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PERIPHERAL SPECIALIZATION FOR FINE ANALYSIS OF DOPPLER-SHIFTED ECHOES IN THE AUDITORY SYSTEM OF THE 'CF-FM' BAT *PTERONOTUS PARNELLII*

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ABSTRACT

Pteronotus parnellii uses the second harmonic (61–62 kHz) of the CF component in its orientation sounds for Doppler-shift compensation. The bat's inner ear is mechanically specialized for fine analysis of sounds at about 61–62 kHz. Because of this specialization, cochlear microphonics (CM) evoked by 61–62 kHz tone bursts exhibit prominent transients, slow increase and decrease in amplitude at the onset and cessation of these stimuli. CM-responses to 60–61 kHz tone bursts show a prominent input-output non-linearity and transients. Accordingly, a summated response of primary auditory neurones (N_1) appears not only at the onset of the stimuli, but also at the cessation. N_1-off is sharply tuned at 60–61 kHz, while N_1-on is tuned at 63–64 kHz, which is 2 kHz higher than the best frequency of the auditory system because of the envelope-distortion originating from sharp mechanical tuning. Single peripheral neurones sensitive to 61–62 kHz sounds have an unusually sharp tuning curve and show phase-locked responses to beats of up to 3 kHz. Information about the frequencies of Doppler-shifted echoes is thus coded by a set of sharply tuned neurones and also discharges phase-locked to beats. Neurones with a best frequency between 55 and 64 kHz show not only tonic on-responses but also off-responses which are apparently related to the mechanical off-transient occuring in the inner ear and not to a rebound from neural inhibition.

INTRODUCTION

There are three types of orientation sounds used by echolating bats: frequency modulated (FM) signals, signals containing a constant frequency (CF) in addition to FM, and noise bursts or clicks. For example, *Myotis lucifugus* uses FM sounds, *Rhinolophus ferrumequinum* and *Pteronotus parnellii* use CF-FM sounds, and *Rousettus amplexicaudatus stresemanii* uses very short noise bursts. 'CF-FM' bats such as *R. ferrumequinum* and *P. parnellii* adjust the frequency of the transmitted signals to receive Doppler-shifted echoes at a particular preferred frequency (Schnitzler, 1968, 1970). *R. ferrumequinum*, for instance, can detect a Doppler-shift as small as 0·05% in echo frequency and compensate for it (Schuller, Beuter & Schnitzler, 1974; Simmons, 1974). In these CF-FM bats, the auditory system is sharply tuned for reception of sounds near the preferred frequency (Grinnell, 1970; Neuweiler, 1970; Pollak, Henson & Novick, 1972; Suga, Simmons & Shimozawa, 1974). The onset and

cessation of an acoustic stimulus evoke summated activity of primary auditory neurones, respectively called N_1-on and N_1-off responses. In *R. ferrumequinum* and *P. parnellii*, the best frequency for N_1-on is slightly higher than for N_1-off (Grinnell, 1970; Neuweiler, Schuller & Schnitzler, 1971; Suga *et al.* 1974). The following basic questions are raised about echolocation and hearing in these CF-FM bats: (1) Whether the sharp tuning of the auditory system is due to mechanical resonance or neural interaction; (2) whether primary auditory neurones are specialized for coding small Doppler-shifts in echo frequency; and (3) whether N_1-off responses arise from mechanical events or rebound from neural inhibition. These questions are answered for the moustache bat *P. parnellii* in the data reported in the present paper. In Part I, we describe properties of the cochlear microphonic (CM) and N_1 responses. We have found that the sharp tuning of the auditory system is due to mechanical resonance in the inner ear and that CM responses show off-transients comparable to N_1-off responses. In Part II, we describe properties of primary and secondary auditory neurones. We have found that these peripheral neurones sensitive to the CF component of the orientation sound have extremely sharp tuning curves and that their off-responses are not due to rebound from neural inhibition. The frequency of a CF component in a Doppler-shifted echo is coded by neurones with exceptionally sharp tuning curves and with action potentials synchronized to beats produced by the overlap of the bat's outgoing sounds and returning echoes.

MATERIALS AND METHODS

Experimental subjects were 15 *Pteronotus parnellii* (previously named *Chilonycteris rubiginosa*) from Panama. For the recording of the cochlear microphonic (CM) and the summated activity of primary auditory neurones (N_1), the bats were anaesthetized with ether only during the surgery, which lasted about 1·5 h. For the recording of action potentials from peripheral auditory neurones, however, the bats were lightly anaesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg of body weight). Ether was used if the animal moved too much. The additional administration of sodium pentobarbital was avoided, if possible. Under anaesthesia, the dorsal part of the skull was exposed. A nail 1·8 cm long was then mounted on the exposed skull with glue (Eastman 910) and dental cement. The nail subsequently was fixed onto a metal rod with a set screw to immobilize the bat's head.

The CM and N_1 responses were recorded with a tungsten-wire electrode 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 soaked in physiological saline solution. Action potentials of peripheral auditory neurones were recorded with a micropipette electrode filled with 3 M-KCl solution. The dorsal cochlear nucleus was exposed after aspiration of the lateral portion of the cerebellum. From the surface of the dorsal cochlear nucleus, the micropipette electrode was inserted ventrally, in an attempt to place it in the modiolus to record action potentials from primary auditory neurones. 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 wall of which was covered with fibre-glass to reduce echoes. The room temperature was maintained at about 35 °C.

The electronic instruments used to generate acoustic stimuli were the same as those used in previous experiments (e.g. Suga, 1968). For studies on the CM and N_1, tone bursts with a 0·2 msec rise–decay time and a 4·0 msec duration were repeatedly delivered at a rate of 1·5 per second, unless described otherwise. Since the durations of the tonal stimuli were short, and the repetition rate of the stimuli was low, the data were not affected by the acoustic middle-ear-muscle reflex, which could occur with a 4·8 msec latency (Suga & Jen, 1975). For studies on responses of single neurones, however, tone bursts with a 0·5 msec rise–decay time and a 15 msec duration were repeatedly delivered at a rate of 1·5 per second, unless otherwise described, to give a closer approximation to the bat's orientation sounds, which average about 15 msec long. The amplitudes of the tone bursts delivered from a condenser loudspeaker were measured with a quarter-inch microphone (Brüel & Kjaer, 4135) placed at the bat's ear. The amplitude was expressed in dB SPL (sound pressure level referred to 0·0002 dyne/cm^2 r.m.s.).

The electronic instruments used to display or store the responses were basically the same as those used in previous experiments (e.g. Suga, 1968). The frequency of the CM response was examined with a specially built frequency-to-voltage converter (e.g. Fig. 1 B). Responses of single neurones to the tone bursts were expressed as the post-stimulus-time (PST) histograms made with a computer (Nicolet, model 1070).

Part I. Properties of cochlear microphonic and summated auditory nerve responses

Threshold curves for CM-on, CM-off, N_1-on, and N_1-off

Cochlear microphonics (CM) usually appeared only during presentation of tonal stimuli, and CM envelopes were usually very similar to those of the stimuli (Fig. 1, A, *a*). However, the envelope of the CM differed from that of the stimulus when the stimulus frequency was about 61 kHz (Fig. 1, A, *b* and *c*). When the stimulus amplitude was small, the amplitude of the CM slowly increased up to a certain level and reached a plateau at the beginning, then slowly decreased down to zero after the cessation of the stimulus, whereas the stimulus had more rapid rise–decay times. Hereafter, the CM after the cessation of the stimulus is called an after-response or *CM-aft*, and the CM at the beginning and during the stimulus is called an on-response or *CM-on*. At certain stimulus frequencies and levels, the CM-aft showed a prominent peak, and then an exponential decay in amplitude. The CM-aft which shows such an envelope peak is hereafter called an off-response or *CM-off*. A summated auditory nerve response (N_1) also appeared at the onset and cessation of each tone burst. Hereafter, these neural responses are called *N_1-on* and *N_1-off*, respectively (Fig. 1, A, *b*). As described later, the N_1-off was not related to the CM-aft, but to the CM-off, so that the terms CM-aft and CM-off are here introduced.

Fig. 2 shows the threshold curves for CM-on, N_1-on, and N_1-off. These curves all indicate that the CM and N_1-responses showed low thresholds at 30–35, 61–64, and 90–100 kHz, which are comparable to the frequencies of the 1st, 2nd, and 3rd harmonics of the CF component in the emitted signals and positively Doppler-shifted echoes. These responses were particularly sharply tuned at about 60–64 kHz. Since the best frequencies of these sharp tuning curves were slightly different over a range of

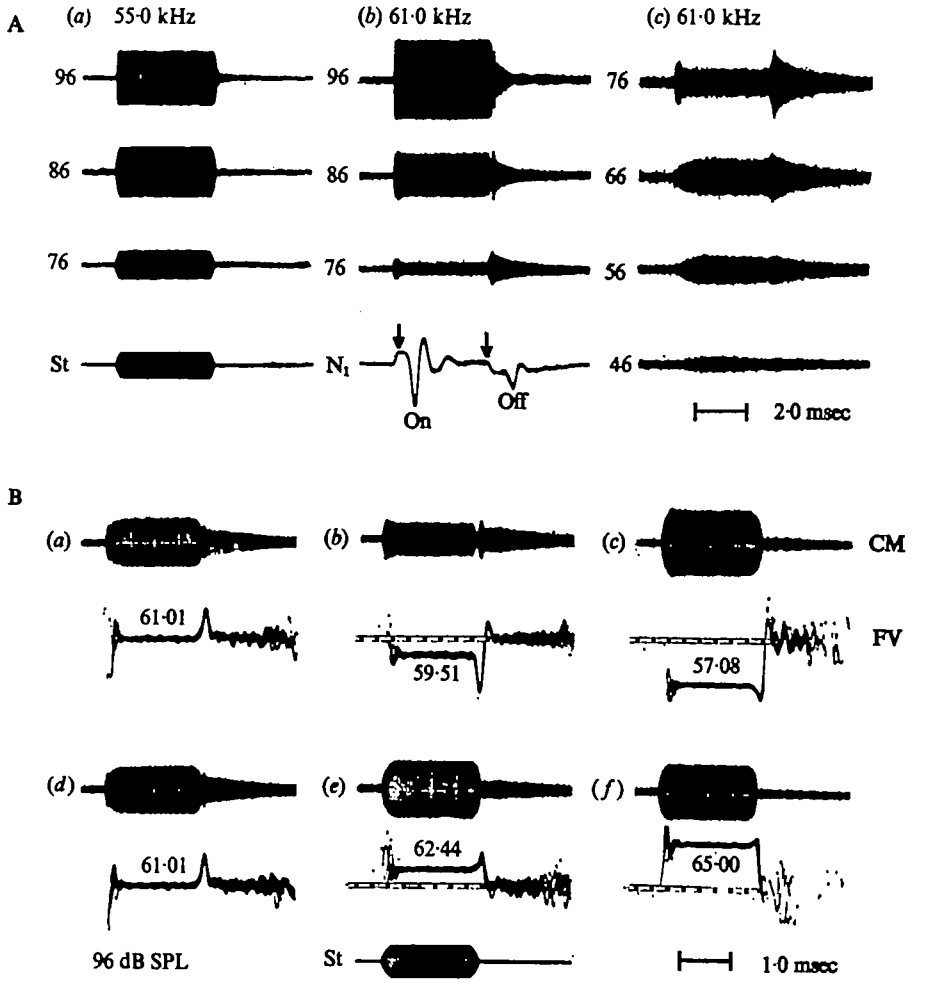

Fig. 1. Cochlear microphonics (CM) recorded from the round window. In A, the frequency of the tonal stimulus is 55·0 kHz for (*a*) and 61·0 kHz for (*b*) and (*c*). The stimulus amplitude is expressed in dB SPL by the number to the left of each CM trace. The stimulus envelope (St) is shown at the bottom of (*a*). The CM responses in (*c*) are shown at twice the amplification of those in (*a*) and (*b*). At the bottom of (*b*), N_1-on, N_1-off, and summating potentials are shown. The beginning and end of the plateau amplitude of the summating potential are indicated by the arrows.

