals with species-specific significance.

MATERIALS AND METHODS

Experimental subjects were 27 *Pteronotus parnellii rubiginosus* (previously named *Chilonycteris rubiginosa*) from Panama. The bats were lightly anaesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg of body weight). To record CM and N_1 responses, the auditory bulla was exposed by removal of the posterior part of the omohyoid muscle, and a tungsten wire electrode was placed at the rim of the round window through a tiny hole made in the auditory bulla. The hole was closed with a piece of sponge moistened with physiological saline solution. To record action potentials of peripheral auditory neurones, the lateral portion of the cerebellum was aspirated and the dorsal cochlear nucleus was exposed. A micropipette electrode filled with 3 M-KCl solution was inserted through the dorsal cochlear nucleus, in an attempt to place it in the modiolus. Recordings were actually made not only from the auditory nerve but also from the posterior ventral cochlear nucleus. The recording of all the above electrical activity was performed in a sound-proofed room, the inner walls of which were covered with foam rubber to reduce echoes. The room temperature was maintained at about 35 °C. A condenser loudspeaker was placed 60 cm in front of the bats. The amplitudes of stimuli were calibrated with a quarter-inch (6·3 mm) microphone (Brüel & Kjaer, model 4135) placed at the bat's ear and expressed in dB SPL (pressure ratio referred to 0·0002 dyne/cm^2 r.m.s.). The thresholds of cochlear microphonic and neural responses ar-

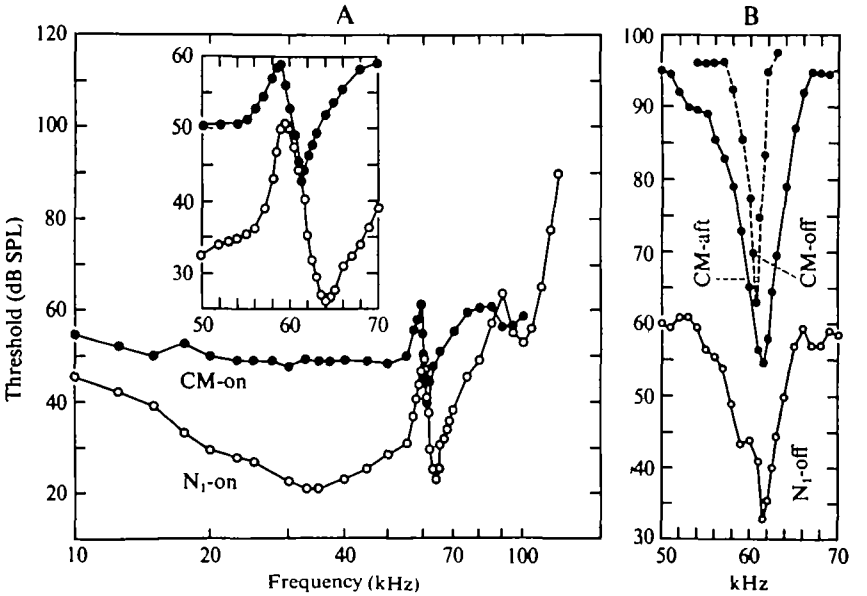


Fig. 1. (A) Threshold curves of CM-on and N_1-on based upon the data obtained from four bats. Since both curves are sharply tuned at either 61 or 64 kHz, the threshold curves between 50 and 70 kHz were measured with 8 bats and the averaged ones are plotted in the expanded coordinates as shown in the inset. (B) Threshold curves of CM-aft, CM-off, and N_1-off based upon the data obtained from 6, 11, and 4 bats, respectively. These threshold curves were measured with a tone burst of 4·0 ms duration and 0·5 ms rise–decay time.

respectively defined as the weakest stimulus which evokes either 15 μV peak-to-peak potential change or 0·1–0·2 impulse/stimulus on the average. The threshold at the best frequency is called the minimum threshold. The details of recording and stimulation instruments have been described in previous papers (Suga, 1968; Suga *et al.* 1975). An instrument which was not described in the previous papers is a Tektronix spectrum analyser (model 1L5), which was set at 100 Hz resolution.

RESULTS

Mechanical tuning curves in terms of cochlear microphonic (CM)

As shown by Pollak *et al.* (1972) in *P. parnellii parnellii*, and Suga *et al.* (1974, 1975) in *P. parnellii rubiginosus*, the threshold curve of the CM of these mustache bats is sharply tuned at about 61 kHz (Fig. 1A). At around 61 kHz, the threshold changes at a rate of −300 dB/octave and +160 dB/octave (the inset of Fig. 1A). Like the degree of sharpness of tuning of an electronic filter, the sharpness of a threshold curve may be expressed as a Q (quality factor), which is the best frequency divided by the bandwidth 3 dB above the minimum threshold. The Q for the CM threshold curve was 89 ± 52. The minimum threshold was 47 ± 9 dB SPL (see Table 1).

The CM usually appeared only during presentation of tonal stimuli, and the CM envelope was usually very similar to that of the stimuli unless the middle-ear muscles contracted (Fig. 2, A1 and A3). For a 61 kHz sound, however, the envelope

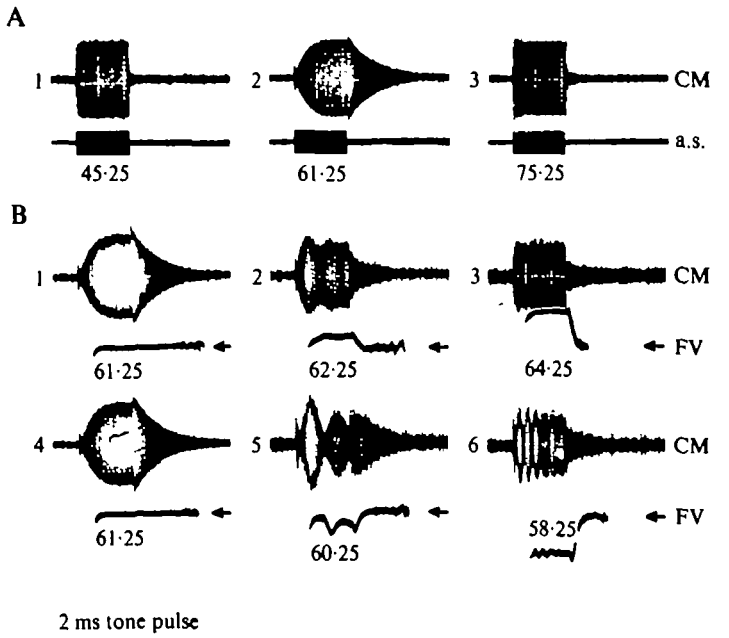


Fig. 2. CM responses recorded from the round window. (A) The envelope of the CM (upper trace) differs from that of an acoustic stimulus (a.s.) at a certain frequency (lower trace). The tone pulse is 45·25 kHz – 68 dB SPL, 61·25 kHz – 66 dB SPL, and 75·25 kHz – 82 dB SPL for 1, 2, and 3, respectively. Its duration and rise–decay time are 2·0 and 0·05 ms, respectively. (B) CMs (upper traces) and their frequencies (FV, lower traces) as analysed with the frequency-to-voltage converter. The tone pulses are 68 dB SPL and 2·0 ms long. Their frequencies are shown in each record in kHz. The arrows indicate 61·25 kHz. Note the prominent transients in the CM responses.

of the CM differed from that of the stimulus (Fig. 2, A2). When the stimulus was small in amplitude and was turned on and off with a 0·2 ms rise–decay time, the amplitude of the CM slowly increased to a certain level and reached a plateau at the beginning, then slowly decreased to zero after the cessation of the stimulus. Hereafter, the CM after the cessation of the stimulus is called the *CM-aft*, and the CM at the beginning and during the stimulus is called *CM-on*. At certain stimulus frequencies and levels, the CM-aft showed a prominent peak, then an exponential decay in amplitude. The CM-aft which shows such an envelope peak is hereafter called *CM-off*. The CM evoked by a 61 kHz sound itself clearly indicates that the mechanical system has a sharp tuning or resonance. It has been found that the transient portions of the CM are due to the mechanical properties of the inner ear (Suga *et al.* 1975). The threshold curves of the CM-on, CM-aft, and CM-off are shown in Fig. 1.

In general, it has been accepted that the CM is the receptor potential and that it is directly related to the displacement of the basilar membrane (Békésy, 1960; Dallos, Cheatham & Ferraro, 1974; Wilson, 1974; Yates & Johnstone, 1976). In *P. parnellii rubiginosus*, the CM evoked by a 61 kHz sound shows a damped oscillation, i.e. the CM-aft. Assuming that the CM-aft is directly related to the damped oscillation of the basilar membrane, comparable to a second order LCR resonator, the Q of the resonator may be calculated with $Q = \pi\lambda f_r$, where f_r is the resonance frequency an

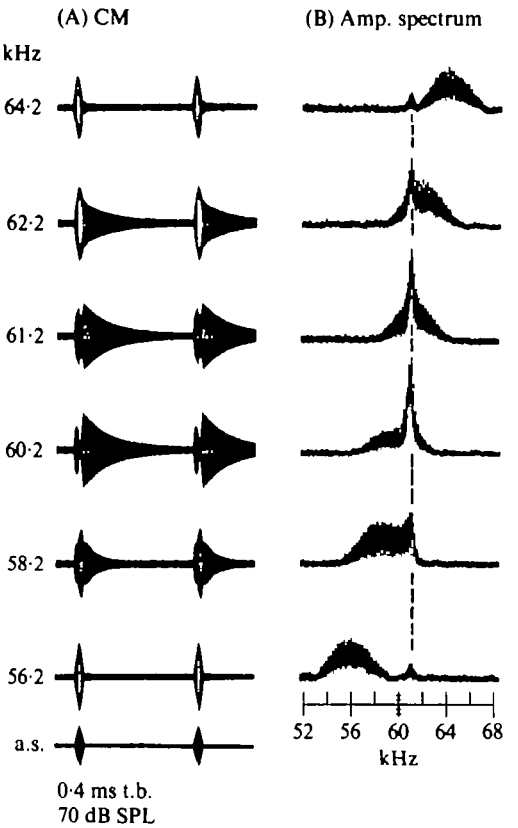


Fig. 3. Analysis of CM responses recorded from the round window with the spectrum analyzer. (A) CM responses to 0·4 ms tone pulses delivered at a rate of 200/s and at a stimulus level of 70 dB SPL. The rise–decay time of the tone pulses was 0·2 ms. The bottom trace marked by 'a.s.' shows the tone pulses. The frequency of the tone pulse is indicated by figures on the left of each CM trace. Note the long decay in CM responses after the stimuli. (B) The power spectra of the CMs shown in A. Note the peak at about 61·0 kHz (dashed line) regardless of stimulus frequency.

