J. comp. Physiol. 106, 111–125 (1976)

Journal of Comparative Physiology · A

Peripheral Auditory Tuning for Fine Frequency Analysis by the CF-FM Bat, *Rhinolophus ferrumequinum*

IV. Properties of Peripheral Auditory Neurons

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Received November 3, 1975

Summary. For echolocation, *Rhinolophus ferrumequinum* emits orientation sounds, each of which consists of a long constant-frequency (CF) component and short frequency-modulated (FM) components. The CF component is about 83 kHz and is used for Doppler-shift compensation. In this bat, single auditory nerve fibers and cochlear nuclear neurons tuned at about 83 kHz show low threshold and very sharp filter characteristics. The slopes of their tuning curves ranged between 1,000 and 3,500 dB/octave and their Q-10 dB values were between 20 and 400, 140 on the average (Figs. 3–5). The peripheral auditory system is apparently specialized for the reception and fine frequency analysis of the CF component in orientation sounds and Doppler-shift compensated echoes. This specialization is not due to suppression or inhibition comparable to lateral inhibition, but due to the mechanical specialization of the cochlea. Peripheral auditory neurons with the best frequency between 77 and 87 kHz showed not only on-responses, but also off-responses to tonal stimuli (Figs. 1, 2, and 6). The off-responses with a latency comparable to that of N_1-off were not due to a rebound from either suppression or inhibition, but probably due to a mechanical transient occurring in the cochlea at the cessation of a tone burst.

Introduction

In terms of orientation sounds, there are three types of echolocating bats: (1) "FM" bats which emit frequency-modulated (FM) signals for echolocation, (2) "CF-FM" bats which emit signals containing a constant frequency (CF) component in addition to FM, and (3) "click" bats which emit click sounds. *Rhinolophus ferrumequinum* (greater horseshoe bat) and *Pteronotus parnellii rubiginosus* (mustache bat) are CF-FM bats which produce a long CF signal followed by a short FM without a gap (Schnitzler, 1968, 1970). *Noctilio leporinus* (fish-

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catching bat) emits CF and CF-FM sounds during cruising and search, but it changes the signals into FM sounds during approach and terminal phases of echolocation (Suthers, 1965). *Rousettus* is only one genus of bat which is known to emit click sounds for echolocation (Grinnell and Hagiwara, 1972; Kulzer, 1956).

R. ferrumequinum and *P. parnellii rubiginosus* show very unique acoustic behavior called "Doppler-shift compensation" (Schnitzler, 1968, 1970). *R. ferrumequinum*, for instance, emits a CF component at about 83 kHz when there are no Doppler-shifted echoes. The frequency of the CF component in this condition is called a "resting frequency." When a positively Doppler-shifted echo comes back, the bat reduces the frequency of the emitted signal to stabilize the echo frequency at a certain preferred frequency. The frequency of the CF component in the stabilized echo is called a "reference frequency." The reference frequency is always a few hundred Hertz higher than the resting frequency regardless of the amount of Doppler-shift (Schuller *et al.*, 1974). In *P. parnellii rubiginosus*, the orientation sound contains three harmonics. The second harmonic is always predominant, and the resting frequency of the CF component in the second harmonic is about 61 kHz.

The auditory systems of these two species of bats are apparently specialized for the detection of echoes stabilized at about 83 kHz in *R. ferrumequinum* (Neuweiler, 1970) and at about 61 kHz in *P. parnellii rubiginosus* (Grinnell, 1970). In *P. parnellii parnellii* and *P. parnellii rubiginosus*, the cochlear microphonic (CM) recorded from either the perilymphatic aqueduct or the round window is sharply tuned at about 61 kHz (Pollak *et al.*, 1972; Suga *et al.*, 1974). This sharp tuning is not due to mechanisms comparable to lateral inhibition, but due to some mechanical specialization of the inner ear. Single peripheral auditory neurons sensitive to 61–62 kHz sounds have unusually sharp tuning curves, and their best frequencies differ slightly from one another. The peripheral auditory system of *P. parnellii rubiginosus* is specialized not only for reception, but also for fine frequency analysis of the CF component in echoes stabilized at about 61 kHz (Suga *et al.*, 1975). In *R. ferrumequinum*, the 16 mm long basilar membrane shows a unique feature, different from that of other mammals: a constriction in width between 4 and 11 mm apical from the round window and a sudden decrease in thickness between 4.3 and 4.7 mm from it. This area, 4.3–4.7 mm from the round window, is concerned with the reception of sounds of 83–84 kHz (Bruns, 1976). The CM threshold curve shows a clearly noticeable notch at about 83 kHz (Schnitzler *et al.*, 1976). It is thus expected that the peripheral auditory system of *R. ferrumequinum* is specialized for the detection and analysis of the CF component of stabilized echoes. The first aim of the present paper is to study the frequency sensitivity of peripheral auditory neurons and to examine whether the sharp tuning at about 83 kHz is due to a mechanism comparable to lateral inhibition.

In *P. parnellii rubiginosus* (Grinnell, 1970) and *R. ferrumequinum* (Neuweiler *et al.*, 1971), prominent summated responses of auditory neurons in the midbrain appear not only at the onset of a tonal stimulus but also at its cessation when its frequency is either about 60 (*P. p. r.*) or 82 kHz (*R. f.*). Since the off-response is very sharply tuned to the sound just below the resting frequency of the

CF component used by these bats and the on-response is not sensitive to sound which is the best for the off-response, the off-response has been considered to be due to the rebound from inhibition or suppression which is a mechanism for sharpening the tuning curve of the on-response. Grinnell (1973) found the prominent auditory nerve response (N_1) at the cessation of a tonal stimulus in *P. parnellii rubiginosus* and concluded that the N_1-off was due to a rebound from non-neural suppression occurring in the cochlea during the stimulus and that the suppression produced the sharp tuning curve of the N_1-on.