B represents CM responses (upper traces) and the CM frequencies as analysed with the frequency-to-voltage converter (lower traces). A 96 dB SPL sound was delivered at 61·01 kHz in (*a*) and (*d*), 59·51 kHz in (*b*), 57·08 kHz in (*c*), 62·44 kHz in (*e*), and 65·00 kHz in (*f*). The envelope of the stimulus is shown at the bottom centre. The dotted horizontal lines show 61·0 kHz in the output of the frequency-to-voltage converter.

a few kilohertz from one individual bat to another, the average curves appeared less sharply tuned than individual curves. Therefore, individual threshold curves are hereafter presented rather than averaged ones. Since the 61–62 kHz CF component (2nd harmonic) in the orientation sound of *P. parnellii* is undoubtedly a principal information-bearing element in echoes (Suga *et al.* 1974), and since the auditory system is sharply tuned at about 61–62 kHz (Grinnell, 1970; Pollak *et al.* 1972; Suga

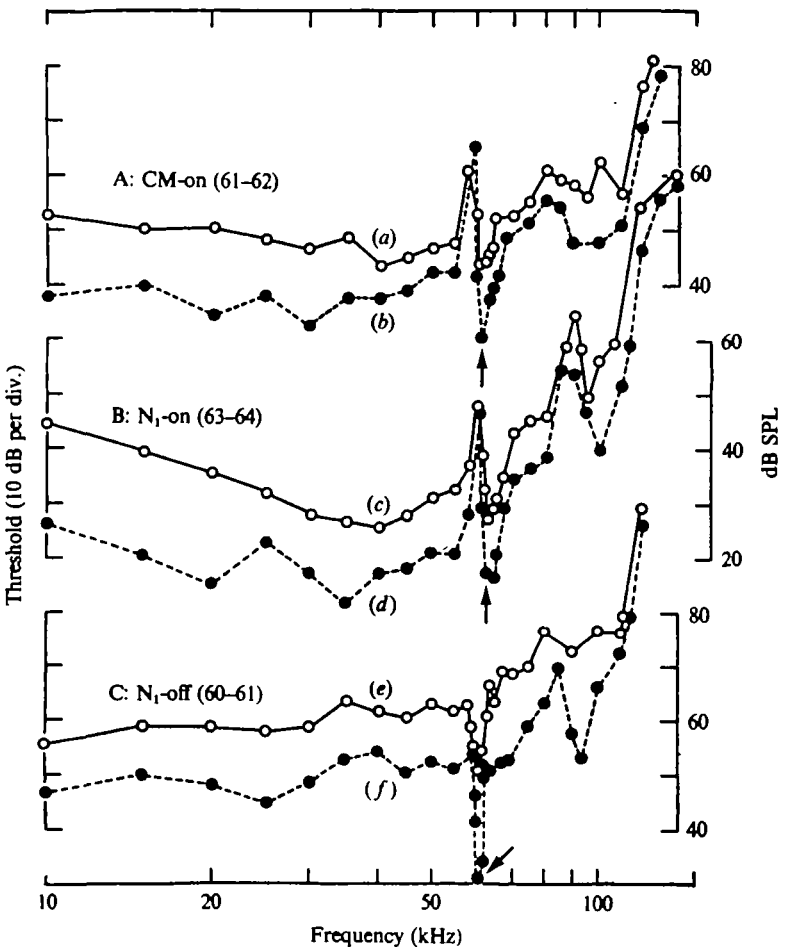

Fig. 2. Threshold curves of CM-on (A), N_1-on (B), and N_1-off (C). The solid curves are the average of the curves obtained from seven bats, while the dashed curves represent the lowest individual curves obtained from any one of the seven bats. The arrows indicate sharp notches in threshold curves. The best frequency, at which this notch appears, was 61–62 kHz for the CM-on, 63–64 kHz for the N_1-on, and 60–61 kHz for the N_1-off. The three ordinates to the right should be seen to overlap.

et al. 1974) the properties of CM and N_1 responses were examined most closely using stimulus sounds between 50 and 70 kHz.

Fig. 3 shows threshold curves for CM-on, CM-off, N_1-on, and N_1-off as measured in two bats. The best frequencies of such curves ranged from 60·5 to 62 kHz for CM-on, 60–61 kHz for CM-off, 63–64 kHz for N_1-on, and 60–61 kHz for N_1-off. The best frequency of CM-off was 0·5–1·0 kHz lower than that of CM-on. On the other hand, the best frequency of N_1-off was 2·0–3·5 kHz lower than that of N_1-on. CM-on and N_1-on had different best frequencies, while CM-off and N_1-off had nearly the same best frequencies. Why were there such differences and similarities in the best frequencies? Why did CM and N_1 both show off responses?

The slopes of the threshold curves away from the best frequencies varied greatly from animal to animal. In the CM-on threshold curve, the threshold decreased at a

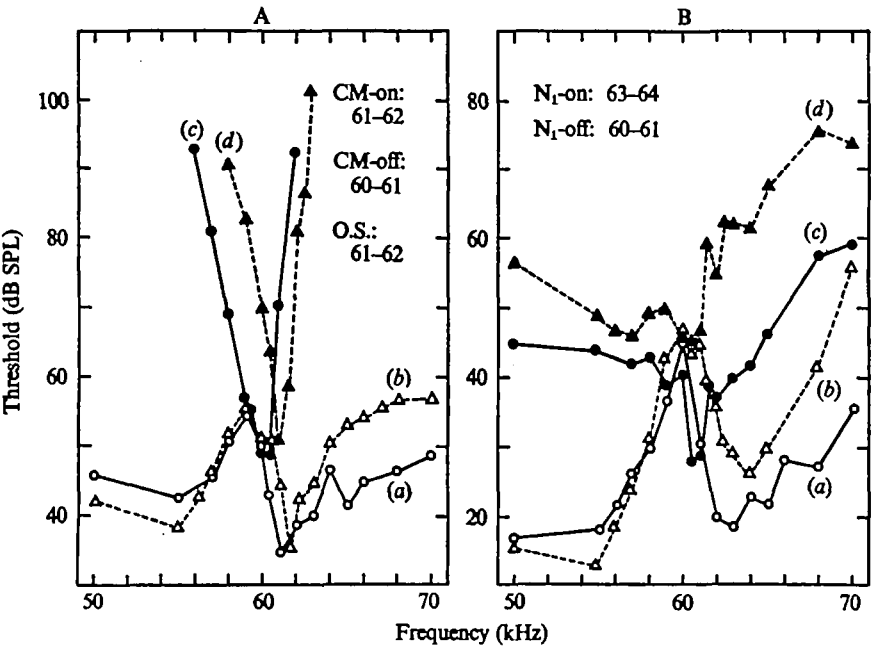

Fig. 3. Threshold curves of CM-on, CM-off, N_1-on and N_1-off obtained from two bats. A, *a* and A, *b*, CM-on; A, *c* and A, *d*, CM-off, B, *a* and B, *b*, N_1-on; B, *c* and B, *d*, N_1-off. All solid curves were obtained from one bat and all dashed ones from the other. Note the difference in best frequency between the solid and dashed curves. The CF component in the 2nd harmonic of the orientation sound (O.S.) ranged between 61 and 62 kHz in both bats.

rate of 200–800 dB/octave for frequencies going upward toward the best frequency and increased at a rate of 200–600 dB/octave going upward beyond it. In the CM-off threshold curve, on the other hand, the threshold decreased at a rate of 300–850 dB/octave going upward toward the best frequency and increased at a rate of about 1200 dB/octave going upward beyond it. Like the degree of sharpness of tuning in an electronic filter, the sharpness of the threshold curve can be expressed by a Q-value, which is the best frequency divided by the bandwidth 3 dB above the minimum threshold. The Q-value for the CM-on threshold curve ranged between 47 and 154, while that for the CM-off threshold curve ranged between 110 and 174. Is this large Q-value for the CM-on threshold curve related to the presence of CM-off and N_1-off responses?

CM and N_1 responses and sharp tuning

As shown in Fig. 1, A, the CM slowly increased and decreased in amplitude when a stimulus close to a frequency of 61 kHz was turned on and off with a 0·2 msec rise-decay time. This phenomenon reveals that such a CM response was evoked in a mechanical system with a sharp tuning curve or resonance. The mechanical system involved in our experiments consisted of the condenser loudspeaker, tympanic membrane, ossicular chain, basilar membrane, and associated structures. The output of the loudspeaker as monitored with a Brüel & Kjaer quarter-inch microphone closely followed the input electrical signal. There was no stimulus artifact comparable to CM-aft. The CM-aft was definitely due to the physical properties of the ear itself.

In order to demonstrate that CM-aft was due to a resonating element in the ear sharply tuned at 61–62 kHz, CM responses to tone bursts were analysed with a frequency-to-voltage converter (Fig. 1, B). When a 61 kHz tone burst was delivered, the CM-on was, of course, at 61·0 kHz, and CM-aft was about 61·4 kHz (Fig. 1, B, *a*). This indicates that 61·4 kHz was the resonance frequency of the sharply tuned element. For stimuli lower than 61·0 kHz, e.g. 59·5 kHz, the CM-on was 59·5 kHz, but the CM-aft was 61·4 kHz (Fig. 1, B, *b*). For sounds higher than 61·0 kHz, e.g. 62·4 kHz, the CM-on was 62·4 kHz, but the CM-aft was 61·4 kHz (Fig. 1, B, *e*). When the frequency of a tone burst was lower than 55 kHz or higher than 65 kHz, the CM-aft became very small. Whenever CM-aft was evoked, however, its frequency was at the resonance frequency, 61·4 kHz. These data clearly indicate that the sharply tuned resonating element in the ear shows forced movements during stimulation at neighbouring frequencies, and then gradually damped movement at its own resonance frequency after cessation of stimulation due to energy stored during the forced movement and after excitation by side-bands in the sound at the cessation.