λ is the time constant of the damped oscillation. f_r can be obtained by the analysis of the CM-aft with a frequency-to-voltage converter or a spectrum analyser. λ can be obtained from the envelope of the damped oscillation after confirming that the CM-aft decays exponentially or that the CM-on increases exponentially at the onset of the response. If one assumes that the 61 kHz tuned area acts as a higher order resonator than the above, its Q would be higher than the Q calculated with the above formula. Since we do not know what order of resonator the 61 kHz tuned area actually corresponds to, the resonator giving the lowest Q is assumed.

Fig. 2B represents examples of the analysis of cochlear microphonics with a frequency-to-voltage converter. When a 61·25 kHz tone burst was delivered, the CM-on was, of course, at 61·25 kHz, and the CM-aft was about 61·25 kHz. This indicates that 61·25 kHz was the resonance frequency of the sharply tuned element. For 62·25 kHz, the CM-on was 62·25 kHz, but the CM-aft was 61·25 kHz. For 58·25 kHz, the CM-on was 58·25 kHz, but the CM-aft was 61·25 kHz. Whenever

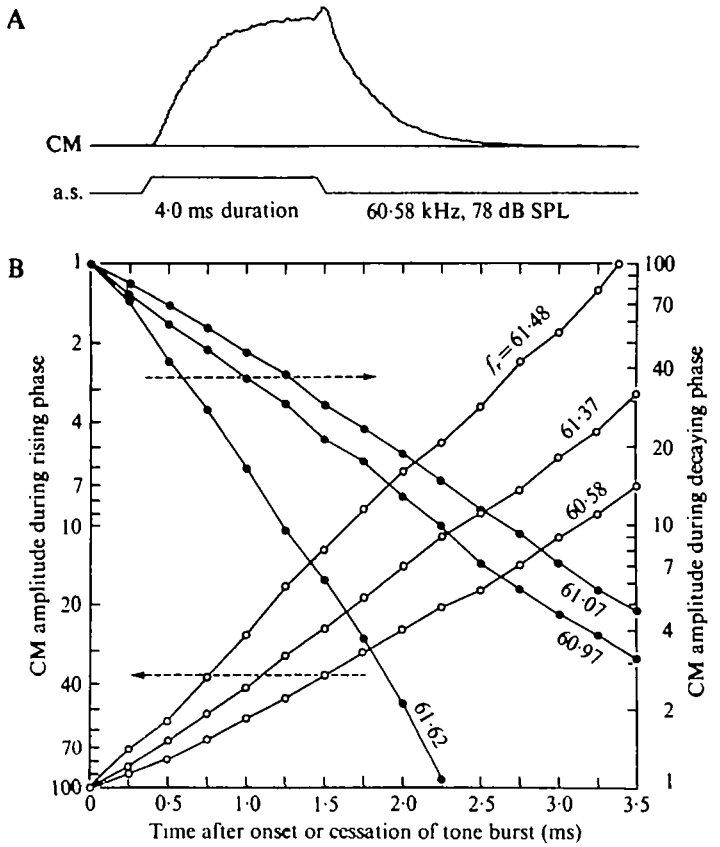


Fig. 4. (A) The envelope of a CM response to a tone pulse of 60·58 kHz and 78 dB SPL. The duration and rise–decay time of the tone pulse were 4·0 and 0·2 ms, respectively (lower trace). Sixty-four CM responses were full-wave rectified and were averaged by a computer (upper trace). (B) The semi-logarithmic plot of CM amplitudes against time after either the onset (open circles) or the cessation (filled circles) of a tone pulse for six cochleas. The resonance frequency (f_r) of each of the six cochleas studied is shown by the figures in the graph. The frequency of the tone pulse was the same as f_r, and its rise–decay time was 0·05 ms. The CM amplitude at the time of λ is indicated by the dashed lines.

CM-aft was evoked, its frequency was at the resonance frequency, 61·25 kHz, in this cochlea.

At the onset of the stimulus which caused CM-aft, the CM-on also showed a transient response comparable to the CM-aft. In this case, the CM due to the stimulus frequency and that due to the resonator activated by the side band generated by the stimulus onset were superimposed upon each other and produced beats. For instance, the CM-on for 60·25 kHz showed 1·0 kHz beats which corresponded to the difference between the stimulus and resonance frequencies (Fig. 2, B5). The CM-on for 58·25 kHz showed 3·0 kHz beats which were due to the difference between the stimulus and resonance frequencies (Fig. 2, B6). These data clearly indicate that the 61 kHz tuned area in the cochlea moved at its resonance frequency independently of the areas tuned at neighbouring frequencies.

The CM showed a prominent non-linearity of its input–output function at frequencies just below its best frequency (e.g. Fig. 6). The side band generated b

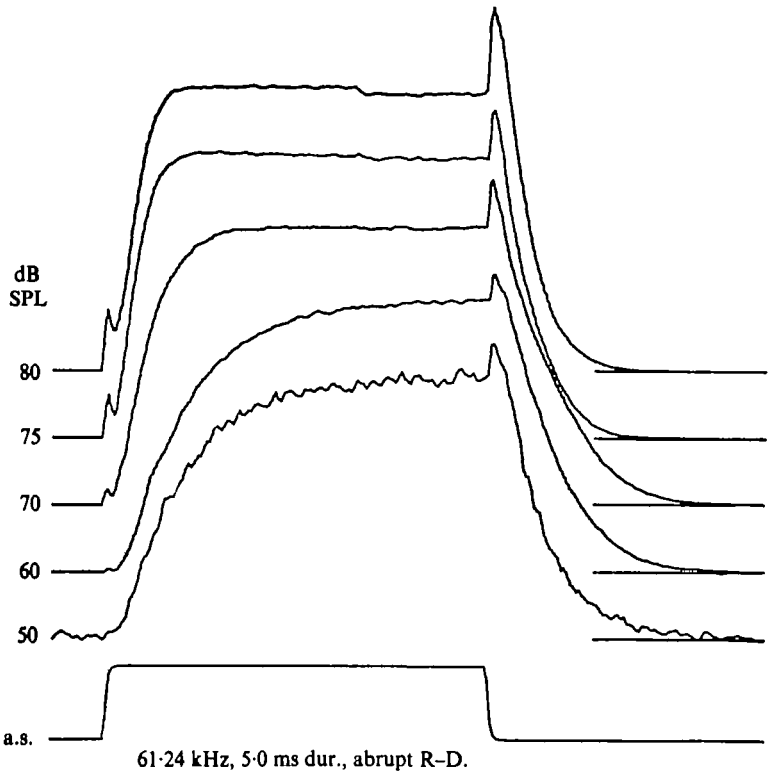


Fig. 5. Non-linearity in the rise and decay phases of CM responses. A 61·25 kHz tone burst with a 5·0 ms duration and a 0·05 ms rise–decay time was delivered at a rate of 2/s. Its amplitude was varied from 50 to 80 dB SPL. Sixty-four CM responses to an identical stimulus were full-wave rectified and averaged by a computer to produce each of the five records shown.

the stimulus onset was larger at frequencies where the non-linearity was more prominent (e.g. Fig. 3). Accordingly, the CM transient and beats were much more prominent at 60·25 and 58·25 kHz than at 61·25 and 64·25 kHz as shown in Fig. 2B.

When CM-aft was small, its frequency was not adequately expressed by the frequency-to-voltage converter. Therefore, the CM was also analysed with a spectrum analyser (Fig. 3). In this case, tone pulses with a 0·4 ms duration and a 0·2 ms rise–decay time were delivered at a rate of 200/s to increase the CM evoked by the side bands relative to that evoked by the carrier. When the frequency of the tone pulse was near 61 kHz, the CM-aft was much larger and longer than the CM-on (e.g. 60·2 and 61·2 kHz in Fig. 3A). CM-aft, however, was smaller for tone pulses of frequencies away from 61·1 kHz. At 56·2 and 64·2 kHz, it nearly disappeared. The amplitude spectrum of the CM always showed a peak of 61·1 kHz in this cochlea (Fig. 3B). In the cochlea from which the data shown in Fig. 3A were obtained, its f_r was also 61·1 kHz when it was measured with the frequency-to-voltage converter. It was noted that the resonance frequency of the CM-aft was slightly different among cochleas of different individuals within a range from 60·3 to 61·9 kHz, and that the frequency range of the tone pulses which evoked the CM-aft was wider on the low-frequency side of the f_r than the high-frequency side (Figs. 1B and 3B).