In cats and monkeys, it has been demonstrated that discharges of auditory nerve fibers are not suppressed or inhibited at all by a tonal stimulus unless the stimulation of the olivo-cochlear bundle or simultaneous two-tone stimulation is introduced. The bats' peripheral auditory system thus would be very special, if the off-response resulted from the rebound from non-neural suppression. In *P. parnellii rubiginosus*, it has been found that the off-response is not due to the rebound, but due to a mechanical transient occurring in the inner ear at the cessation of a tonal stimulus (Suga *et al.*, 1975). It is likely that the off-response of the midbrain auditory nuclei in *R. ferrumequinum* also originates from the mechanical transient occurring in the inner ear. The second aim of the present paper is to study whether single peripheral auditory neurons show off-responses and, if so, whether the off-responses result from suppression or inhibition during a tonal stimulus.

Materials and Methods

Experimental subjects were eight *Rhinolophus ferrumequinum* (greater horseshoe bat) from eastern France. These bats were lightly anesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg of body weight). Ether was used if the animal moved too much. The dorsal part of the skull was exposed. A nail, 1.8 cm long, was then mounted on the exposed skull with glue and dental cement. The nail subsequently was fixed onto a metal rod with a set screw to immobilize the bat's head. The dorsal cochlear nucleus was exposed after aspiration of the lateral portion of the cerebellum. In order to record action potentials of peripheral auditory neurons, a micropipette electrode filled with 3M-KCl solution was inserted ventrally from the surface of the dorsal cochlear nucleus, in an attempt to place it in the modiolus. Recordings were made from the cochlear nuclei and auditory nerve. The distance between a condenser loudspeaker and the head of the bat was 60 cm. The chamber in which the loudspeaker and the bat were placed was made with particle board and its inner wall was covered by 3.0 cm thick foam rubber in order to reduce echoes. The chamber was heated up to 30–33 °C.

Instruments used to deliver acoustic stimuli and to record action potentials were basically the same as described in Neuweiler (1970). Responses of single neurons were expressed by post-stimulus-time (PST) histograms with a PDP-12 computer. Threshold curves of single neurons were measured by watching the screen of a cathode-ray oscilloscope on which action potentials were displayed and by listening to an audio monitor from which clicking sounds were delivered whenever neurons discharged action potentials.

Results

Response Patterns of Single Neurons to Tone Bursts

Responses to acoustic stimuli were studied in 180 single neurons. In 55 neurons, latencies of responses were measured which ranged between 1.3 and 3.5 msec.

60% of the 55 showed a latency shorter than 2.0 msec. In terms of the latency of the single unit responses and location of the tips of the recording electrodes used, at least half of the neurons studied were recorded in the cochlear nuclei rather than the auditory nerve. Excitatory responses were commonly tonic on-responses which were followed by post-excitatory suppression of background activity, if any. The PST histograms of such excitatory responses showed a prominent peak at the beginning of the response and then a decay toward a plateau (Fig. 1A, left column). When the best frequency of a neuron was between 77 and 87 kHz, the neuron showed not only tonic on-responses, but also phasic on- and off-responses. Each of the phasic on- and off-responses always lasted less than 5 msec and consisted of less than three impulses (Fig. 1A, right column). At higher stimulus levels, the tonic on-response was strong and the post-excitatory suppression immediately following it also was strong. Thus the off-response, which would follow the tonic on-response, was unclear or was cancelled out by the post-excitatory suppression (Fig. 1A, 90 to 70 dB SPL). At weaker stimulus levels, the tonic on-response and the post-excitatory suppression were weak, so that the off-response was clear (Fig. 1A, 60 to 50 dB SPL).

The phasic on- and off-responses were not associated with inhibition or suppression, but these were probably due to mechanical transients occurring at the onset and cessation of a tone burst. This conclusion was obtained by the following three observations: (1) no suppression of background activity prior to the off-response (Fig. 2A), (2) a tonic on-response immediately prior to the off-response (Figs. 1A and 2B), and (3) no suppression of a response to a test tone pulse delivered prior to the off-response (Fig. 2, C and D). When two tones are simultaneously delivered in certain combinations, "two-tone suppression" occurs in primary auditory neurons [e.g. Nomoto *et al.* (1964) in monkeys; Sachs and Kiang (1968) in cats; Suga (1964b) in *M. lucifugus*]. In *R. ferrumequinum*, two-tone suppression was also observed. The response to a certain short test tone could be suppressed when it was delivered prior to the off-response. But the response to the other short test tone could not be suppressed (Fig. 2, C and D). In other words, the off-response did not result from the suppression. The suppression observed was the two-tone suppression, which has been explained by nonlinearity in the cochlea mechanisms (Pfeiffer, 1970).

About 10% of the neurons studied showed not only excitatory responses, but also inhibitory ones. The inhibition was easily identified by a reduction of background discharges during a tonal stimulus and/or a phasic on-response and after discharges. The inhibition usually lasted 5–15 msec longer than the duration of the stimulus. Then off-discharges appeared as a rebound from the inhibition. This off-response usually lasted more than 10 msec and consisted of several impulses (Fig. 1B, 100 dB SPL). Thus the off-response due to the rebound was easily discriminated from the off-response due to the mechanical transient.