At the onset of the stimulus which caused CM-aft, the CM also showed a transient response comparable to the CM-aft. This CM-on transient was prominent for a moderately weak stimulus (e.g. Fig. 1, A, *c*) and sometimes was associated with beats due to the difference between the resonance frequency (61–62 kHz) and the frequency of the tonal stimulus. It was, however, not prominent for an intense stimulus (e.g. Fig. 1, A, *b*). This may be due to forced movement, occurring over a large area on the basilar membrane.

Assuming that the inner ear incorporates a simple resonating element, the Q-value can be calculated from $Q = \pi\lambda f$, where λ is the time constant of damped oscillation and f is the resonance frequency. f was about 61 kHz. λ was about 0·7 msec in CM responses to 80–90 dB sounds, but it was about 1·3 msec in the CM responses to 60–70 dB sounds (e.g. Fig. 1, A, *c*). The Q-value thus ranged between 134 and 250, which is larger than the Q-value (47–154) calculated with the CM-on tuning curve. As described above, the time constant of the decay of the CM-aft became smaller with an increase in stimulus level. Furthermore, the envelope of the CM-aft deviated from an exponential decay when the stimulus level was high (Fig. 1, A, *b* and *c*). These two phenomena were probably due to a larger contribution to the CM-aft of activities of hair cells with tuning curves slightly different from those of the hair cells with the sharpest tuning curves. It was expected that single hair cells tuned at about 61 kHz would have very sharp tuning curves. Recording of the receptor potential from single hair cells, however, appeared to be very difficult, so that tuning curves of single auditory nerve fibres tuned at 61 kHz were studied (see part II).

Both CM-on and CM-aft showed an identical best frequency (Fig. 4A), indicating that the same sharply tuned resonating element is responsible for evoking both. The best frequency of CM-off was the same as that of N_1-off (Fig. 3), which indicates that N_1-off is related to CM-off.

The difference in the best frequency between the CM-on and N_1-on appeared to be related to the slow rise in the amplitude of the CM at the resonance frequency of the sharply tuned element (e.g. Fig. 1, A, *c*). The synchronization of the action potentials of primary auditory neurones at the onset of a stimulus became poor with a slow

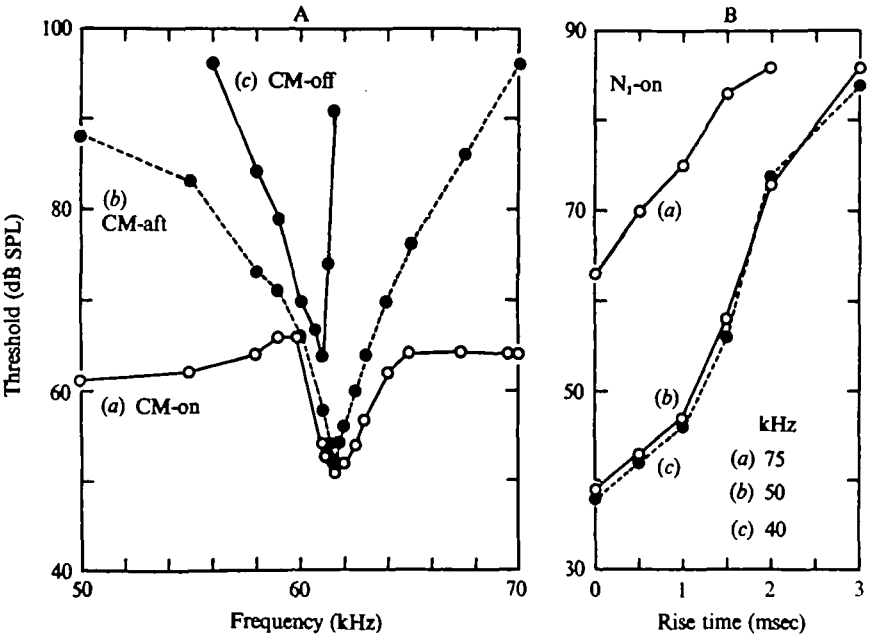

Fig. 4. Graph A shows threshold curves of CM-on, CM-aft, and CM-off obtained from a single bat. Graph B shows the change in threshold of N_1-on with the change in rise time of a 10 msec long tone burst. The N_1-on was evoked by a tone burst of either 75 (*a*) or 50 (*b*) or 40 (*c*) kHz.

stimulus rise time. The mechanical resonance effectively slowed the rise time of the CM response and had the same effect on the synchronization as would a slower stimulus rise time, so that the N_1-on threshold for the sound at the resonance frequency would become higher. In Fig. 1, A, *c*, the rise time of the CM for a 66 dB SPL, 61·0 kHz stimulus is about 1·0 msec. If the stimulus level at 61·0 kHz is much lower than 66 dB SPL, the rise time of the CM may become even longer and may be comparable to the decay time of CM-aft, which is about 4 msec. To examine the validity of the explanation that the high threshold of N_1-on at 61–62 kHz is due to poor synchronization of impulses of primary auditory neurones, we measured the extent to which the threshold of N_1-on changed with tone bursts of different rise times. The frequencies of these tone bursts with variable rise times were kept away from 61 kHz, so that the envelope of the CM response was comparable to that of the acoustic stimulus.

As shown in Fig. 4B, when the rise time is 1·0 msec the N_1-on threshold is 8 dB higher than when the rise time is abrupt (0·01 msec). When the rise time is 3·0 msec, the threshold is 47 dB higher. The N_1-on threshold greatly increased with increasing rise time of the stimulus, regardless of its frequency. These data thus indicate that, if the envelope of the CM (i.e. the movement of the basilar membrane) at the onset of a sound at 61–62 kHz were the same as the envelope of the CM at other frequencies, the N_1-on threshold at 61–62 kHz would be much lower than that measured, even lower than that at frequencies of 63–64 kHz. One may conclude that the best frequency of this bat's auditory nervous system is the same as the resonance frequency of the

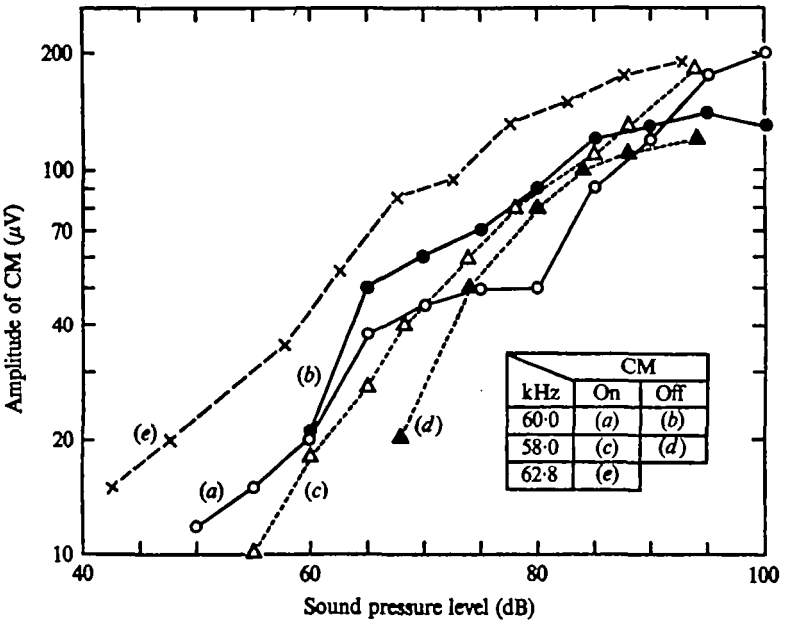

Fig. 5. Response-amplitude functions of the CM-on (curves *a*, *c*, and *e*) and CM-off (curves *b* and *d*). The frequency of a 4 msec long tone burst was 60·0 kHz for (*a*) and (*b*), 58·0 kHz for (*c*) and (*d*), and 62·8 kHz for (*e*). The CM-off was nearly absent at 62·8 kHz.

ear (61–62 kHz). In fact, studies on single primary auditory neurones demonstrate that minimum thresholds of neurones tuned at 61–62 kHz are much lower than those tuned at 63–64 kHz (Fig. 14). Vocal responses to acoustic stimuli (i.e. behavioural responses) are also sharply tuned at about 62 kHz (Suga *et al.* 1974).

Response-amplitude functions of CM-on and CM-off

The amplitudes of the CM-on and CM-off responses varied differently as a function of stimulus level (Figs. 1, A, and 5). At the best frequency of CM-off, the amplitude of CM-on (for a steady state) reached a plateau for stimuli between 70 and 80 dB SPL, and then increased further with stimulus levels higher than 80 dB SPL.The CM-on response thus showed a very prominent non-linear response-amplitude function (Fig. 5, curve *a*). On the other hand, the peak amplitude of the CM-off response monotonically increased and then reached a plateau for stimuli between 80 and 90 dB SPL (Fig. 5, curve *b*). CM-off was larger than CM-on for stimuli between 60 and 90 dB SPL, but it was always smaller than CM-on for stimuli at 100 dB SPL. At stimulus frequencies other than 60–61 kHz, CM-on monotonically increased in amplitude (Fig. 5, curves *c* and *e*) and was larger than N_1-off regardless of stimulus level.