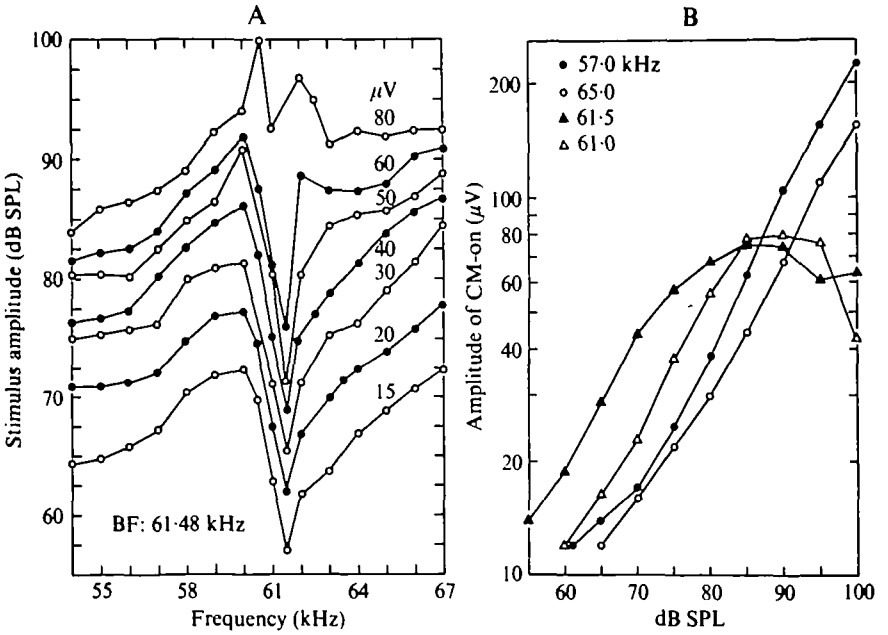


Fig. 6. The amplitude of CM-on as a function of stimulus frequency and level. In (A), the stimulus amplitude which evoked a certain CM amplitude (e.g. 80 μV) is plotted as a function of stimulus frequency. The figure above each curve represents the CM amplitude in microvolts. The best frequency was 61·48 kHz. In (B), the CM amplitude is plotted against stimulus amplitude. Each curve is obtained for a different stimulus frequency which is shown in kilohertz following the symbol used for the curve. The CM amplitude is approximately linearly related to the stimulus level, except at about 61 kHz.

The threshold curve of the CM-aft is shown in Fig. 1B. Its high-frequency slope is steeper than its low-frequency slope. These may be due to the travelling wave envelope limiting the propagation of the higher frequency energy to more basal sections of the basilar membrane.

Since the CM showed some non-linearity for 60–63 kHz stimuli higher than 70–80 dB SPL (Fig. 6), stimuli weaker than that were mainly delivered to measure the time constant of the damped oscillation. Then the CMs evoked by these short tone bursts were often small in amplitude. It was thus necessary first to full-wave rectify the CMs and then average with the computer. Figs. 4A and 5 show the CMs after full-wave rectification and averaging. Semi-logarithmic plots of the amplitudes of CMs as a function of time (after either the onset or cessation of the stimulus) indicated that these amplitudes increased or decreased exponentially (Fig. 4B). Thus, λ for each cochlea could be easily obtained from such a plot. The slope of the curve, however, was quite different depending on stimulus levels (Fig. 5). This non-linear property of the transient response was difficult to analyse further, so that we simply used the longest time constants obtained from individual cochleas to determine the extent to which very sharp neural tuning curves at 61 kHz depended upon a mechanical tuning of the structure. The mean and standard deviation of the longest time constants obtained from 8 cochleas were $1{\cdot}15 \pm 0{\cdot}31$ ms for CM-on and $0{\cdot}99 \pm 0{\cdot}28$ ms for CM-aft. Since there was no significant difference in time constant between the CM-on and CM-aft, the data for both were pooled, yieldi

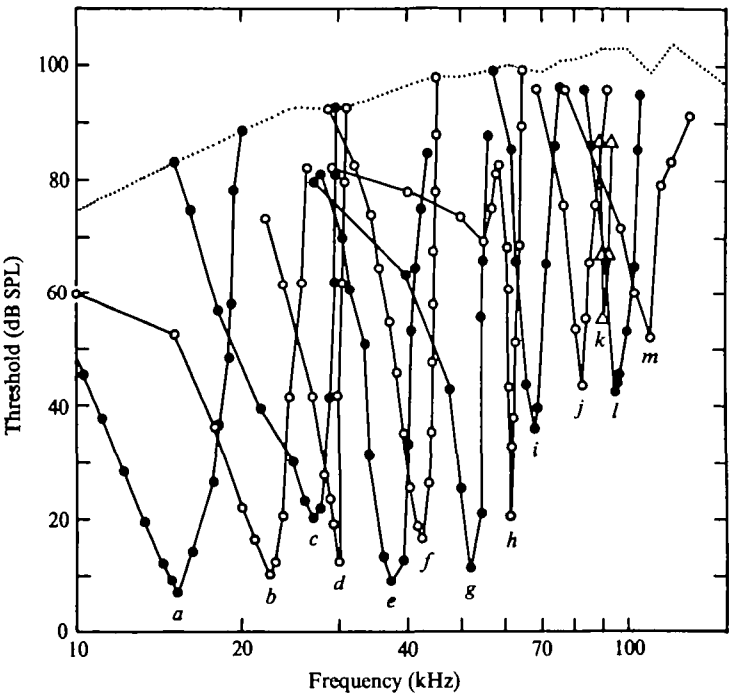


Fig. 7. Threshold curves of 13 peripheral neurones. Note the sharpness of the threshold curve tuned at either 30 or 61 or 92 kHz.

a time constant of $1{\cdot}07 \pm 0{\cdot}30$ ms. The mean and standard deviation of the resonance frequencies of these 8 cochleas were $61{\cdot}10 \pm 0{\cdot}43$ kHz, which yielded a Q value of 204 ± 57. The stimulus levels used to obtain these data were 65 ± 12 dB SPL.

As shown in Fig. 5, the CM shows a prominent non-linearity at the beginning and the end of its response to a tone burst. A non-linearity was also observed at the plateau amplitude (i.e. steady state) of the response (Fig. 6). Since the animals were anaesthetized and the tone bursts used in these experiments were brief (2–5 ms), the non-linearity of the CM was not due to the acoustic reflex of the middle-ear muscles. Fig. 6A and B show the CM plateau amplitude as a function of the frequency and amplitude of a tone burst. At below 60 kHz and above 63 kHz, the CM amplitude increases rather linearly with the stimulus level over a wide range. However, it showed a prominent non-linearity between 60 and 63 kHz when the stimulus level was higher than 70 dB SPL.

Neural tuning curves

Physiological properties of 370 single neurones were studied in the auditory nerve and ventral cochlear nucleus. Eight per cent of them showed excitatory and inhibitory responses to single tonal stimuli. Ninety-two per cent showed only excitatory responses; these tuning (or threshold) curves are described below.

Fig. 7 shows tuning curves of 13 single neurones. The area above each curve represents an excitatory (or excitatory response) area. As described in a previous paper (Suga *et al.* 1975), the excitatory areas of neurones tuned at about 30 or 61

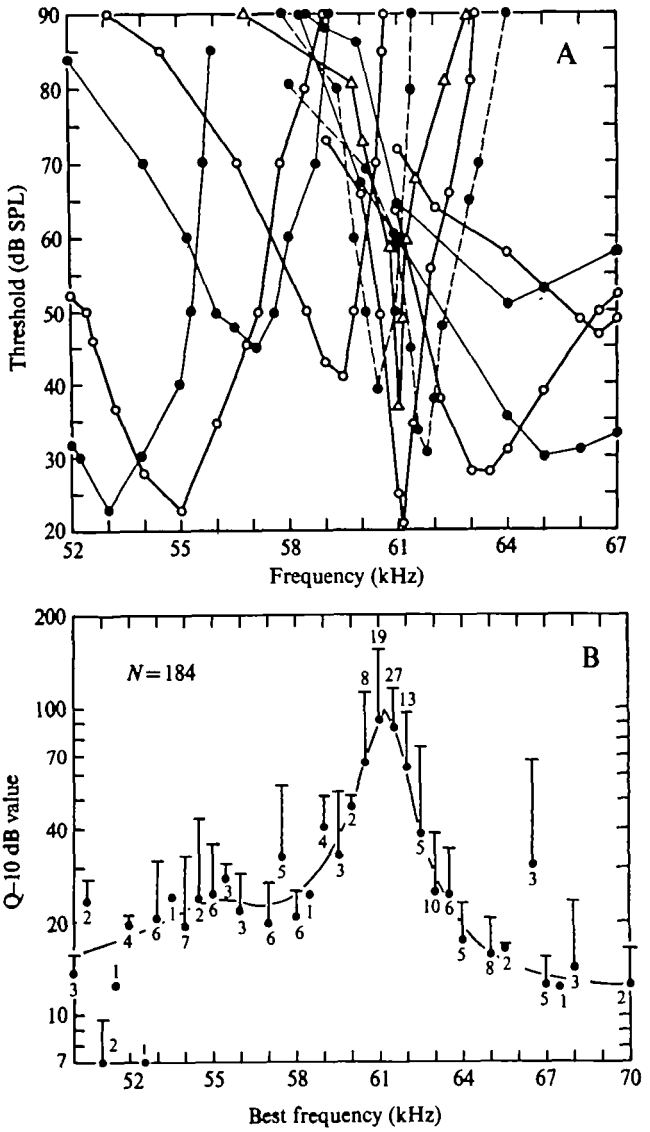
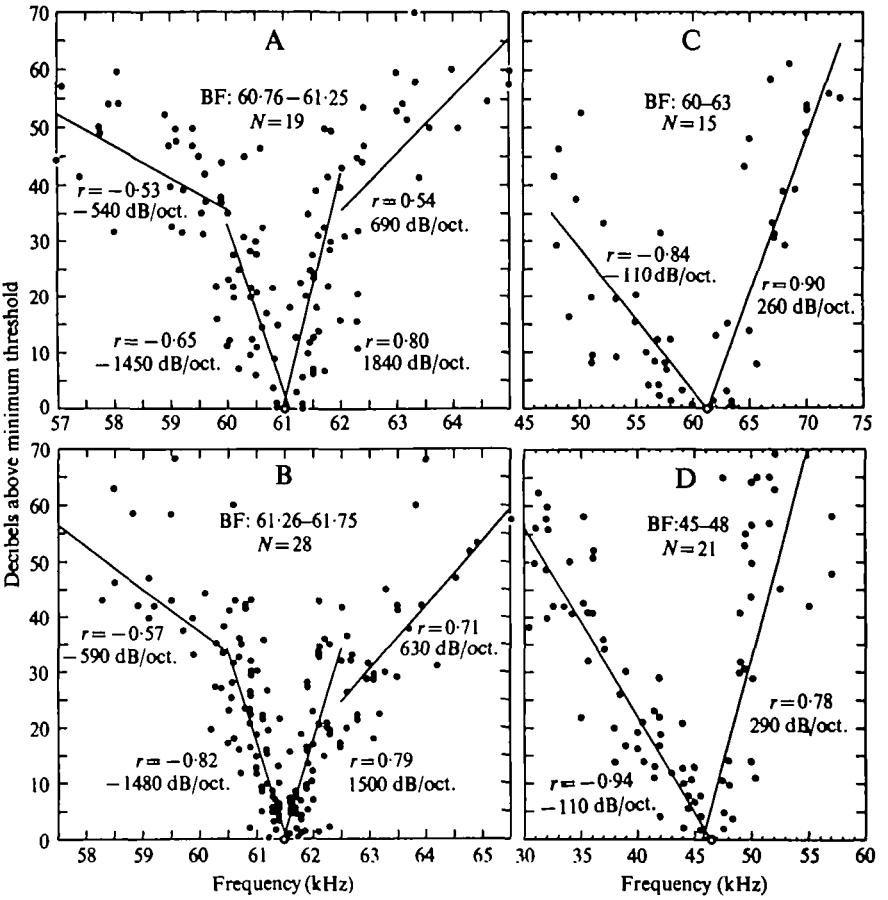