When inhibition occurred in neurons with the best frequency between 77 and 87 kHz, these response patterns were complex. Fig. 1B shows response patterns of one such neuron. At 100 dB SPL, the response pattern is character-

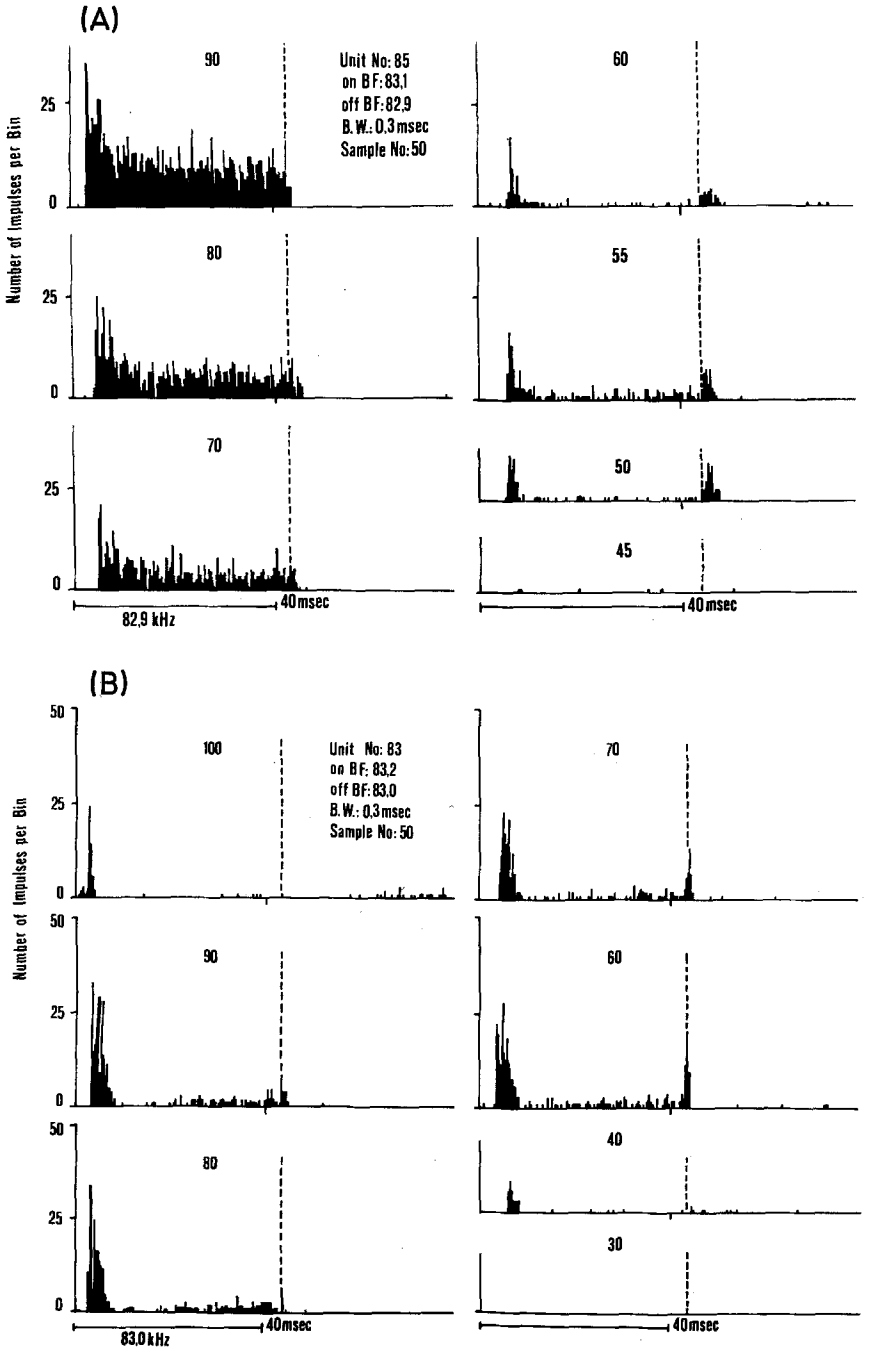

Fig. 1. Post-stimulus-time (PST) histograms showing response patterns of two single neurons (A and B). The tonal stimulus is 82.9 kHz for (A) and 83.0 kHz for (B). Its duration and rise-decay time are 40 and 0.5 msec, respectively. The stimulus amplitude in dB referred to 0.0002 dyne/cm^2 r.m.s. is given by the figures at the upper center of each histogram. The sample number is 50 in each histogram. The bin width of the histograms is 0.3 msec. The end of the on-response, if any, is indicated by a vertical dashed line. The on and off-best frequencies are respectively 83.1 and 82.9 kHz for A and 83.2 and 83.0 kHz for B