Middle-ear muscles and sharp tuning

If the sharp tunings observed in CM and N_1 responses were due to the ossicular chain, they should be affected by contraction of the middle-ear muscles because of the resulting increase in stiffness of the ossicular chain. Pollak *et al.* (1972) observed that

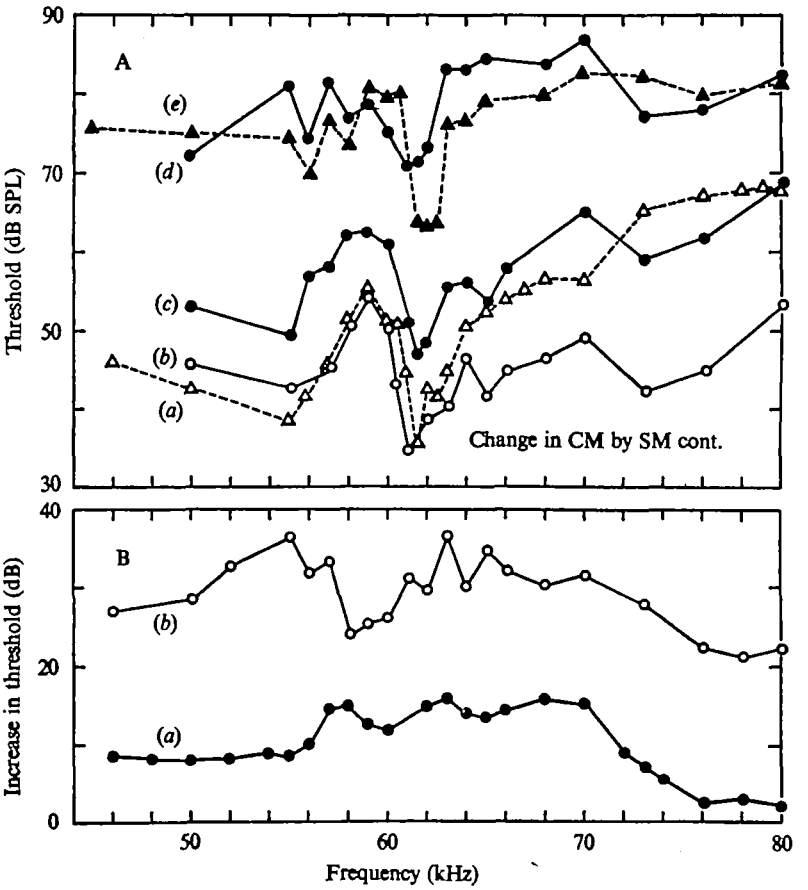

Fig. 6. Effect of the contraction of the stapedius muscle (SM) on the threshold of the CM-on. In A, (*a*) and (*b*) represent the threshold curves measured without the SM contraction, while (*c*), (*d*), and (*e*) show those measured during the SM contraction evoked by electric stimuli at different intensities. The solid and dashed curves indicate that the curves were obtained from two different bats. B represents the frequency-attentuation curves of the SM, i.e. the increase in threshold of the CM-on response by the SM contraction as a function of frequency of a tone burst. These curves were the average of the data obtained from two bats. (*a*) and (*b*) were obtained during the weak and strong contractions of the SM, respectively.

CM-on was much less sharply tuned in a deeply anaesthetized *P. parnellii* than in an awake one. If this is true, it is conceivable that either an increase in tension of the middle-ear muscles and/or an increase in activity of the olivo-cochlear bundle sharpened the tuning. In echolocating bats, the stapedius muscle is highly developed and controls incoming signals more than the tensor tympani muscle does (Wever & Vernon, 1961; Henson, 1970; Suga & Jen, 1975). Accordingly, a tungsten wire electrode was placed on the stapedius muscle and 0·1 msec electric pulses were delivered to the muscle at a rate of 350 per second in order to determine the effect of its tetanic contraction on the threshold curve of CM-on (Fig. 6).

When the stapedius muscle was weakly stimulated, the CM threshold increased by 10–15 dB for sounds between 58 and 70 kHz, while it sometimes decreased by about 5 dB for sounds around 80 kHz. When intense electrical stimuli were delivered to the

tapedius muscle, not only the stapedius muscle but also the tensor tympani muscle contracted. In this case, such a decrease in threshold around 80 kHz was never evoked. When the threshold for 50–70 kHz sounds increased by 25–35 dB, the threshold for an 80 kHz sound increased about 22 dB. The best frequency of the CM-on and the sharpness of its threshold curve around the best frequency were not significantly changed either by the contraction of the stapedius muscle alone, or by the contraction of the stapedius and tensor tympani muscles together (Fig. 6). Therefore, it seems very unlikely that the sharp tuning at 61–62 kHz is due to resonance in the middle ear, and that it is modified by the contraction of the middle-ear muscles. The sharply tuned element appeared to be in the inner ear.

Ossicular chain, middle-ear cavity, and sharp tuning.

In order to confirm that the sharp tuning at 61–62 kHz was not due to a resonating element in the middle-ear, but rather to one in the inner ear, the threshold curve of the CM-on was measured before and after the elimination of the ossicular chain and the tympanic membrane (Fig. 7). Since it was impossible to eliminate the ossicular chain without making a wide opening in the auditory bulla, and likewise impossible to nicely close this wide opening, we first examined whether there was a difference in threshold curve before and after opening the auditory bulla. As far as measurements between 45 and 80 kHz were concerned, no noticeable change in threshold curve was evoked by widely opening the auditory bulla. The sharp tuning was apparently not due to a resonance of the middle-ear cavity.

After the removal of the ossicular chain and the ear drum, the oval window was usually covered by a small amount of lymph and/or coagulated blood. The following data were obtained in this condition. Without the ossicular chain, the threshold of the CM-on increased 10–20 dB for sounds at the best frequency (61–62 kHz), while the threshold for other sounds between 45 and 80 kHz increased 30–40 dB (Fig. 7, B). Thus, the threshold curve after the elimination of the ossicular chain and tympanic membrane was sharper than that measured before the elimination (Fig. 7, A). The best frequency was not changed by the elimination of the ossicular chain. The envelope of the CM was quite different from that of the tone burst at 61–62 kHz, as observed before the elimination of the ossiculi (Fig. 1). CM-on, CM-aft, and CM-off still occurred. These data clearly indicate that the mechanical element sharply tuned at 61–62 kHz is in the inner ear.

FM components and off-responses

The orientation sound of *P. parnellii* always consists of a long CF component followed by a short FM component. In the predominant second harmonic, the frequency stays nearly constant at 61–62 kHz for 5–30 msec, and then it sweeps down to about 50 kHz during a few milliseconds. If the CF component produces an off-response which lasts for a few milliseconds, the off-response may interact with an on-response evoked by the FM component. Suga *et al.* (1974) have found that N_1-off and LL-off (LL: lateral lemniscal evoked potential) evoked by the CF tone burst are greatly suppressed by the addition of an FM sound at the end of the CF sound. We examined here how the CM-aft evoked by a CF tone burst was affected by the addition of an FM sound at the end of the CF (Fig. 8).

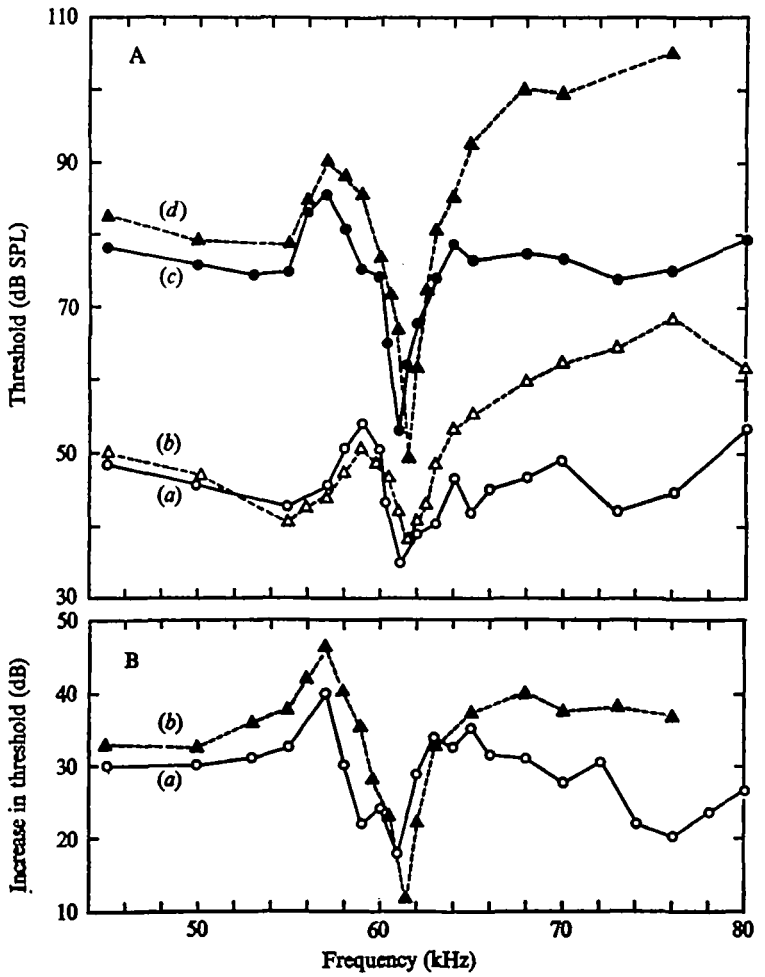

Fig. 7. Effect of the elimination of the ossicular chain on the threshold curve of the CM-on. In A, (*a*) and (*b*) represent the threshold curves measured before the removal of the ossiculi, while (*c*) and (*d*) show those measured after the removal. B represents the increase in threshold of the CM-on by the removal of the ossicular chain as a function of frequency. All solid curves were obtained from one bat, while all dashed ones, from the other.

When a 61·7 kHz tone burst was delivered, the CM-aft lasted about 4·9 msec, and the N_1-off was prominent (Fig. 8, A). By adding a 0·72 msec FM component sweeping from 61·7 to 58·7 kHz to the end of the 61·7 kHz tone, the CM-aft (slow decay in CM amplitude) was reduced in amplitude and duration. The large CM at the end of the stimulus was the response to the FM component.The summated neural activity at the end of the stimulus was much smaller than N_1-off and showed a peak latency longer than that of the N_1-off (Fig. 8, B). It appeared to consist predominantly of responses to the FM component. In other words, N_1-off to the CF component was greatly reduced by the addition of the short FM component to the CF component. The CM-off further decreased by an increase of the FM sound in duration and frequency sweep. When the FM component was 2·2 msec in duration and swept from

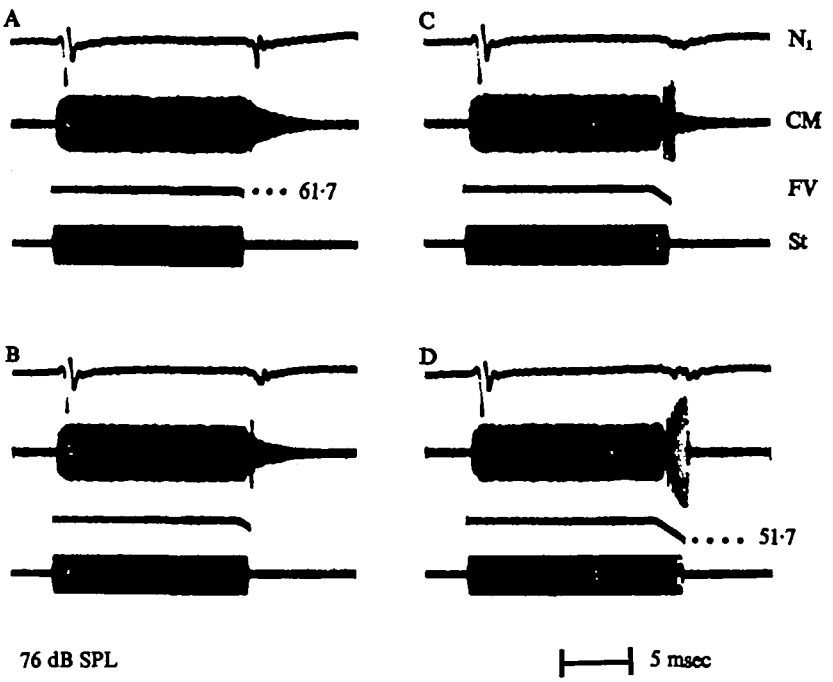

Fig. 8. The effect of an FM sound on the CM-aft and N_1-off evoked by a CF tone. The CF tone is 61·7 kHz and 15 msec long, while the FM tone varies in frequency and duration. The duration and sweep range of the FM sound are respectively 0·3 msec and 61·7 to 59·7 kHz in A, 0·7 msec and 61·7 to 58·7 kHz in B, 1·3 msec and 61·7 to 56·4 kHz in C, and 2·2 msec and 61·7 to 51·7 kHz in D. In each photograph, the four traces represent N_1, CM, the output of the frequency-to-voltage converter (FV), and stimulus (St), respectively. The FV converter was used to show the frequency sweep in the stimuli. The stimulus amplitude was 76 dB SPL regardless of frequency, but the CM amplitude varied with stimulus frequency.