Fig. 8. (A) Threshold curves of 12 peripheral neurones indicating a drastic change in sharpness with best frequencies. (B) Distribution of Q-10 dB values of 184 single neurones against their best frequencies. The data points are grouped with a 0·5 kHz interval, and the means and one standard deviation were as indicated with the filled circles and vertical bars. The number of data points (i.e. neurones) in each group is indicated with a figure. Note the very large Q-10 dB values at 61·0 and 61·5 kHz.

or 92 kHz (*d*, *h* and *k*) were narrower than those tuned at other frequencies. In particular, the areas tuned at about 61 kHz were extremely narrow. Since the shape of the tuning curves greatly changes as a function of the best frequency at around 61 kHz, twelve tuning curves are plotted on an expanded frequency axis in Fig. 8A. The tuning curves are very sharp for the best frequency at about 61 kHz, but they become broader for the best frequencies either higher or lower than 61 kHz.

Tuning curves may be described in terms of their sharpness and slopes. Th



(A) MT = 46 ± 17 dB SPL Q–10 dB = 83 ± 64 (C) MT = 64 ± 13 dB SPL Q–10 dB = 9·0 ± 4·1

(B) 45 ± 20 84 ± 34 (D) 42 ± 16 9·3 ± 5·2

Fig. 9. Average slopes of threshold curves obtained from *Pteronotus parnellii rubiginosus* (A and B) and *Myotis lucifugus* (C and D). The data points in (A) were obtained from 19 peripheral neurones with best frequencies between 60·76 and 61·25 kHz, and those in (B), from 28 neurones with best frequencies between 61·26 and 61·75 kHz. The data points in (C) were obtained from 15 peripheral neurones tuned at between 60·0 and 63·0 kHz, and those in (D), from 21 single neurones tuned at between 45·0 and 48·0 kHz. The data are normalized to the mean best frequency of each group. The mean best frequency is expressed by the open circle, which represents the data points which are the same as the number of neurones in each group. The slopes of the regression lines and the regression coefficients are given in each graph. The minimum thresholds and Q-10 dB values of the neurones are given with the mean and standard deviation for each group on the bottom of the figure. Note a difference in abscissa between (A–B) and (C–D).

sharpness of a tuning curve for a single neurone has been expressed by a Q-10 dB value, which is the best frequency divided by the bandwidth at 10 dB above the minimum threshold (e.g. Kiang, 1965). Fig. 8B shows how the Q-10 dB value changes as the function of the best frequency between 49·9 and 70·0 kHz. The Q-10 dB value is greatest from 60·8 to 61·8 kHz and is 84 in this region.

The slopes of these sharp tuning curves range between 1000 and 4000 dB/octave.

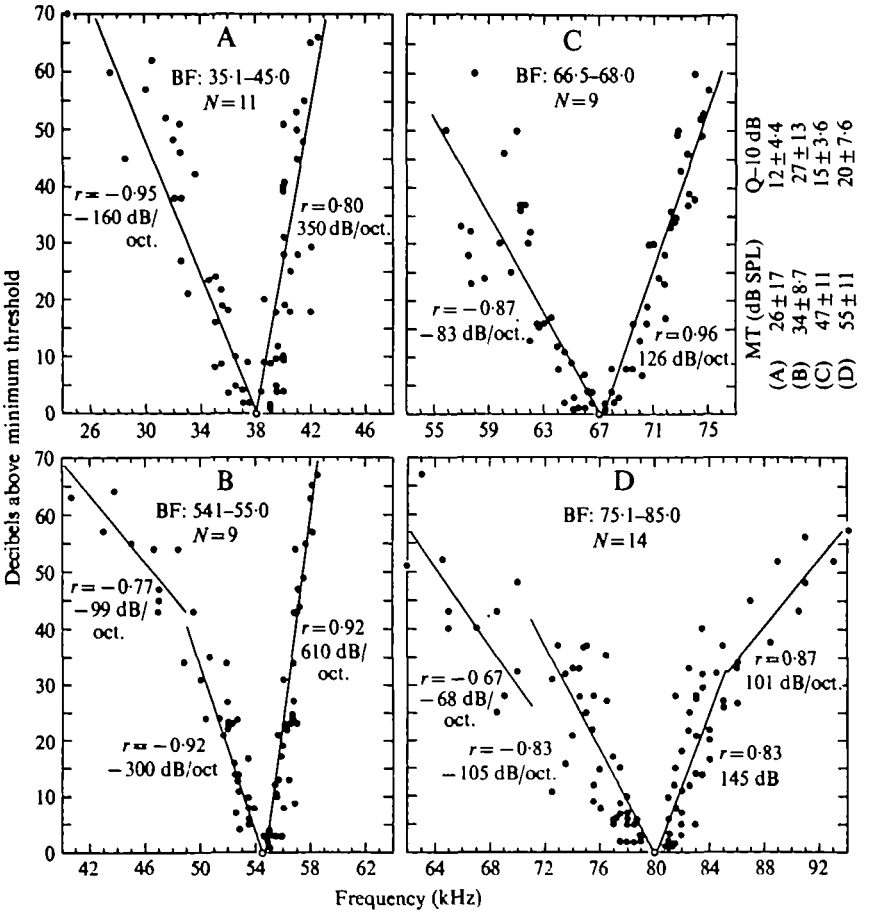


Fig. 10. Average slopes of threshold curves obtained from *Pteronotus parnellii rubiginosus*. The number of neurones and their best frequencies, from which the data points were obtained, are indicated in each graph. As in Fig. 9, the data are normalized to the mean best frequency of each group. See the legend of Fig. 9.

In order to obtain their average slopes, data points obtained from several neurones with nearly the same best frequencies were plotted in a single graph and regression lines were calculated. Fig. 9A represents the data obtained from 19 single neurones tuned between 60·76 and 61·25 kHz. The low-frequency and high-frequency slopes are respectively −1450 and +1840 dB/octave between the minimum threshold and 30 dB above it. Beyond 30 dB above the minimum threshold, these slopes are respectively −540 and +690 dB/octave. Slopes comparable to those in Fig. 9A are also obtained from neurones tuned to 61·26–61·75 kHz sounds (Fig. 9B). Since the slopes of these tuning curves were so sharp and the best frequencies of the neurones were slightly different from one another, it is expected that *P. parnellii rubiginosus* is able to discriminate a very minor difference in frequency within this narrow range. As shown later, the sharply tuned single neurones can code a frequency shift as small as ±0·01 %.