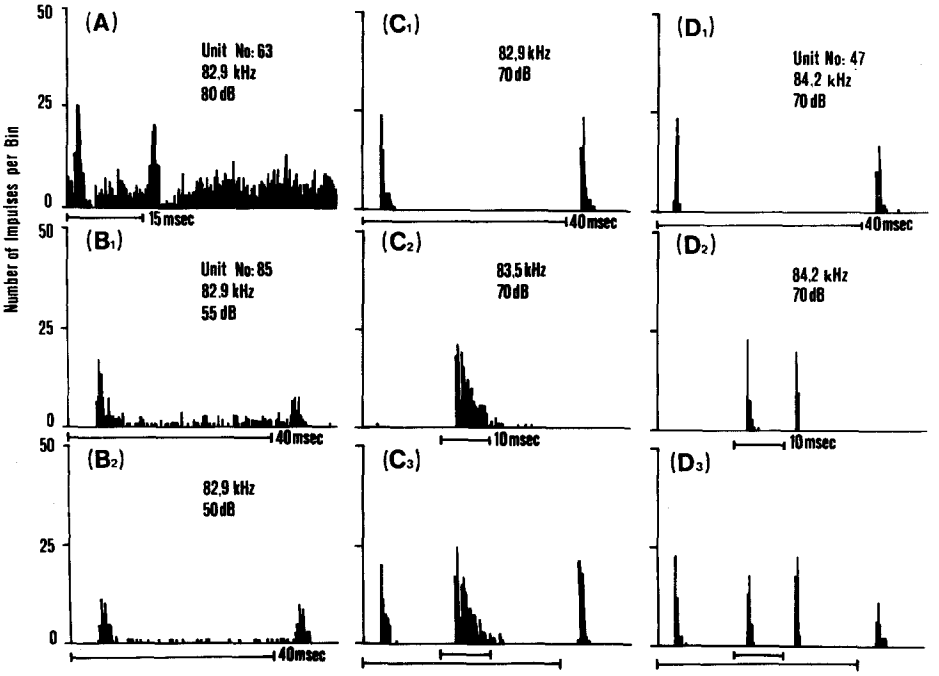

Fig. 2. PST histograms of responses of four single neurons (A, B, C and D), indicating no relationship of off-responses to suppression or inhibition. In (A), background activity is not suppressed prior to the off-response. Post-excitatory suppression is, however, clear immediately after the phasic on and off-responses. The tonal stimulus is 82.9 kHz, 80 dB SPL, and 15 msec long. In (B), the neuron is spontaneously inactive. The off-response immediately follows the tonic on-response. The tonal stimulus is 82.9 kHz, 55 (B_1) or 50 dB SPL (B_2), and 40 msec long. In (C and D), the neurons are not spontaneously active and show phasic on and off-responses to either 82.9 (C_1) or 84.2 kHz sound (D_1) which is 40 msec long. The response to a 10 msec long test tone burst of either 83.5 (C_2) or 84.2 kHz (D_2) is not inhibited between the on and off-responses to the 40 msec long tone burst (C_3 and D_3). The stimulus amplitude is 70 dB SPL for both the short and long tone bursts. The sample number is 50, and the bin width is 0.3 msec

ized by a very phasic on-discharge and long-lasting off-discharges which started to appear about 15 msec after the cessation of the stimulus. These off-discharges are apparently due to a rebound from inhibition and are not at all contributing to N_1-off described by Schnitzler *et al.* (1976) and LL (or N_4)-off described by Neuweiler *et al.* (1971). In this neuron, the threshold for inhibition at 83.0 kHz was apparently higher than that for excitation, because a tonic on-response appeared for stimuli weaker than 100 dB SPL. At 70 and 60 dB SPL, the neuron showed phasic and tonic on-responses and also an off-response which was different from off-discharges due to a rebound from inhibition in latency and time course.

Tuning Curves

Tuning (or threshold) curves of 116 single neurons were measured in terms of excitatory responses, i.e., phasic and tonic on-responses. The area above

the tuning curve is called the excitatory (or excitatory response) area. The best frequencies of these neurons ranged between 10 and 100 kHz. When a best frequency was below 70 kHz or above 90 kHz, the sharpness of an excitatory area was somewhat comparable to that in an "FM" bat, *Myotis lucifugus*, which emits only FM sounds for echolocation and does not compensate the signals for Doppler-shift. However, the excitatory areas of the neurons tuned to sounds between 70 and 90 kHz were significantly narrower than those in *M. lucifugus*. In particular, the areas tuned around 83 kHz were extremely narrow (Figs. 3A, 4B). The high and low frequency slopes were as steep as 3,500 and 2,000 dB/octave, respectively.

The sharpness of the tuning curve of a single neuron has been expressed by a Q-10 dB value, which is the best frequency divided by the band width of the tuning curve at 10 dB above the minimum threshold. In order to show the change in the sharpness with the best frequency and also the difference in the sharpness of the tuning curve between *R. ferrumequinum* and *M. lucifugus*, Q-10 dB values were plotted against best frequencies of single neurons obtained from these two species of bats (Fig. 4). In *M. lucifugus*, the Q-10 dB value was less than 20 except for 3% of the neurons studied, regardless of the best frequencies (Fig. 4A). In *R. ferrumequinum*, on the other hand, the Q-10 dB value was greatly different depending upon the best frequencies. It was less than 20 for best frequencies lower than 70 kHz and higher than 90 kHz. For 80 and 90 kHz best frequencies, the Q-10 dB values of a majority of the neurons were much higher than 20 (Fig. 4B). In particular, the Q-10 dB value was as high as 400 between 83 and 84 kHz, 140 on the average.

The resting frequency of *R. ferrumequinum* studied differs individually within a range from 83 to 85 kHz and the Q-10 dB value varied drastically according to the best frequency, particularly at 80–86 kHz, so that the data shown in Fig. 4B were plotted with an expanded frequency axis, in which the best frequencies were expressed in reference to the resting frequency of each bat used for the single unit study (Fig. 5). The Q-10 dB value was greatest for the resting frequency, and it was smaller at best frequencies either lower or higher than the resting frequency. It is evident that the peripheral auditory system of *R. ferrumequinum* is specialized for fine frequency analysis of sounds which are predominantly contained in its non-Doppler-shift-compensating orientation sound and stabilized echoes.

Threshold curves of N_1 (Schnitzler *et al.*, 1976) and LL (Neuweiler, 1970) are sharply tuned at 83–84 kHz and are broadly tuned at 20–30 and 50–60 kHz. The thresholds at these frequencies are very low, -5 to 5 dB SPL, but the threshold at 81–82 kHz is very high, 40 to 60 dB SPL. The threshold curves of these evoked potentials are dependent upon not only the sensitivity of the ear, but also synchronization of single unit activity and the size of the population of neurons simultaneously activated by sound. It was thus examined whether a distribution of minimum thresholds against best frequencies matched the threshold curves of the evoked potentials. As shown in Fig. 3B, the minimum thresholds of the neurons tuned at 83–84 kHz are much lower than those of the neurons tuned to neighboring frequencies. The behavioral threshold curve obtained by Long and Schnitzler (1975) also shows such a change in threshold