61·7 to 51·7 kHz (as in the orientation sound), the CM evoked by the FM was prominent and the CM-aft disappeared (Fig. 8, D). The complex wave of the neural response at the end of the stimulus was mainly due to successive excitation of primary auditory neurones with different best frequencies evoked by the FM component, and not to the N_1-off evoked by the CF component. It may even be possible to conclude that this complex wave did not include any significant N_1-off, because the CM-aft disappeared with the addition of the FM component.

Since the CM reflects the motion of the basilar membrane to a great extent, the above data indicate that the suppression of the CM-aft and N_1-off by the addition of the FM component was not due to any neural inhibition, but was simply due to a mechanical event in the inner ear. That is, the resonating element (i.e. a particular part of the basilar membrane) sharply tuned at 61–62 kHz was driven during the acoustic stimulus. When the stimulus of 61–62 kHz was suddenly stopped, the sharply tuned element showed damped oscillation at the resonance frequency, i.e. the after-response or off-response. When the frequency of the stimulus moved away from the resonance frequency, as is the case with the addition of the FM component to the end of the CF component, the tuned element was driven by the stimulus sweeping

down and out of its response range. The vibration amplitude of the resonator decreased according to the shape of its tuning curve. Thus, little energy was stored in the tuned element, and the damped oscillation did not occur.

Part II. Properties of peripheral auditory neurones

Discharge patterns of single neurones

Responses to acoustic stimuli were studied in 216 single neurones. These neurones were located mainly in the auditory nerve and posterior ventral cochlear nucleus. Out of the 216, 196 neurones showed only excitatory responses to tonal stimuli regardless of stimulus frequency and amplitude, while 20 neurones changed their response patterns from excitatory to inhibitory responses with stimulus frequency and also sometimes with stimulus amplitude. Excitatory responses were commonly tonic on-responses which were followed by post-excitatory suppression of background activity, if any. The post-stimulus-time (PST) histograms of excitatory responses showed a prominent peak at the beginning of the response and then a decay toward a plateau because of adaptation (Fig. 10, B1). These histograms were comparable to those of primary auditory neurones sensitive to high frequencies in cats and monkeys. In keeping with the fact that the envelope of the CM did not follow that of the acoustic stimulus between 56 and 63 kHz and showed prominent transient responses at stimulus onset and cessation (Figs. 1 and 3), single neurones sensitive to these sounds showed not only tonic on-responses, but also off-responses which were comparable to the CM-off and CM-aft. Between the phasic on- and off-responses, background activity, if any, was not inhibited (Fig. 9). The latencies of on- and off-responses were comparable to those of N_1-on and N_1-off, so that responses of these neurones apparently could contribute to N_1-on and N_1-off.

Fig. 9 shows examples of responses of a single neurone tuned at 63·0 kHz. At 65·0 kHz, the PST histograms showed that the neurone responded with a relatively constant latency and tonically discharged during the stimulus. There were no after discharges, but there was post-excitatory suppression of background activity (D). At 63·0 kHz, the neurone showed prolonged after discharges comparable to the CM-aft, but no peak of discharges corresponding to the CM-off (C). For stimuli at 59·5 and 60·5 kHz, however, the neurone showed after-discharges which clearly corresponded to the CM-off (A and B). These may be called off-responses to distinguish them from the after discharges which showed no peak as shown in C. When tonic on-responses were prominent at high stimulus levels, the off-responses were not clear. With a decrease in stimulus amplitude, the tonic on-responses became less prominent and only the phasic part of the on-response remained. The off-response was then very prominent (A and B). As already described, background activity was not inhibited prior to off-responses; that is, the off-responses were not due to rebound from neural inhibition. Since the threshold of tonic on-responses of the neurone in Fig. 9 was 91 dB SPL for a 59·5 kHz tone and 88 dB SPL for a 60·5 kHz tone, the phasic on- and off-responses to sounds below the thresholds were obviously responses to transients occurring in the inner ear at the onset and cessation of the stimuli (Fig. 1, A).

No neurones were found which showed only off-responses as a rebound from neural inhibition, regardless of the frequency and amplitude of the tone burst. These data on

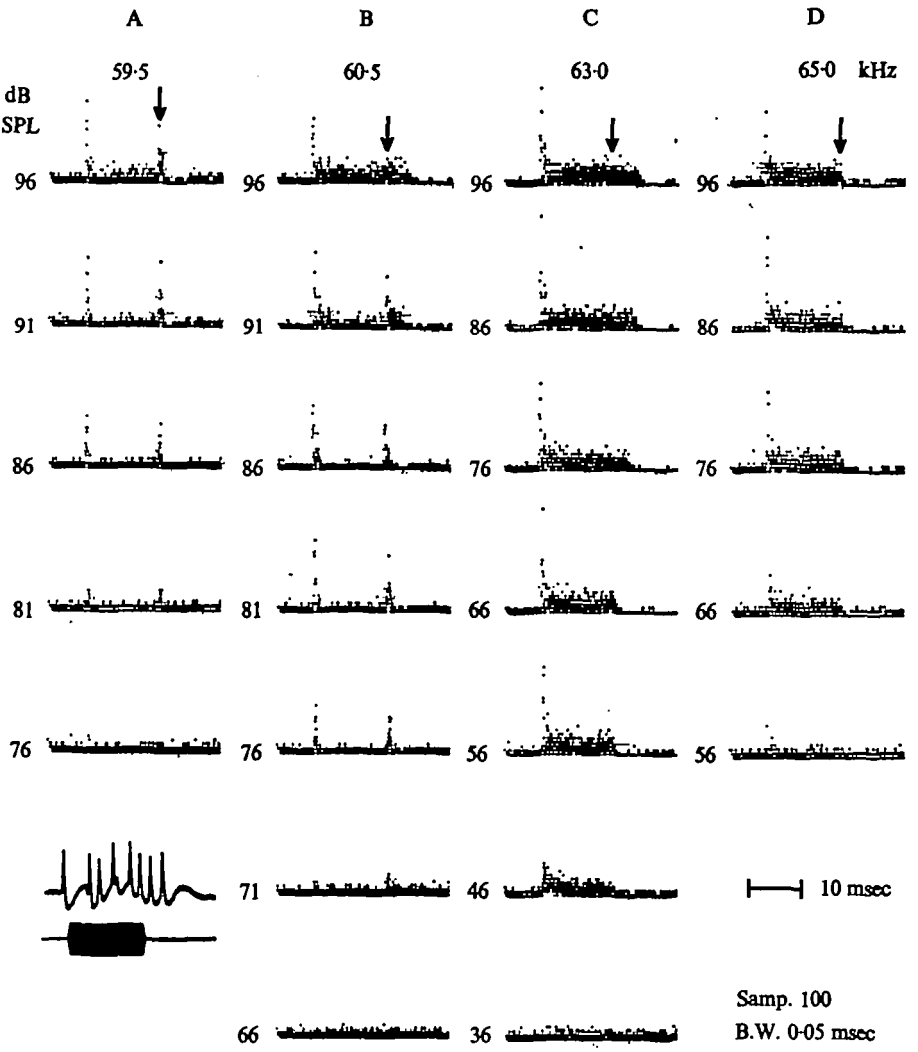

Fig. 9. Post-stimulus-time (PST) histograms representing responses of a single neurone to tone bursts of 59·5 (A), 60·5 (B), 63·0 (C), and 65·0 kHz (D). The stimulus level is indicated by the figures to the left of each histogram. The duration of the tone burst and its rise–decay time are 15 and 0·5 msec, respectively. Each stimulus was delivered 100 times. The bin width of the histogram is 0·05 msec. The beginning of after (or off)-discharges is indicated by the arrow. On the bottom left, a response to a tone burst is shown as an example.

response patterns in neurones clearly indicate that the N_1-off was due to off-discharges of single neurones sharply tuned between 55 and 64 kHz, and that the N_1-off was not due to rebound from neural inhibition following cessation of the stimulus.

In our extracellular recordings, inhibitory responses were observed only when neurones were spontaneously active, unless a pair of sounds was delivered. In the 20 neurones which showed either excitatory (tonic on) or inhibitory responses to single tone bursts depending upon stimulus frequency, inhibition of background discharges lasted more than 10 msec after the cessation of the stimulus when it was

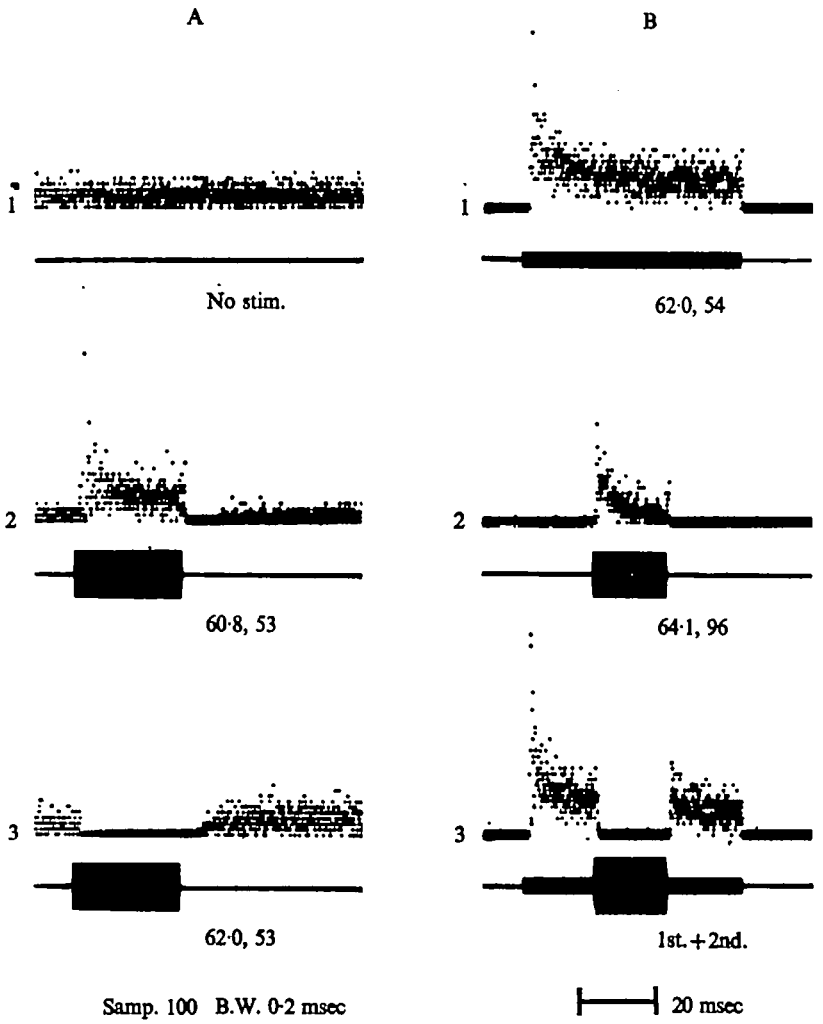

Fig. 10. PST histograms of responses of two neurones (A and B). A1, background discharges; A2, excitatory response to a 60·8 kHz, 53 dB SPL sound; A3, inhibitory response to a 62·0 kHz, 53 dB SPL sound; B1, excitatory response to a 62·0 kHz, 54 dB SPL sound; B2, excitatory response to a 64·1 kHz, 96 dB SPL sound; B3, two-tone suppression by a simultaneous delivery of the 62·0 and 64·1 kHz sounds. Note the difference in time course at the end between neural inhibition and two-tone suppression. Each stimulus was delivered 100 times. The bin width is 0·2 msec.