Unlike *P. parnellii rubiginosus*, *Myotis lucifugus* (little brown bat) uses frequency

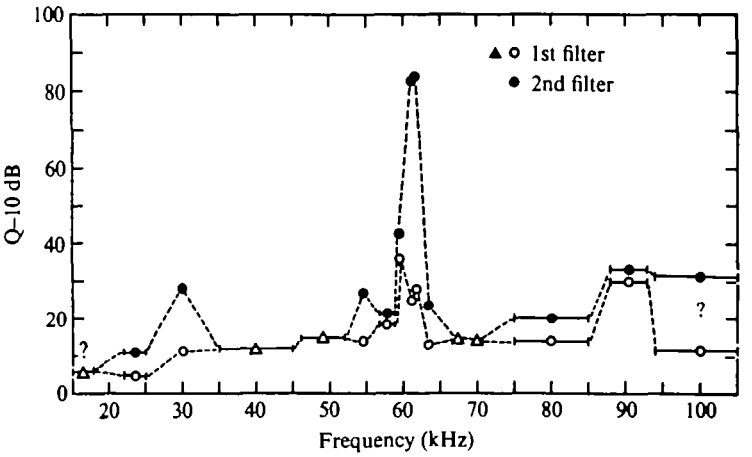


Fig. 11. Relationship between best frequencies and Q-10 dB values of the low and high intensity regions of tuning curves from *P. Parnellii rubiginosus*. As shown in Figs. 9 and 10, neurones with best frequencies within a certain range are pooled and the average slopes of their timing curves are calculated. Then a mean Q-10 dB value is obtained of the first (open circle) and second (filled circle) filters (i.e. for the high and low intensity regions of their mean tuning curve). When each skirt of the tuning curve consists of a single slope, the Q-10 dB value is expressed by an open triangle (see text). The range of the best frequencies of the neurones in each pool is given by a horizontal bar. When there is no horizontal bar across a circle or triangle, the range of best frequencies is nearly the same as the diameter of the circle or the length of the edge of the triangle. The question mark is placed for the Q-10 dB value(s) at the best frequency of both 16·5 and 100 kHz, because the sample number of neurones with one of these best frequencies is small and either the low or the high frequency skirt of their tuning curves is not adequately measured to obtain the average slope.

modulated orientation sounds, in which the frequency sweeps downward about one octave. The comparison of the tuning curves of *M. lucifugus* with those of *P. parnellii rubiginosus* highlights the specialization of the peripheral auditory system of the latter. Fig. 9C and D represent the data obtained from peripheral auditory neurones of *M. lucifugus* by Suga (1973). In C, the data obtained from 15 single neurones tuned between 60 and 63 kHz are plotted. The low-frequency slope is −110 dB/octave and the high-frequency slope is +260 dB/octave. Neurones tuned to 45–48 kHz sounds also showed slopes comparable to these (D). There is thus a remarkable difference between the slopes of the tuning curves of *P. parnellii rubiginosus* and *M. lucifugus*. Note that these averaged tuning curves are not exactly inverted triangles, because the tips of the tuning curves are rounded. Thus, their Qs and Q-10 dBs are much larger than those calculated for the simple triangular tuning curves.

In *P. parnellii rubiginosus*, the slopes beyond 30 dB above the minimum threshold are different from those below it. Such a difference in slope is not evident in *M. lucifugus*. Why do the tuning curves with the best frequency at about 61 kHz show the 'double slopes' on each side of the tuning curve? Do the tuning curves with other best frequencies also show such double slopes? Fig. 10 shows the tuning curves of single neurones tuned to different frequencies. The neurones with the best frequency between 35·1 and 45·0 kHz had very similar tuning curves. The slope of each side of these tuning curves could be fitted to a single regression line (A). This was also true for the tuning curves with the best frequency between 66·5 and 68·0 kHz (C). The tuning curves with the best frequency between either 54·1 and

Table 1. *Tuning of CM and single peripheral neurones*

Type of resp.	BF or f_r (kHz)	N	Formula	Q or Q-10 dB	MT (dB SPL)
CM	61·10 ± 0·67	19	BF/3 dB BW	89 ± 52	47 ± 9
CM	61·10 ± 0·43	16	$nf_r\lambda$	204 ± 57	65 ± 12
Neurones	60·76 ± 61·75	27	BW/3 dB BW	210 ± 89	49 ± 19
Neurones	60·76 ± 61·75	47	BF/10 dB BW	85 ± 48	45 ± 19

MT, minimum threshold.

55·0 kHz or 75·1 and 85·0 kHz showed double slopes on one or both sides of the curves (B and D). The Q-10 dB values of these four tuning curves ranged between 12 and 27 (see Fig. 10). The double slopes indicate that there are two filters. The Q-10 dB values calculated above are only for the low intensity region of the tuning curve, which is hereafter defined as that produced by the second filter. To determine the sharpness of the filter, called hereafter the first filter, responsible for the high intensity region of the tuning curve, the upper regression line(s) is extrapolated and a Q-10 dB value is calculated. When a tuning curve shows no double slopes, it is assumed that only the first filter determines the neural tuning curve. Fig. 11 shows a relationship between the Q-10 dB values of the first and second filters and their best frequencies. The Q-10 dBs of the first filters range between 5 and 14, but are nearly twice as large at best frequencies of about 60 and 91 kHz. The Q-10 dBs of the second filters are much larger than those of the first filters at about 30 and in particular, 61 kHz, but are nearly the same at other frequencies. Here the terms first and second filters are simply used for convenience in description. The possible origin of each of the two filters will be discussed later.

Mechanical vs. neural tuning curves

It is important to determine whether the very sharp tuning of single neurones is due to a mechanical feature of the cochlea and/or some sharpening mechanism comparable to lateral inhibition. As in the previous experiments (Suga *et al.* 1975), sharply tuned neurones showed no inhibition of background activity by single tones just outside of their sharp tuning curves, so that the sharp tuning did not result from inhibition, but is probably a result of mechanical specialization. When two tone bursts were delivered simultaneously, 'two-tone suppression' was observed as in cats and monkeys (Suga *et al.* 1975). However, no suppression was evoked by the successive delivery of the two tone bursts.

To compare the neural tuning curve with the mechanical tuning curve in terms of the damped oscillation of CM at 61 kHz, Q-10 dB and Q values of single neurones tuned to sounds between 60·76 and 61·75 kHz were calculated. The mean and standard deviation in Q-10 dB values of the 47 single neurones were 85 ± 48. Their minimum thresholds were 45 ± 19 dB SPL. In 27 neurones out of the 47, the tuning curves were good enough for calculation of the Q value. Their Q values were 210 ± 89. The mean value is nearly the same as the Q value in terms of the damped oscillation of the CM (see Table 1). For a critical comparison, however, the standard deviation is much too large. If the assumption that the CM is directly related to the mechanical motion of the basilar membrane is correct, one may conclude that the mechanical tuning alone is adequate to explain the very sharp neural tuning curve at 61 kHz.

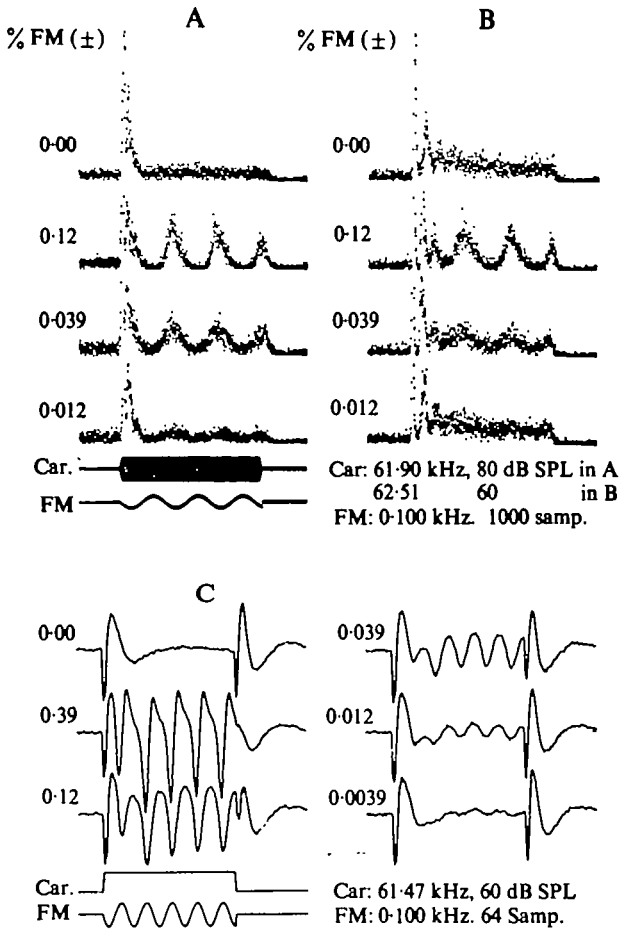


Fig. 12. Responses to frequency-modulated tone bursts which simulate echoes from beating insect wings. (A) and (B) Post-stimulus-time (PST) histograms of responses of two neurones with the best frequency of either 61·80 (A) or 61·74 kHz (B). The carrier sound was 61·90 kHz, 80 dB SPL in (A) and 62·51 kHz, 60 dB SPL in (B). The modulation frequency was 0·10 kHz. Per cent frequency modulation, which is the peak frequency deviation from the carrier expressed by percent, is shown by the figure to the left of each histogram. The sample number was 1000. The bin width was 50 μs. (C) Summated auditory nerve responses (N_1s) to frequency-modulated tone bursts. The carrier sound was 61·47 kHz, 60 dB SPL. The modulation frequency was 0·10 kHz. The sample number was 64. The bin width was 100 μs.

Coding of frequency modulated signals of biological significance

In *P. parnellii rubiginosus*, the tuning curves of the neurones which have a best frequency of about 61 kHz are extremely sharp and their best frequencies are slightly different from one another. As described in the Discussion, such an auditory system has great advantages in echolocation. One of the advantages is detection of frequency modulation (FM) due to the wing beat of a flying insect (Johnson, Henson & Goldman, 1974). In reflecting a CF orientation sound, beating wings will produce an echo which may contain modulations in amplitude and frequency. In our limited measurements with 30 ms tone bursts, impulse-count functions of neurones with 61–62 kHz best frequencies showed an average slope of about 11 impulses/s/dB and a plateau

at 75–85 dB SPL. A comparable slope has also been obtained from cats (e.g. Nomoto, Suga & Katsuki, 1964). The ability of the sharply tuned neurones of *P. parnellii rubiginosus* to code amplitude modulation is thus probably not superior to that of neurones in cats. However, the coding of FM is especially superior in the 61 kHz tuned neurones of *P. parnellii rubiginosus*, because of their unusually sharp tuning curves.