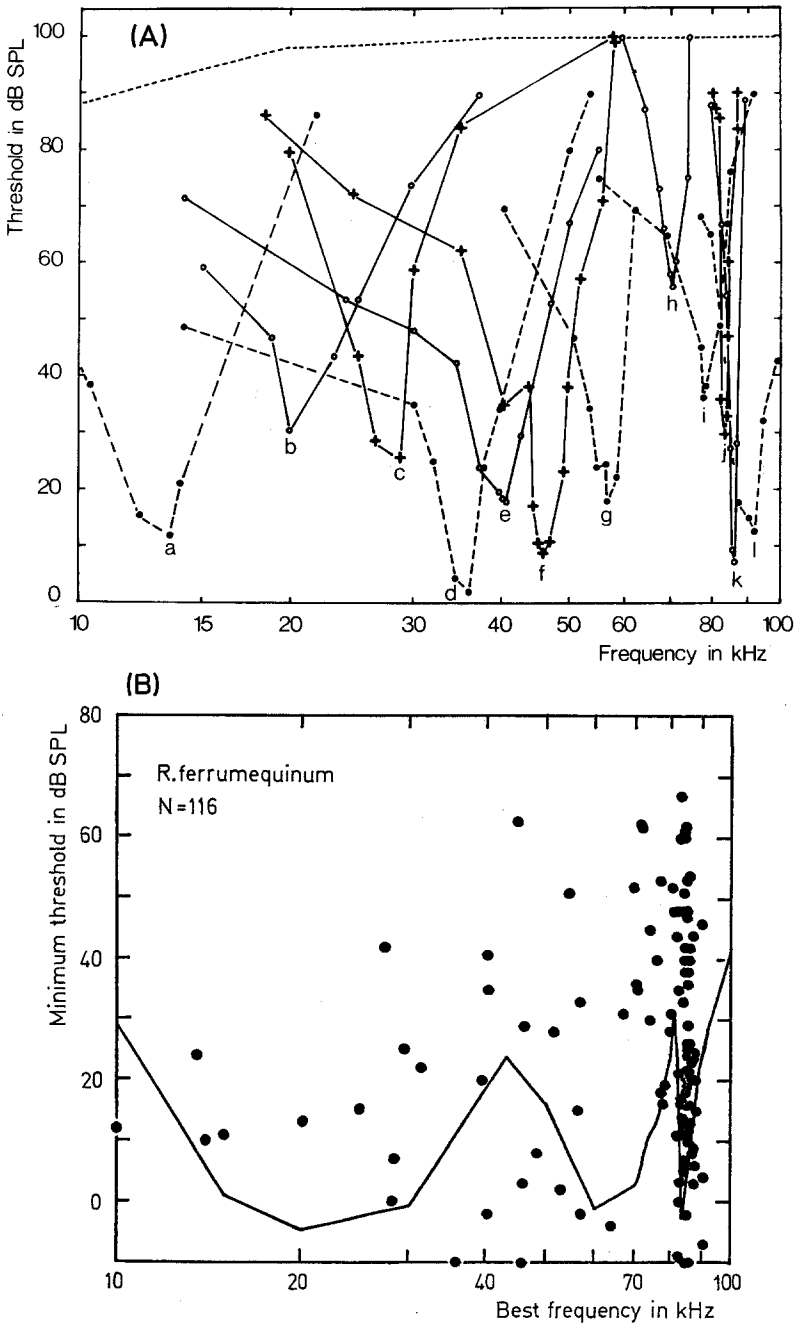

Fig. 3. (A) Tuning curves of 12 single neurons (a–l). The ordinate and abscissa respectively represent threshold in dB SPL and frequency in kHz. (B) A distribution of minimum threshold as a function of on-best frequencies. The curve is the behavioral threshold curve obtained by Long and Schnitzler (1975)