an intense inhibitory sound. Since the inhibition slowly disappeared, off-discharges were absent or were not prominent (Fig. 10, A). Off-discharges, if any, appeared with a latency longer than 10 msec after the cessation of an intense inhibitory tone, so that these off-discharges, which were undoubtedly a rebound from neural inhibition, did not contribute at all to N_1-off. Since several primary auditory neurones converged upon a single cochlear nuclear neurone, some of the cochlear nuclear neurones tuned to sounds between 55 and 64 kHz showed complex response patterns. For instance, some of the PST histograms showed a phasic on-response followed by tonic inhibition, and then a phasic off-response followed by inhibition. Background activity showed

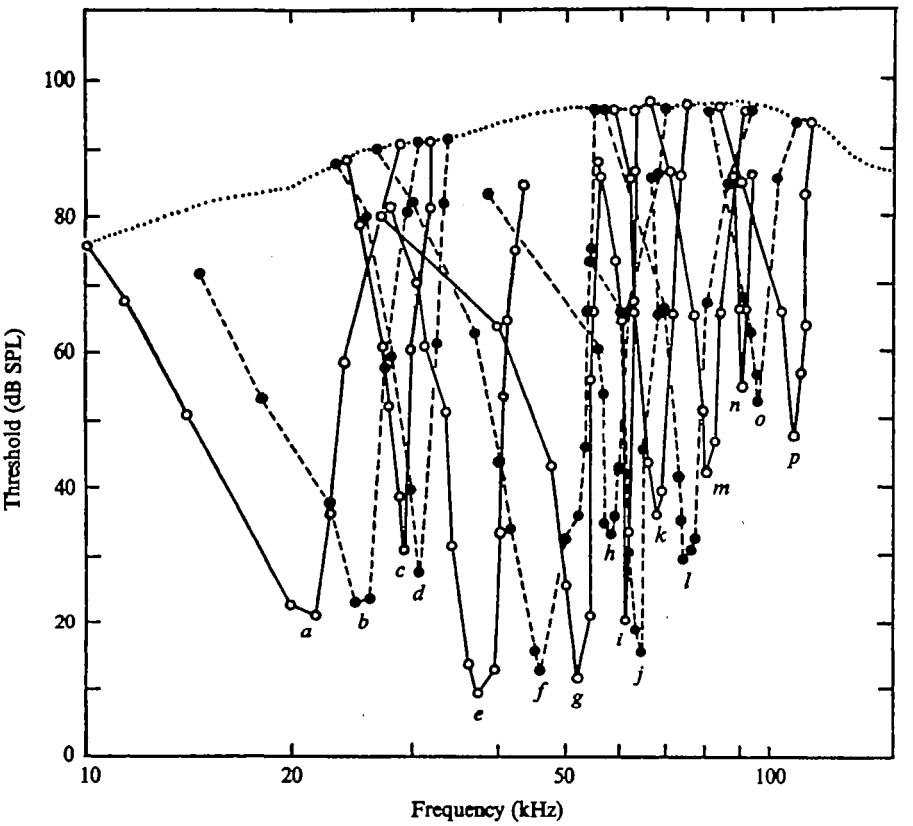

Fig. 11. Tuning curves (or excitatory areas) of sixteen single neurones (*a–p*). The uppermost dotted line is the frequency-response curve of the loudspeaker. Note the change in sharpness of the curves with best frequencies.

a small rebound from the inhibition. Such a complex response pattern, however, can be explained by a simple interaction of excitatory and inhibitory neurones.

As in cats (Sachs & Kiang, 1968) and monkeys (Nomoto, Suga & Katsuki, 1964) peripheral auditory neurones in *P. parnellii* showed 'two-tone suppression' (or inhibition). One of the two presented tones was excitatory for a given neurone, and the other tone by itself evoked either excitation or no change in the background activity of the neurone. When these two sounds were simultaneously delivered, however, the excitation from one tone was suppressed. The time course of two-tone suppression was quite different from that of neural inhibition. As shown in Fig. 10, B, off-discharges immediately occurred at the cessation of the short second tone.

Tuning curves of single neurones

Tuning (or threshold) curves of 168 single neurones were measured in terms of their excitatory responses, i.e. tonic and phasic on-responses. The area above the tuning curve is called the excitatory (or excitatory response) area. The best frequencies of these neurones ranged between 22 and 110 kHz (Figs. 11–14). We noticed that the excitatory areas for neurones tuned approximately at 30, 61 or 92 kHz were narrower than for those tuned at frequencies other than near 30, 61, or 92 kHz (Fig. 11). In

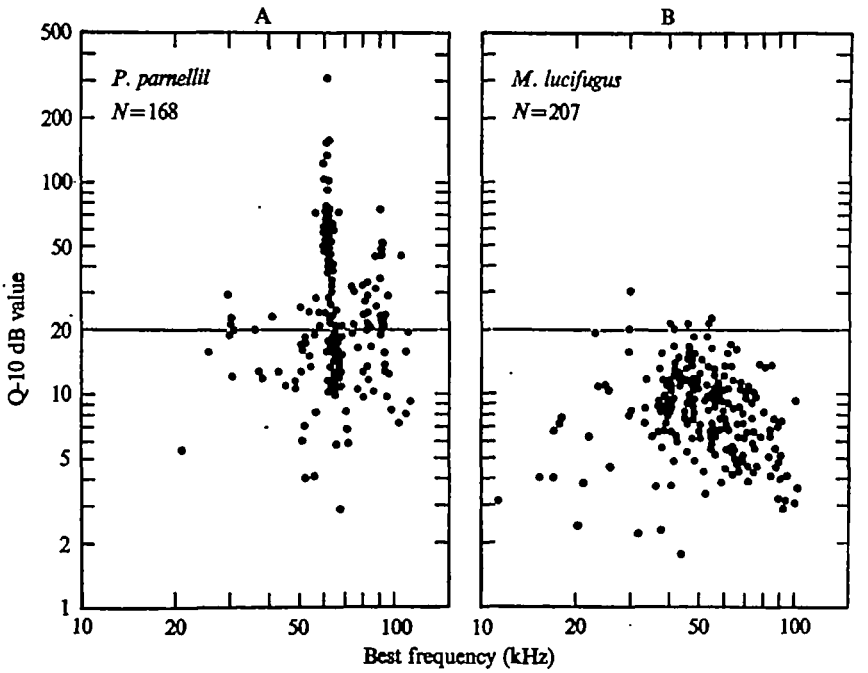

Fig. 12. Distribution of Q-10 dB values of excitatory areas against best frequencies. (A) 168 neurones were sampled from *P. parnellii*. (B) 207 neurones were sampled from *M. lucifugus* (based on data obtained by Suga, 1973).

particular, the areas tuned around 61 kHz were extremely narrow, even narrower than the narrowest excitatory area of neurones found in the inferior colliculus or auditory cortex of *Myotis lucifugus*. In these extremely sharp tuning curves, the threshold decreased at a rate of 1200–2700 dB/octave with an increase in frequency toward the best frequency. Above the best frequency, the threshold increased at a rate of 1700–3900 dB/octave (e.g. Fig. 11, *i*, and Fig. 15, D and E). Thus, a change in frequency of only a few percent around the best frequency of such neurones caused a 50–60 dB change in the threshold of their responses. Such extremely sharp tuning curves were obtained only from neurones tuned between 60 and 62 kHz and also about 92 kHz. The best frequencies of neurones tuned at 60–62 kHz differed slightly from one another. A minor difference in frequency within this range could thus be coded by activation changing from one group of neurones to another.

The sharpness of the 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 (Kiang, 1965). Fig. 12, A and B, respectively show distributions of Q-10 dB values as a function of best frequency in *P. parnellii* and *M. lucifugus*. For *M. lucifugus*, Q-10 dB values were less than 20 except for a few neurones (Suga, 1973). For *P. parnellii*, on the other hand, about half of the Q-10 dB values were greater than 20. Thus, at the periphery, the excitatory areas of *P. parnellii* were substantially narrower than those of *M. lucifugus*. For best frequencies around 61 or 92 kHz, the Q-10 dB value exceeded 40. The largest Q-10 dB value obtained was 310. Since the Q-10 dB value varied drastically according to best frequency, particularly