Fig. 12 shows post-stimulus-time (PST) histograms of responses of two single neurones to FM tone bursts. In A, the carrier is 61·90 kHz and 80 dB SPL. When the carrier was not frequency modulated, a prominent phasic on-response followed by a weak tonic on-response was evoked. When the carrier was sinusoidally frequency-modulated at a rate of 0·1 kHz, however, the discharge rate during the stimulus clearly and synchronously varied with the FM (see ±0·12% and ±0·039% modulation; here a per cent modulation is the peak frequency deviation from the carrier expressed by per cent). At a ±0·012% modulation, the synchronous discharges with the FM were still recognizable. To study the coding of FM by a group of primary neurones, the same experiment was conducted recording the summated auditory nerve response called N_1 (Fig. 12C). The N_1 synchronized with an FM was very clear even at a ±0·012% FM, but it was very small at a ±0·0039% FM. This very high sensitivity to FM is apparently related to the sharp tuning curves of peripheral auditory neurones.

Difference in best frequency between CM-on and N_1-on

As pointed out in the previous paper (Suga *et al.* 1975), on the average the CM-on is tuned at 61·1 kHz, while the N_1-on is tuned at 64·0 kHz (Fig. 1). This large difference in best frequency may result from the slow rise in the amplitude of the CM at the resonance frequency of the sharply tuned element. The N_1-on amplitude depends upon the number of neurones which synchronously fire action potentials at the onset of a stimulus. The synchronization obviously becomes poor with a slow stimulus rise time. The mechanical resonance effectively slows the rise time of the CM response and has the same effect on the synchronization that a slower stimulus rise time would have.

To demonstrate that the N_1-on tuning curve is distorted by the slow rise of the CM amplitude, the rise time of the CM envelope was kept constant and the N_1-on tuning curve was measured. The time constant of the damped oscillation is not yet known for each location along the basilar membrane. However, single unit data indicate that the Q-10 dB value changes with the best frequency (Fig. 8B) and that the sharpness of the neural tuning curve at 61 kHz is comparable to that of the mechanical tuning curve in terms of the damped oscillation of the CM (Table 1). If one assumes that the neural tuning curves are entirely based upon the mechanical tuning curves regardless of their best frequencies and that the system is comparable to a second order resonator, the time constants of the damped oscillations of different places along the basilar membrane can be calculated from the Q-10 dB values. The Q value was found to be about 2·6 times larger than the Q-10 dB value by calculating both the values from the tuning curves which had sufficient data points for such a calculation.

The time constant of the damped oscillation of the basilar membrane (λ_B) wa

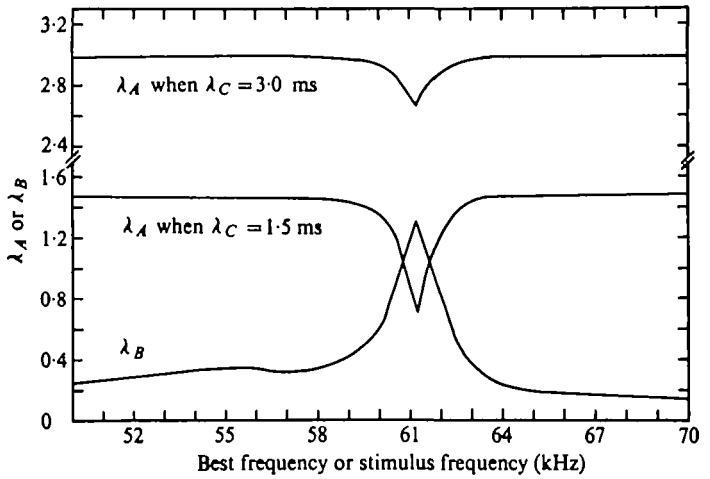


Fig. 13. The time constants of the damped oscillation of the basilar membrane (λ_B) and the rising phase of a tone burst (λ_A) as a function of either the best frequency (for λ_B) or stimulus frequency (for λ_A). λ_B was obtained with the formula $2{\cdot}6 \times$ Q-10 dB/πf_r from Fig. 8B. λ_A was calculated with $\sqrt{(\lambda_C^2 - \lambda_B^2)}$, in which λ_C was the time constant of the rising phase of the cochlear microphonic and was either 1·5 or 3·0 ms.

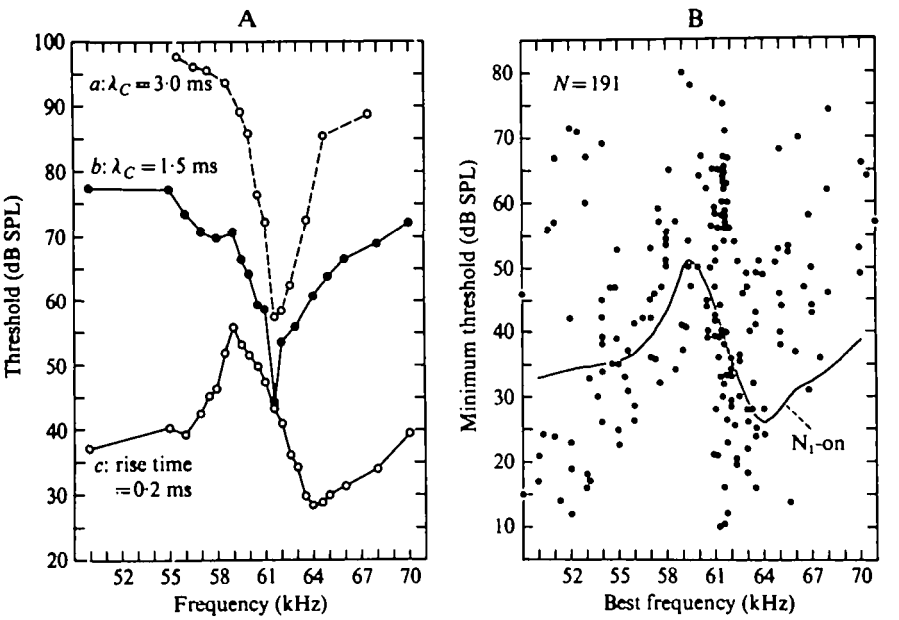


Fig. 14. (A) Threshold curves of N_1-on measured with tone bursts having different rise times. λ_A was varied to keep λ_C at either 3·0 (curve *a*) or 1·5 ms (curve *b*) as a function of stimulus frequency. The resulting N_1-on best frequency was 61·5 kHz. Curve *c* with the best frequency at 64·0 kHz was obtained with tone bursts with a 0·2 ms rise time regardless of their frequencies. Curves *a*, *b*, and *c* are the averages of data obtained from 3, 6, and 6 bats, respectively. (B) The distribution of minimum thresholds of 191 peripheral neurones as a function of their best frequencies. For comparison, the N_1-on threshold curve is placed, which is the average of the data obtained from 12 bats.

calculated as $2{\cdot}6 \times$ Q-10 dB/πf, as a function of the best frequency (f) (Fig. 13). To keep the time constant of the CM or the input to primary auditory neurones (λ_C) constant regardless of stimulus frequencies, the time constant of the rising phase of a tone burst (λ_A) should be varied with frequency. λ_A is given by $\sqrt{(\lambda_C^2 - \lambda_B^2)}$ in the first order approximation. In Fig. 13, λ_A necessary to keep λ_C either at 1·5 or 3·0 ms is plotted as a function of the stimulus frequency. It is evident that the change in λ_A with stimulus frequency becomes smaller for longer λ_C.

In Fig. 14A, curve *c* is the N_1-on threshold curve measured with a tone burst having a constant rise time, 0·2 ms. Its best frequency is 64 kHz, as already described. Curves *a* and *b* respectively are the N_1-on threshold curve measured with tone bursts in which λ_A was adjusted to keep λ_C at either 3·0 or 1·5 ms. The best frequencies of these curves are 61·5 kHz, as expected. We may thus conclude that the best frequency of the auditory nervous system is the same as the average resonance frequency of the CM at 61·1 kHz.

Additional support for this conclusion comes from our single unit data. In Fig. 14B, the minimum thresholds of 191 single neurones are plotted against their best frequencies. The thresholds of the single neurones are high at 59–60 kHz and above 64 kHz, but they are low at 61–62 kHz. The distribution of the minimum thresholds between 59 and 64 kHz thus fits to the N_1-on tuning curve obtained with the time constant adjustment (Fig. 14A, *a* and *b*), rather than the N_1-on tuning curve measured with a tone burst with a constant rise time of 0·2 ms (Fig. 14A, *c*). The N_1-on best frequency at 64 kHz instead of 61·1 kHz obviously results from the unsynchronized activation of the peripheral auditory neurones tuned at about 61 kHz. However, a discrepancy is noticed between the N_1-on tuning curve with the time constant adjusted and the distribution of minimum thresholds at frequencies below 59 kHz (see Discussion).

DISCUSSION

Mechanical vs. neural tuning curves

One of the essential and persistent problems in the peripheral auditory system is whether there is a difference between the mechanical tuning curve of the basilar membrane and the neural tuning curve of primary auditory neurones. In the guinea pig (Evans, 1972), the cat (Evans & Wilson, 1975), and the squirrel monkey (Geisler, Rhode & Kennedy, 1974; Rhode, 1973), the mechanical tuning curve is significantly wider than the neural tuning curve. Evans (1972) thus proposed that there is a 'second filter' in addition to the mechanical one, and this second filter is 'private' for individual primary auditory neurones. The nature of this 'private second filter' is totally unknown.

In *P. parnellii rubiginosus*, 61–62 kHz sensitive neurones have unusually sharp tuning curves, and such sharp tuning curves are not a result of either mechanical systems peripheral to the inner ear or a mechanism comparable to lateral inhibition which would sharpen a tuning curve (Suga *et al.* 1975). Furthermore, the mechanical tuning curve in terms of its damped oscillation (i.e. the CM-aft) is as sharp as the neural tuning curve tuned to 60·76–61·75 kHz sounds. If our assumptions are correct that the CM is directly related to the mechanical motion of the basilar membrane and that the local resonator sharply tuned to about 61 kHz is comparable

to a second-order resonator, we may conclude that the unusually sharp neural tuning curves depend totally upon the mechanical tuning curves.