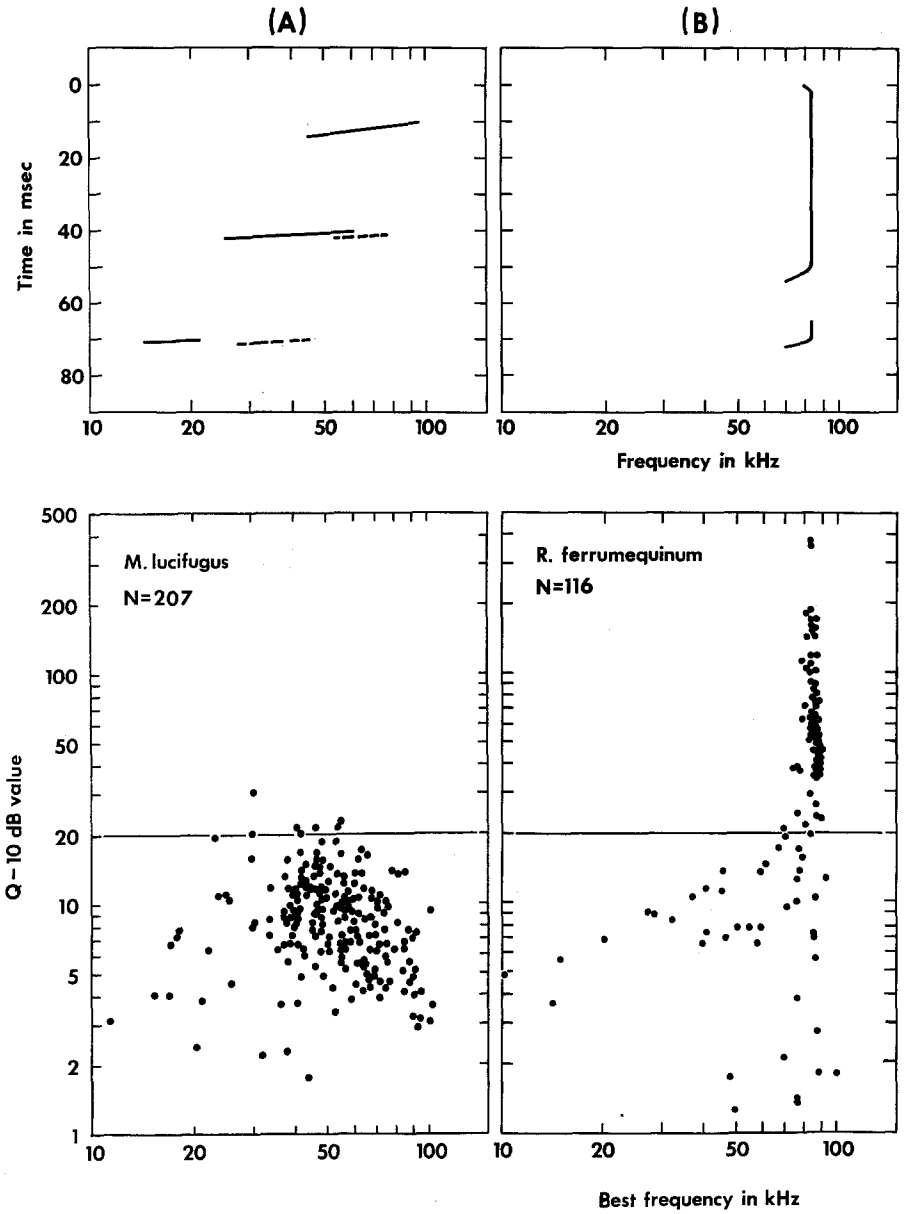

Fig. 4. Sonagrams of orientation sounds (upper graphs) and distributions of Q-10 dB values as a function of on-best frequencies (lower graphs) in *M. lucifugus* (A) and *R. ferrumequinum* (B)

with sound frequency. Our data clearly indicate that the peripheral auditory system of *R. ferrumequinum* is specialized not only for fine frequency analysis of sounds which are predominantly used for echolocation, but also for detection of them.

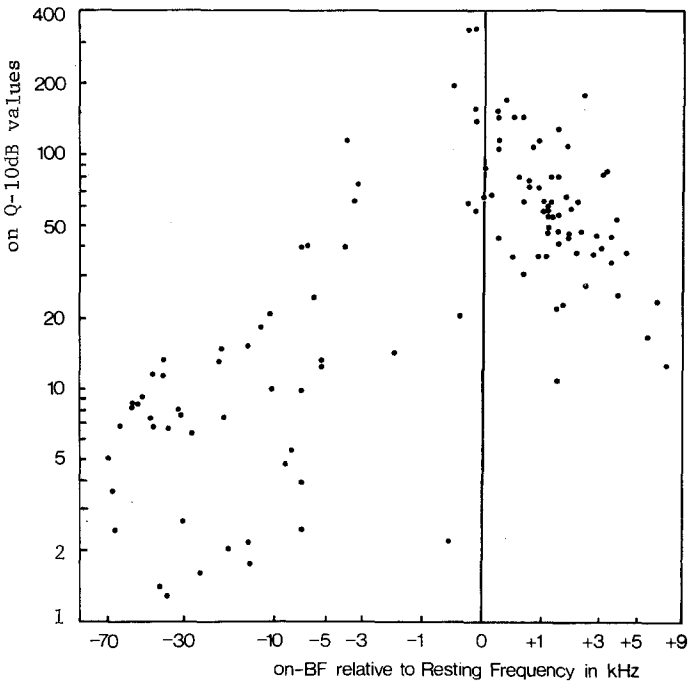

Fig. 5. Distributions of Q-10 dB values as a function of on-best frequencies. The best frequencies are expressed in kHz referred to resting frequencies of individual bats from which single unit activity was recorded. The resting frequency is 83.3 kHz on the average

Off-tuning Curve

Since the single neurons with the best frequency between 77 and 87 kHz showed off-responses which were not related to suppression or inhibition, "off-tuning curves or areas" were measured. The best frequency for an on-response is hereafter called the "on-BF," and that for an off-response is called the "off-BF." Fig. 6 shows examples of on and off-tuning curves of five single neurons. The on-area or excitatory area was narrowest when the best frequency was 83 kHz, as already described. The on-BF ranged between 77 and 87 kHz, while the off-BF was about 83 kHz regardless of the on-BF. Thus the on-BF would be either lower (A) or higher (C to E) than the off-BF, or nearly the same (B). The relationship between the on and off-BFs is plotted in Fig. 6F, in which the BFs are expressed referring to the resting frequency of each bat from which single neurons were recorded. Regardless of the on-BF, the off-BF was about 500 Hz lower on the average than the resting frequency.

Discussion

Specialization of the Peripheral Auditory System for Reception and Analysis of Species-specific Orientation Sounds and Echoes

Since the auditory system has developed together with vocalization, it is expected that the auditory system is specialized for processing acoustic signals which