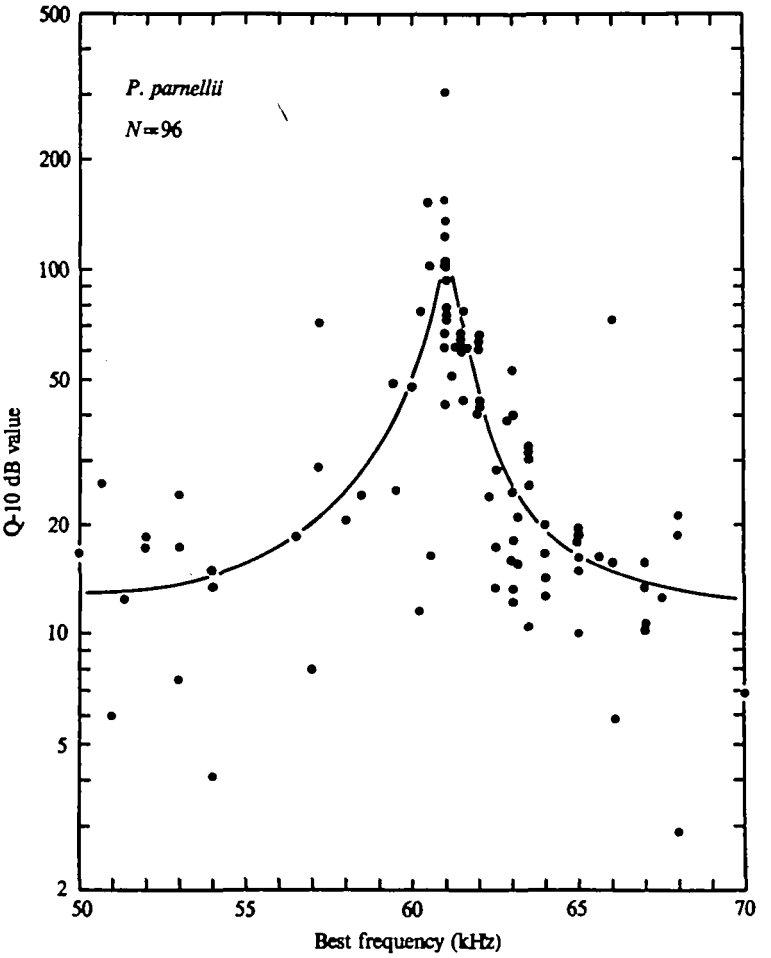

Fig. 13. Distribution of Q-10 dB values of excitatory areas of 96 single neurones tuned at sounds between 50 and 70 kHz. The ordinate and abscissa represent Q-10 dB values and best frequencies, respectively.

at about 61 kHz, these data from *P. parnellii* were plotted with an expanded frequency axis (Fig. 13). The Q-10 dB value was greatest at 61·0 kHz, and it was smaller at best frequencies either lower or higher than 61·0 kHz. Fig. 15 also shows such changes in the sharpness of tuning curves with changes in best frequency. The curve in Fig. 13 represents the approximate Q-10 dB value as a function of the best frequency. Such a difference in Q-10 dB values with differences in best frequency indicates that the peripheral auditory system of *P. parnellii* is highly specialized for frequency analysis within the region of frequencies present in CF components of orientation sounds. Changes in Q-10 dB value with changes in best frequency appear to exist in *M. luci-fugus* (Fig. 12, B). Q-10 dB values at best frequencies between 30 and 60 kHz tend to be larger than those at best frequencies away from this range.

The Q-10 dB values of the neurones tuned at 61 kHz ranged between 42 and 305 (103 on the average), which corresponds to Q values ranged between 92 and 670. The

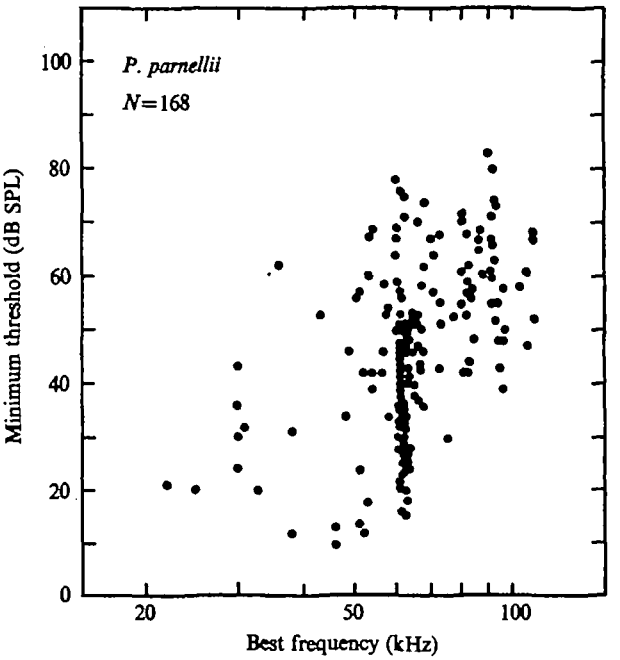

Fig. 14. Distribution of minimum thresholds of excitatory responses against best frequencies. The sample number is 168.

Q value calculated with both the time constant and frequency of the CM-aft, i.e. the damped oscillation of the CM, ranged between 134 and 250.

In our experiments, neurones tuned to sounds higher than 50 kHz were frequently recorded, although we looked for neurones sensitive to sounds lower than 50 kHz. In particular, neurones sensitive to 61–62 kHz were most frequently encountered. The minimum thresholds of these neurones were generally lower than those of neurones tuned at 63–64 kHz (Fig. 14). The distribution of minimum threshold fits the CM-on threshold curve tuned at 61–62 kHz rather than the N_1-on threshold curve tuned at 63–64 kHz (Figs. 2 and 3), so these data indicate that the difference in best frequency between the threshold curves of the CM-on and N_1-on is not due to an actual difference in a threshold curve between the receptor potential and neural activity, but rather to slow rise times for energizing the resonator at 61–62 kHz, as already described.

We paid particular attention to finding neurones which showed only off-responses to single tone bursts and, if any, to measure an 'off-turning curve' or 'off-area'. There were no such neurones among the 216 sampled. However, neurones tuned at between 55 and 64 kHz showed off-responses to tone bursts at certain amplitudes and frequencies. As already described, these off-responses were not related to neural inhibition, because background discharges were not at all inhibited during tonal stimuli (Fig. 9). The best frequencies for the off-responses (hereafter called off-best frequency) ranged between 59·5 and 61·5 kHz, regardless of the best frequency for on-responses (hereafter called on-best frequency), as shown in Fig. 15. Thus, the on-best frequency would be either lower (A and B) or higher (D to H) than the off-best frequency, or nearly the same (C). Since CM-off was tuned at 60–61 kHz, the off-

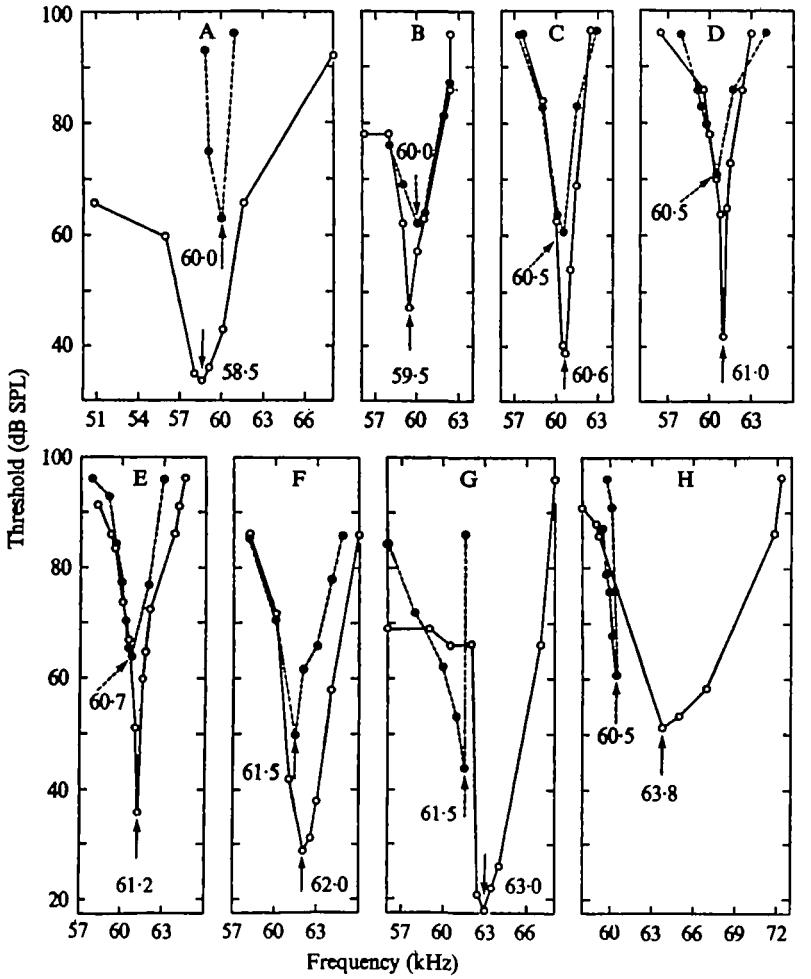

Fig. 15. Excitatory and off-areas of eight single neurones (A–H). The solid and dashed curves represent the excitatory and off-areas, respectively. The solid and dashed arrows indicate the best frequencies of the excitatory and off-responses, respectively. Here, the excitatory response means tonic and phasic on-discharges, and the off-response means after and off-discharges. These best frequencies are also given by the figures near the arrows. These neurones did not show inhibitory responses at all.

responses of these single neurones are obviously related to CM-off which is evoked by a mechanical event in the inner ear.

About 10% of the neurones studied showed not only excitatory responses, but also inhibitory responses to single tonal stimuli (Fig. 10, A). The inhibitory area was often very large on the side higher than the best frequency (Fig. 16, A). Some neurones showed an upper-threshold, so that these neurones responded very poorly to sounds above 90 dB SPL. In the example shown in Fig. 16, A, the neurone had an upper-threshold for sounds slightly higher than its best frequency. The upper-threshold was due to neural inhibition, because the area above the upper-threshold was occupied by the inhibitory area. Since the inhibitory areas effectively narrowed the excitatory area

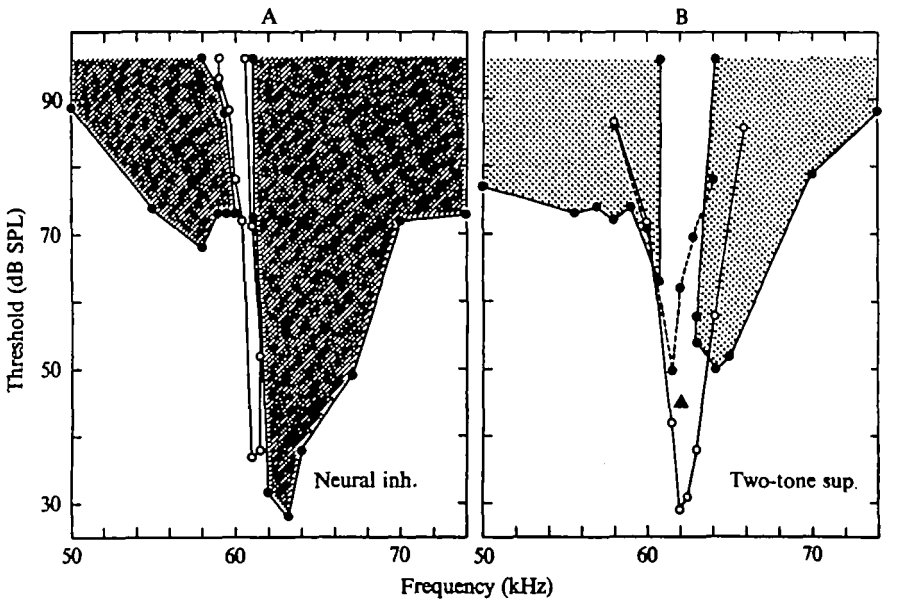

Fig. 16. A, the excitatory (unshaded) and inhibitory (shaded) areas of a single neurone. In the shaded area, a single sound caused inhibition of background activity. B, the excitatory (unshaded) and suppressive (dotted) areas of a single neurone. In the dotted areas, a single sound simultaneously delivered with the sound at the best frequency (solid triangle) caused suppression of the response to the latter. In this neurone, the off-area was also measured (dashed curve). The background activity of the neurone was not at all inhibited by a single tone.

at high stimulus levels, the excitatory area tuned at 61 kHz was very narrow for all stimulus levels, unlike that of a neurone without inhibitory areas.