There are, however, three things which should be discussed in relation to the above conclusion: (1) the Q of the CM tuning curve and the Q in terms of CM damped oscillation, (2) large standard deviations in Q and Q-10 dB values, and (3) the first and second filters.

As shown in Table 1, the Q of the CM threshold curve is much smaller than that in terms of the damped oscillation of the CM. The CM threshold is based upon the activity of many sensory hair cells with different best frequencies, while the longest CM-aft used for the calculation of the time constant of the damped oscillation is probably mainly due to the sharply tuned resonator. This appears to be the main reason for the discrepancy between the Qs.

The standard deviation of the Q, both in terms of the CM-aft and in terms of the neural tuning curve, is large. According to Pollak *et al.* (1972), the Q of the CM threshold curve of *P. parnellii parnellii* is very high, about 620 in one of their data. They found that the sharp tuning of the CM threshold curve at 61 kHz is extremely sensitive to anaesthetics, which broaden the tuning curve. According to a recent personal communication from Pollak, the anaesthetics themselves have no effect on the CM threshold curve, but the decrease in body temperature after anaesthesia alters the sharpness of the curve. Our CM threshold curves were measured from 0·5 to 14 h after the cessation of administration of anaesthetics, which were either ether or sodium pentobarbital (20 mg/kg body weight), or both. We did not notice such a change. In our experiments, the temperature of the sound-proofed room was kept between 32 and 35 °C. We did not monitor the body temperature or the temperature of the cochlea which was exposed to the air. If the sharp tuning is really drastically affected by a change in the body temperature as found by Pollak, one of the possible origins of the large standard deviation would be the fluctuation in body temperature during our experiments. Without systematic studies on the effect of temperature on the tuning curves, it is, however, impossible to provide a convincing explanation for the above problem. Another possible origin is the variation in the innervation mode of primary auditory neurones to the hair cells. It is worth remembering that certain parts of the organ of Corti are highly innervated (Henson, 1973). In our experiments, single unit activity was recorded not only from the cochlear nerve, but also from the ventral cochlear nucleus on some occasions. This may also provide an explanation for the large standard deviation in the Q of the neural tuning curve.

In the tuning curves with the best frequency between 60·76 and 61·75 kHz, each skirt showed the double slopes which were quite different from each other (Fig. 9A and B). A possible explanation is that the low intensity region of these tuning curves (the second filter) is due to the sharply tuned local resonator and the high intensity region (the first filter) is due to the travelling wave. In other words, the travelling wave travels toward the apex from the round window and activates the sharply tuned local resonator. The Q-10 dB of the first filter is about 25, while that of the second filter is about 84. The second filter is about 3·4 times sharper than the first filter. If one assumes that neural tuning curves with single slopes are solely or predominantly due to the first filter, one can obtain the Q-10 dB values of both the

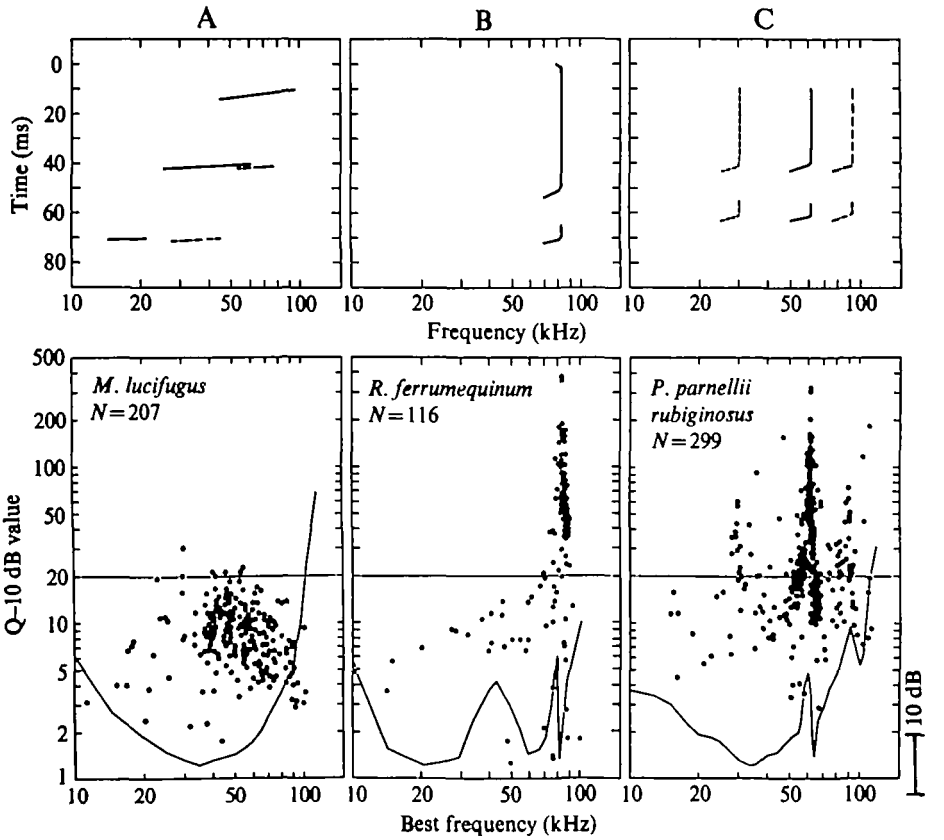


Fig. 15. Sonagrams and distributions of Q-10 dB values of peripheral neurones of *M. lucifugus* (A), *R. ferrumequinum* (B), and *P. parnellii rubiginosus* (C). The distribution of Q-10 dB values as a function of best frequency shows a clear peak in *R. ferrumequinum* and *P. parnellii rubiginosus*. Note that the peak(s) appears at the frequency of the predominant components of the echolocation signal. The curves in the lower graphs represent the threshold curve (or audiogram) of these bats. The data shown in (A) were obtained by Suga (1973) and those in (B), by Suga *et al.* (1976). The curve in (B) was measured by Long & Schnitzler (1975). The 10 dB scale for the threshold curves is shown near the lower right corner.

first and second filters as a function of best frequency (Fig. 11). Fig. 11 indicates that the first filter is nearly the same regardless of frequency, except for about 61 and 92 kHz, where it becomes slightly sharper than the others and that the second filter is much sharper than the first filter at about 61 kHz. It should be noted that the second filter described above is probably different in properties from that found in guinea pigs and cats (Evans, 1972; Evans & Wilson, 1975).

Peripheral specialization for the reception and analysis of a predominant component(s) in species-specific signals

Since the auditory system has evolved in parallel with the vocalization system, the audiograms of animals match reasonably well to the power spectra of the biologically significant sounds used by them. In other words, the ear is built for the effective detection of biologically significant signals. For instance, the audiograms or the neural threshold curves of mammals are tuned to dominant components in the species-specific acoustic signals. However, the tuning is not sharp at all and is usually not drastically different from species to species, because their commu-

sation sounds are commonly broad-band signals. In *P. parnellii rubiginosus* and *R. ferrumequinum*, each orientation sound consists of a long CF component and a short FM component (Grinnell, 1970; Neuweiler, 1970; Pollak *et al.* 1972; Suga *et al.* 1974), while non-orientation sounds so far studied are broad-band signals (Long & Schnitzler, 1975; Gordon, O'Neill & Suga, unpublished). Since the orientation sound is indispensable for their survival and its CF component is overwhelmingly predominant, the ears of these bats show dramatic specialization for the reception of this component. That is, the peripheral auditory system is broadly tuned to communication signals and is very sharply tuned to the CF component in the orientation sounds (Fig. 15). This sharp tuning does not result from mechanisms comparable to lateral inhibition, but is due to the mechanical specialization of the cochlea (Bruns, 1976*a*, *b*; Suga *et al.* 1975, 1976). The audiograms of several species of rodents show two sensitivity peaks. The high frequency peak at 45–60 kHz is for the effective detection of ultrasonic pulses from their young (Brown, 1973).

The specialization of the ear for the effective detection of biologically significant sounds has been shown in mammalian species, but, except for bats, it has not yet been demonstrated that the ear is also specialized for the frequency analysis of these signals. For instance, it has not yet been found that the critical ratio or the frequency difference limen becomes smaller or the Q-10 dB value becomes larger at the frequencies of sounds which are biologically important. In several different types of mammals, the critical ratio and the frequency difference limen become monotonically larger with frequency in nearly the same function regardless of species (Gourevitch, 1970; Fay, 1974). The Q of the mechanical tuning curve increases with frequency (Békésy, 1960). Thus, the Q-10 dB value of primary auditory neurones increases monotonically with frequency (in cats, Kiang, 1965). The changes in the critical ratio or the Q-10 dB value, however, have not been correlated with or discussed in relation to the frequency analysis of biologically significant acoustic signals. The data presented in the present and previous papers (Suga *et al.* 1975, 1976) are thus quite unique, and it is worthwhile to assemble the data obtained from three different species of bats, *M. lucifugus*, *R. ferrumequinum*, and *P. parnellii rubiginosus* (Fig. 15).

M. lucifugus emits FM orientation sounds in which the frequency sweeps downward about an octave. The range of the frequency sweep varies according to phases of echolocation, covering frequencies from 10 to 100 kHz (Griffin, 1958). The centre frequency is about 50 kHz. Studies thus indicate that *M. lucifugus* does not use CF–FM signals for echolocation. The auditory system of the animal is broadly tuned to 30–50 kHz. The Q-10 dB values of peripheral auditory neurones of *M. lucifugus* are less than 20 except for a few neurones. There appears to be a slight tendency for the Q-10 dB values to be larger at 40–50 kHz than at other frequencies (Suga, 1973; Fig. 15A).