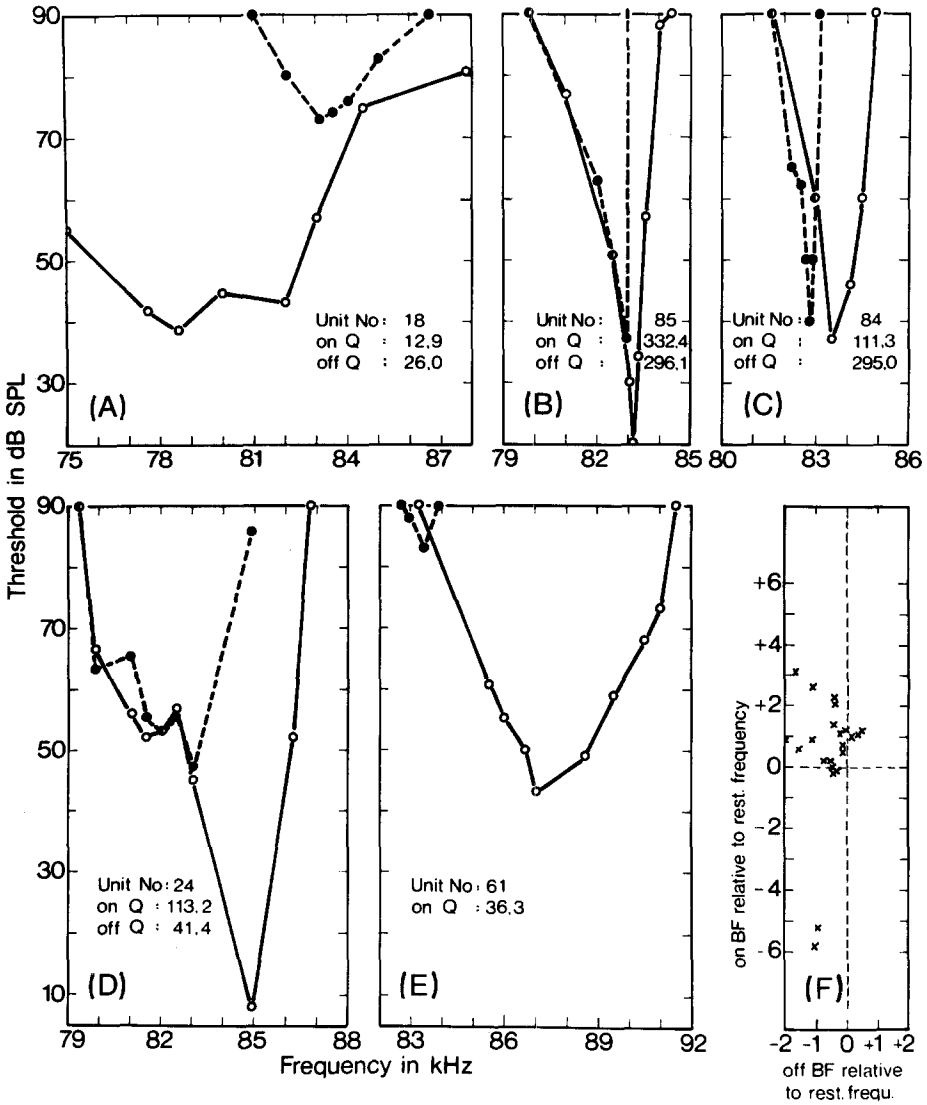

Fig. 6. On and off-tuning curves of five single neurons (A–E) and the distribution of on-best frequencies against off-best frequencies (F). In (A) to (E), on- and off-tuning curves are shown by the solid and dashed lines, respectively. In (F), the best frequencies are expressed in kHz referred to resting frequencies of individual bats, from which these tuning curves were obtained. The resting frequency is 83.3 kHz on the average

are most frequently used by an animal. In humans, monkeys, and cats, energy of communication sounds is not concentrated at a narrow frequency band. This is also true of *Myotis lucifugus*, which always emits FM signals for echolocation. In these animals, the ear is broadly tuned to sounds within a certain range, indicating its matching to acoustic signals used by each species of them. Each of the orientation sounds of *R. ferrumequinum*, on the other hand, always contains a long CF component at about 83 kHz in addition to short FM com-

ponents, so that its power spectrum shows a prominent peak at about 83 kHz. The behavioral and electrophysiological threshold curves of this animal indicate that the ear is very sharply tuned at about 83 kHz and is broadly tuned to the sounds of 20–30 and 50–60 kHz. The sensitivity peak at 50–60 kHz is not prominent in the electrophysiological threshold curve (Neuweiler, 1970; Neuweiler *et al.*, 1971; Schnitzler *et al.*, 1976), as it is in the behavioral one (Long and Schnitzler, 1975). The high sensitivity at 20–40 kHz is probably for the reception of communication sounds, while the remarkably sharp and high sensitivity at about 83 kHz is obviously for the reception of the predominant component in the orientation sounds and stabilized echoes. Our present experiments indicate that the peripheral auditory system of *R. ferrumequinum* is specialized not only for the reception of the predominant component, i.e., CF component, but also its fine frequency analysis. The single neurons tuned to the sounds between 83 and 84 kHz show unusually sharp tuning curves. Such specialization is not based upon any suppression or lateral inhibition, but it is probably due to mechanical specialization occurring in the cochlea (Bruns, 1976).

The same results have also been obtained from *P. parnellii rubiginosus* (Suga *et al.*, 1974, 1975). Unlike *R. ferrumequinum*, the orientation sound of *P. parnellii rubiginosus* contains the first, second and third harmonics. The second harmonic is always predominant. Its CF component is about 61 kHz. The electrophysiological threshold curve shows three sensitivity peaks at about 30, 61, and 92 kHz (Grinnell, 1970; Suga *et al.*, 1974, 1975). The single neurons tuned at either 30 or 61 or 92 kHz show sharp tuning curves. In particular, the tuning curves of the neurons tuned at about 61 kHz are unusually sharp (Suga *et al.*, 1975). The cochlear microphonic is sharply tuned at about 61 kHz (Pollak *et al.*, 1972) and shows a prominent off-response at about 61 kHz (Suga *et al.*, 1975). The ear of *P. parnellii rubiginosus* is thus apparently specialized for both the reception and fine frequency analysis of the CF components in its orientation sounds and stabilized echoes. This specialization is not due to any suppression or lateral inhibition, but it is probably due to mechanical specialization of the cochlea.

Sharply Tuned Neurons and Echo-Detection

In addition to fine frequency analysis of the CF component in a stabilized echo, a group of neurons sharply tuned to sound between 80 and 86 kHz appears to have advantages in echo-detection. Since the CF component of the orientation sound (hereafter, emitted CF) is 10 to 50 msec long, it is an ideal signal for echo-detection. However, the CF component of an echo (hereafter, echo CF) always overlaps with the emitted CF and its detection would be interferred with by the emitted CF if both CFs activated one and the same group of neurons. Since the tuning curves of the neurons with the best frequency between 80 and 86 kHz are so sharp, the Doppler-shifted echo CF activates the neurons which may not be excited or only poorly excited by the emitted CF. This selective excitation of the neurons should be ideal for echo detection. During the Doppler-shift compensation, the emitted CF becomes lower than

83 kHz and the echo CF becomes closer to 83 kHz. The auditory system is about 30–60 dB less sensitive to 81–82 kHz sound than to 83–84 kHz sound (Neuweiler, 1970; Neuweiler *et al.*, 1971; Long and Schnitzler, 1975; Schnitzler *et al.*, 1976). Such a difference in sensitivity with frequency makes the stabilized echo CF relative to the emitted CF larger in amplitude at sensory hair cells than it actually is. The peripheral auditory system of *R. ferrumequinum* is apparently developed for the effective detection of the echo CF by passively reducing the masking effect of vocal self-stimulation. It has been demonstrated that the middle-ear muscles (MEMs) contract synchronously with vocalization and attenuate vocal self-stimulation (Henson, 1965, 1967; Suga and Jen, 1975; Suga *et al.*, 1974). This "vocal MEM contraction" reduces the masking effect of the vocal self-stimulation on echoes and can improve echo-detection in FM bats, such as *M. lucifugus* and *Tadarida braziliensis*. In *P. parnellii*, the vocal MEM contraction is strong during the emission of the FM component at the end of its orientation sound (Henson, 1967). In *R. ferrumequinum*, however, it remains to be studied whether echo detection is improved by it. The detection of an echo FM may be improved, but that of the echo CF may not be, because both the emitted and echo CFs always overlap to a significant extent.

Hanging down from a ceiling of a cage, *M. lucifugus* usually does not emit orientation sounds unless it is going to fly, while *R. ferrumequinum* delivers orientation sounds very frequently, at a rate of about 4 sounds per sec. When a hand is moved toward them outside the wire mesh cage, *M. lucifugus* usually shows no noticeable reaction to it, but *R. ferrumequinum* quite often reacts by spreading the wings or flying away. *R. ferrumequinum* obviously can ignore strong echoes from stationary objects such as the floor and walls, and can focus on weak echoes from moving objects. The CF component in the orientation signal of *R. ferrumequinum* has the properties suited for the detection of a relative velocity of a moving target. Doppler-shift compensation found by Schnitzler (1968) clearly indicates that *R. ferrumequinum* can detect a Doppler shift of an echo. The acoustic behavior of *R. ferrumequinum* suggests that it can prey upon insects with a strategy which is not employed by *M. lucifugus*. That is, *R. ferrumequinum* hangs down from a branch of a tree, emits CF-FM orientation signals at a certain rate and listens to Doppler-shifted echoes, ignoring many strong echoes from leaves. When a flying insect comes into its "auditory field," the bat would be able to selectively detect its echo for hunting it. Non-Doppler-shifted echoes would activate the group of sharply tuned neurons which are excited by a vocal self-stimulation, while the Doppler-shifted echo would activate the other group of sharply tuned neurons which are not excited or are poorly excited by a vocal self-stimulation. Thus, a series of sharply tuned neurons would be elements necessary for the selective detection of Doppler-shifted echoes.

In the CF-FM signal, sound energy is highly concentrated at the frequency of the CF component, so that the CF is theoretically a better signal for target detection than the FM. When a positively Doppler-shifted echo returns, *R. ferrumequinum* reduces the frequency of the emitted CF to stabilize the echo CF at the frequency to which the auditory system is the most sensitive and has the highest frequency resolution. The stabilized echo CF is the best for frequency

analysis and is the most tolerant to masking effect of emitted sounds, other echoes and background noise. However, the Doppler-shift compensation may not only be for the effective detection of Doppler-shifted echoes and the measurement of a relative velocity of a target, but also for the improvement of the information processing necessary for echolocation. Simmons (1974) has proposed that the ambiguity in echo ranging introduced by a relative movement of a target is reduced by the Doppler-shift compensation.

Sharply Tuned Neurons and Coding of Frequency-modulated Sounds

An echo CF returning from a flying insect may be modified in amplitude and frequency by wing beat (Johnson *et al.*, 1974). Coding of amplitude modulation by peripheral auditory neurons in *R. ferrumequinum* appears to be comparable to that in other mammals, because these impulse-count functions did not seem to be particularly different from that of other mammals. Coding of frequency modulation by single neurons in *R. ferrumequinum*, however, should be superior to that in other mammals, because of the very sharp tuning curves of neurons with the best frequency of 83–84 kHz. As a matter of fact, Schuller (1972) found that the lateral lemniscal evoked potential was very sensitive to a frequency shift.

Off-Responses

As already described, we could not find single neurons which showed only off-responses. The neurons, which showed off-responses, also showed on-responses to tonal stimuli without a single exception. Off-responses which were comparable to the N_1-off in terms of latency were not due to a rebound from suppression or inhibition but probably due to a structural discontinuity occurring in the 16 mm long basilar membrane. Bruns (1975) found that the thickness of the basilar membrane suddenly decreases from 35 to 10 μm between 4.3 and 4.7 mm from the round window, and that this area is concerned with the reception of sounds of 83–84 kHz. Because of this discontinuity, the prominent side bands may appear at the onset and cessation of a tone burst which causes the vibration of this area and the immediately adjacent area. The data presented in Fig. 6 indicate that the side bands are particularly prominent at the place tuned at 82–83 kHz and that the neurons with the best frequency between 77 and 87 kHz are excited by the side bands.

In *P. parnellii rubiginosus*, peripheral auditory neurons with the best frequency between 55 and 64 kHz show off-responses. The off-best frequencies range between 60.5 and 61.6 kHz regardless of the on-best frequencies. These off-responses are not due to a rebound from suppression or inhibition, but due to a mechanical transient occurring in the cochlea at the cessation of a tone burst, because background activity was not suppressed or inhibited prior to them and the cochlear microphonic showed the most prominent off-transient for the tone burst between 60.5 and 61.5 kHz (Suga *et al.*, 1975). The origin of the off-response thus appears to be the same in *R. ferrumequinum* and *P. parnellii rubiginosus*.

We thank Alexander von Humboldt Stiftung, Deutsche Forschungsgemeinschaft (Grant No. Ne146/6–8), Stiftung Volkswagenwerk (Grant No. 111858), and American National Science Foundation (Grant No. 40018 and BMS 75-17077) for their support for our cooperative work.

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