In the orientation sound of *R. ferrumequinum*, the CF component is about 83 kHz and is predominant because of its long duration, 10–50 ms, while the FM component is short, 2–3 ms (Schnitzler, 1968). The ear is very sharply tuned at 83 kHz. The distribution of the Q-10 dB values for single peripheral auditory neurones of *R. ferrumequinum* shows a clear, sharp peak at about 83 kHz. Q-10 dB values for other best frequencies are less than 20 (Suga *et al.* 1976; Fig. 15B).

In *P. parnellii rubiginosus*, each orientation sound consists of a long CF component (10–15 ms) and a short FM component (2–3 ms), as already described. The second harmonic is always predominant. The first and third harmonics are often about 18 and 12 dB weaker than the second, respectively. The frequencies of the CF components are about 30·5, 61·0, and 91·5 kHz for these three harmonics. Since the CF component is much longer than the FM, the 61·0 kHz component in particular is far higher in total energy than the other components of the orientation sounds. In *P. parnellii rubiginosus*, about half of the Q-10 dB values are greater than 20. For a 61 kHz best frequency, the Q-10 dB value is extremely large and goes up to 310. For best frequencies around 92 and 30 kHz, the Q-10 dB value is also higher than those at other frequencies.

Thus, for the frequencies of sounds which are predominant in their orientation signals, the tuning curves of single peripheral neurones in *R. ferrumequinum* and *P. parnellii rubiginosus* are substantially narrower than those of *M. lucifugus*. Since the peaks in the distribution of the Q-10 dB values nicely match those in the power spectrum (i.e. the predominant components in the orientation signals) it is evident that the peripheral auditory systems of these CF–FM bats are remarkably specialized for the fine analysis of the CF component in the species-specific signals.

In *R. ferrumequinum*, the structural basis of fine frequency analysis of sound at 83 kHz has been found by Bruns (1976*a*, *b*). Since there is a lack of studies comparable to that of Bruns, B. R. Schaffer, B. A. Bohne, and N. Suga have started to examine the cochlear structure of *P. parnellii rubiginosus*. So far we have the surface preparations of three cochleas. We have found the following: (1) the basilar membrane is 12·2 mm long and is 60 μm wide at the basal end and 130 μm wide at the apical end. The width, which is the distance from an inner hair cell to the outer edge of a Henson's cell, monotonically increases from the basal end to the apical end, but its increment is very small between about 4·5 and 7·0 mm, 1·7 μm/mm; (2) the density of cochlear hair cells varies along the basilar membrane. It is lowest between about 3·7 and 5·5 mm from the round window, about 11% less than the neighbouring area. The density is high between about 5·5 and 6·9 mm and between 10·1 and 11·2 mm, about 11% more than the neighbouring area. Between 3·3 and 5·7 mm, the cochlear wall is very thick; (3) the high density areas are richly innervated by spiral ganglion cells, while the low density area is poorly innervated. Such non-uniform innervation is also found in *P. parnellii parnellii* (Henson, 1973). Pye (1967), who examined the transverse section of the cochlea, noticed that the spiral ligament is hypertrophied in the basal turn. Further studies on the cochlea will be necessary to explore the anatomical basis for the sharply tuned local resonator.

Functional significance of sharply tuned neurones

The functional role of the CF component in the orientation sound of *R. ferrumequinum* and/or *P. parnellii rubiginosus* in echolocation has been extensively discussed by Schnitzler (1968, 1970) and Simmons (1974). It has also been discussed in relation to neurophysiological data (Grinnell, 1970; Johnson *et al.* 1974; Neuweiler, 1970; Suga *et al.* 1975, 1976). Since a set of comprehensive neurophysiological data was obtained in the present experiments, it may be worthwhile to discuss briefly this problem in terms of the echolocation in *P. parnellii rubiginosus*, although

it overlaps with the discussion in the papers cited above, particularly with that in Suga *et al.* (1976).

As already described, the peripheral auditory system is sharply tuned at about 61 kHz. The neurones tuned at about 61 kHz are much more sensitive than neurones sensitive to neighbouring frequencies. Their tuning curves are extremely sharp, and their best frequencies are slightly different from one another. With a long CF signal and such a group of sharply tuned neurones, *P. parnellii rubiginosus* gives certain advantages in echo-detection and frequency analysis.

The emitted CF is 10–50 ms long, so that its total energy is very high within the orientation signal. Accordingly it is a signal suited for echo-detection. The auditory filter sharply tuned to the signal improves the signal-to-noise ratio. If the filter were too sharp, however, the Doppler-shifted echo CF might not be detected. Since the bat is interested in the echo CF Doppler-shifted upward, i.e. an approaching echo source, the high-frequency slope of the auditory filter should not be too steep. As a matter of fact, we have found that the slope of the CM threshold curve on the high-frequency side is half as steep as the slope on the low-frequency side (Fig. 1). Once a Doppler-shifted echo CF is detected, the bat reduces the frequency of the emitted CF and lowers the echo CF to about 61 kHz within the band of fine frequency resolution in the cochlea. The echolocation system is obviously functioning to eliminate clutter and to detect a moving target effectively.

Since the emitted CF is 10–50 ms long, the echo CF always overlaps with the emitted CF to a great extent. If the echo CF were received by the neurones which were activated by the emitted CF, its detection would be greatly interfered with by the emitted CF. Therefore the echolocation system employing a long CF signal is obviously not adapted for the detection of non-Doppler-shifted echoes, but is excellent for the detection of Doppler-shifted echoes. The 'vocal middle-ear-muscle contraction' functions to reduce vocal self-stimulation, but it does not improve the detection of the echo CF (Henson, 1967; Suga *et al.* 1974). The tuning curves of the neurones with best frequencies between 59 and 63 kHz are sharp, so that the Doppler-shifted echo CF activates the neurones which may not be excited or are only very poorly excited by the emitted CF. This relatively selective excitation of the neurones is absolutely necessary for CF echo-detection in *P. parnellii rubiginosus*. During the Doppler-shift compensation, the emitted CF becomes lower than 61 kHz and the echo CF becomes closer to 61 kHz (Schnitzler, 1970; it should be noted that the frequency of the CF differs among specimens to some extent). The peripheral auditory system in terms of the CM threshold curve is about 20 dB less sensitive to a 59 kHz sound than to a 61 kHz sound. Such a difference in sensitivity with frequency makes the stabilized or compensated Doppler-shifted echo CF relative to the emitted CF larger in amplitude at the sensory hair cells than it is in the air. In other words, the emitted CF does not effectively stimulate the ear, but the Doppler-shifted echo CF does. The peripheral auditory system of *P. parnellii rubiginosus* is apparently adapted for the effective detection of the echo CF by reducing the masking effect of vocal self-stimulation.

The group of sharply tuned neurones is directly related to the fine frequency analysis of the CF component of orientation signals. These neurones were very sensitive to frequency shift, because they showed phase-locked responses to frequency

modulation as small as ±0·01 %. In terms of the summated neural response, a barely recognizable phase-locked response was found even with ±0·004 % FM. It has also been demonstrated that the evoked potential of the inferior colliculus of *R. ferrumequinum* is very sensitive to a frequency shift (Schuller, 1972). Considering that humans can detect a 0·3 % frequency shift of a 1·0 kHz sound, the above neurophysiological data indicate that the minimum detectable per cent frequency difference in *P. parnellii rubiginosus* is much smaller than that of humans. The fine frequency analysis is probably used for accurate measurement of the relative velocity of a moving target and also for detection of wing beats of an insect. A 0·02 % Doppler-shift is 12 Hz for a 61 kHz sound and is equivalent to a target moving at a speed of 3·4 cm/s relative to the bat. A 0·008 % frequency shift is 5 Hz for a 61 kHz sound. When a 1 cm long moth moves its wings at a rate of 50 times/s, it would, under favourable conditions, produce a Doppler-shift of about 350 Hz. It is likely that the bat utilizes information in the form of an FM evoked by a few wing beats for identification of insects and discrimination among insects. Wing beats modulate an echo not only in frequency, but also in amplitude. An impulse-count function of peripheral auditory neurones of *P. parnellii rubiginosus* appears to be not steeper than that of cats (e.g. Nomoto *et al.* 1964), so that the sharply tuned neurones do not indicate the superiority in coding of amplitude modulation. There is a possibility that a Doppler-shifted echo CF activates the neurone which is also excited by an emitted CF to some extent. In this case, the per cent modulation in the beat produced by the overlap of the echo CF with the emitted CF may be significant, although the beat is hardly noticeable acoustically at the external ear and in CM responses. Single neurones can code a beat frequency up to 3 kHz (Suga *et al.* 1975). It is not possible for individual neurones to discriminate between the FM and AM due to an insect wing beat and the beat due to the overlap between the emitted CF and the echo CF Doppler-shifted by the movement of an insect relative to the bat, because they simply change their discharge rate according to the change in the effectiveness of a stimulus. However, the FM causes mainly a change in activity across neurones with different best frequencies, while the AM causes primarily a change in activity within neurones with similar best frequencies. The brain presumably processes the FM separately from the AM.

Behavioural experiments have demonstrated that *R. ferrumequinum* shows compensation behaviour for a Doppler-shift as small as 50 Hz (Schuller, Beuter & Schnitzler, 1974; Schnitzler, 1968; Simmons, 1974). Flieger's recent preliminary experiments indicate that *R. ferrumequinum* can discriminate an object moving in such a way so as to cause less than a 50 Hz Doppler-shift from a non-moving object (personal communication). Behavioural data about frequency discrimination have not yet been obtained from *P. parnellii rubiginosus*, but we guess that the minimum detectable frequency difference is nearly the same in *P. parnellii rubiginosus* (at about 61 kHz) and *R. ferrumequinum* (at about 83 kHz) because the distribution of Q-10 dB values shows a comparable specialization in both animals.

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