In some of the neurones which did not show neural inhibition, areas for two-tone suppression were measured. The 'suppressive area' was usually overlapped with the excitatory area and was larger for frequencies higher than the best frequency than for lower frequencies (Fig. 16, B). There appeared to be no basic difference in two-tone suppression between *P. parnellii* and other mammals (cats and monkeys).

FM components and off-discharges

CM-off and N_1-off were greatly reduced by adding an FM sound at the end of a CF tone near 61 kHz (e.g. Fig. 8); and a comparable phenomenon was observed in PST histograms of responses of single neurones. Fig. 17, A, shows responses of a single neurone to a 60·5 kHz tone delivered at three different amplitudes. Off-responses (to the right of the solid bars in Fig. 17) were most prominent with a weak sound (A3) than with an intense sound (A1). This is comparable to what was observed in CM responses (Fig. 1, A, *b*). When a 3·0 msec FM sound sweeping from 60·5 to 55·0 kHz was added at the end of the CF tone, the off-discharges were clearly reduced (Fig. 17, B). Discharges during the FM sound (i.e. those between the solid and dashed bars) were probably responses to the FM signal rather than after-discharges evoked by the CF tone, since the FM signal swept in frequency away from the resonance frequency at 61·0 kHz, which was very close to the best frequency of the neurone, 61·3 kHz. After the termination of the FM signal (the dashed bars in Fig. 17) there was no excitation, but only post-excitatory suppression (B1 and B2).

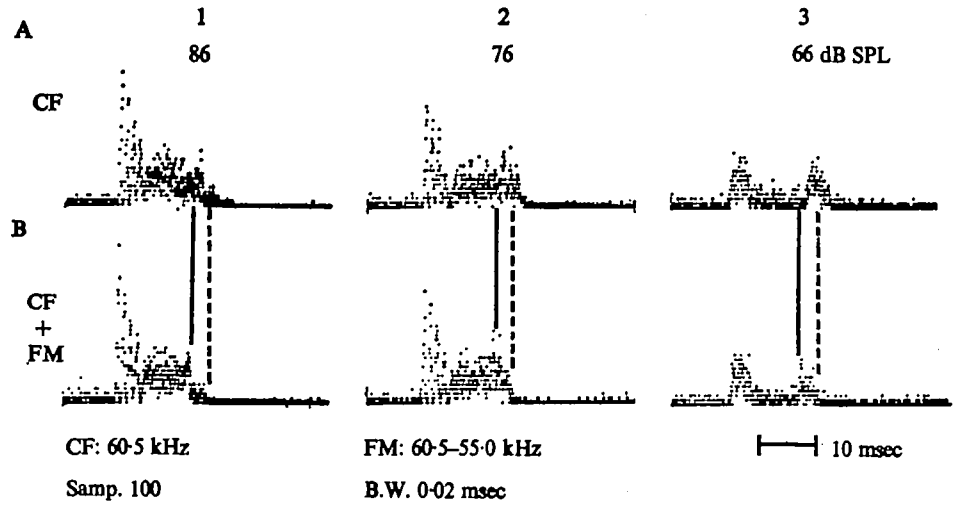

Fig. 17. The effect of an FM sound on the off-discharges evoked by a CF tone. Responses of a single neurone to a CF tone at 60·5 kHz (A) and to the CF tone immediately followed by an FM tone (B) are expressed by PST histograms. The best frequency of the neurone was 61·3 kHz. The FM tone swept from 60·5 to 55·0 kHz and was 3·0 msec long. The solid and dashed perpendicular lines indicate the ends of the CF and FM tones, respectively. In A and B, the sound pressure level is 86 dB for 1, 76 dB for 2, and 66 dB for 3. Each stimulus was delivered 100 times and responses to them are shown by the PST histograms. The bin width is 0·02 msec.

Coding of beats

Because of the long duration (5–30 msec) of the emitted orientation signal in *P. parnellii*, an orientation sound and its echo usually overlap in the bat's cochlea and produce beats. The percent modulation in the beat may be very small acoustically at the external ear and in terms of CM responses, but it may be very large at a particular place on the basilar membrane where the effective amplitude of a Doppler-shifted echo can be comparable to that of the vocal self-stimulation. The beats may carry information about the Doppler shift, i.e. a frequency difference between emitted sounds and echoes. In *R. ferrumequinum*, it has been demonstrated that the animal compensates for a Doppler shift only when echoes significantly overlap with the emitted signals (Schuller, 1974). Thus, the beat appears to carry important information for the compensation behaviour. Accordingly, the extent to which the beats were coded by peripheral auditory neurones was studied. Two tone bursts, slightly different in frequency, were simultaneously delivered at a repetition rate of four per second. The amplitudes of these stimuli were adjusted to be an equal number of decibels higher than the thresholds of the neurone at the frequencies of the two stimuli. Since excitatory areas tuned at about 61 or 92 kHz were very sharp, the amplitudes of the two sounds could differ from each other by as much as 10–60 dB, depending on the slope of the tuning curve and the separation of the sounds in frequency. The phase relation between the sounds was kept constant from delivery to delivery, and responses to the paired signals were expressed as PST histograms. In Fig. 18, two intense sounds are delivered in certain combinations in order to evoke many discharges and clearly show phase-locked responses to beats.

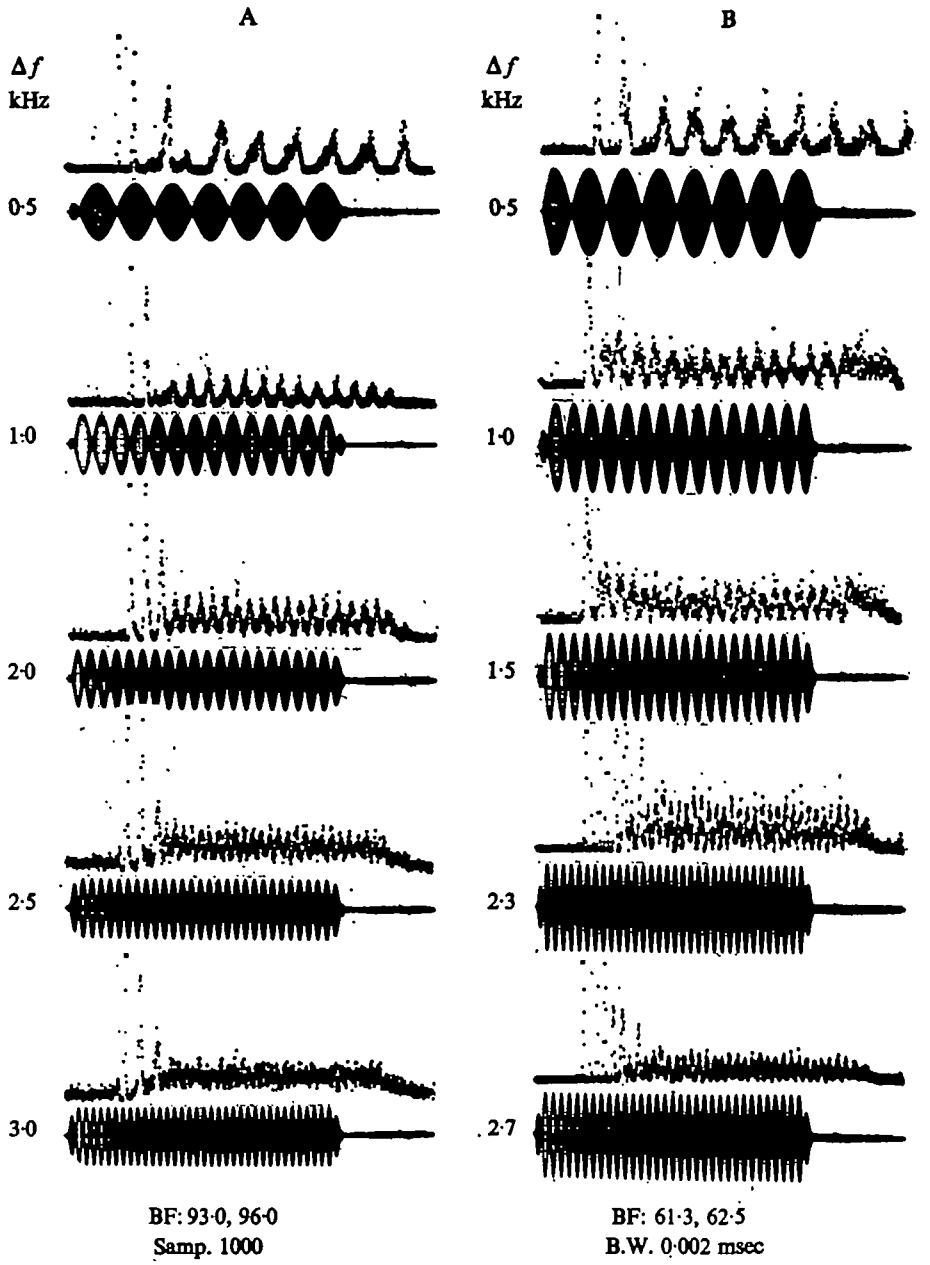

Fig. 18. Responses of single neurones to beats. A, PST histograms of responses of two neurones which were tuned at either 93·0 (photo 1·0–3·0) or 96·0 kHz (photo 0·5). B, PST histograms of responses of two neurones which were tuned at either 61·3 (photo 1·0 and 1·5) or 62·5 kHz (photo 0·5, 2·3 and 2·7). Two sounds with a 15 msec duration and 0·5 msec rise–decay time were phase-locked and delivered 1000 times. The frequencies and amplitudes of these sounds were 95·6 kHz, 76 dB and 96·1 kHz, 76 dB for A-0·5; 93·0 kHz, 77 dB and 94·0 kKz 77 dB for A-1·0; 92·0 kHz, 77 dB and 94·0 kHz, 77 dB for A-2·0; 91·5 kHz, 77 dB and 94·0 kHz, 77 dB for A-2·5; 91·0 kHz, 77 dB and 94·0 kHz, 77 dB, for A-3·0; 63·0 kHz, 96 dB and 63·5 kHz, 96 dB for B–0·5; 60·5 kHz, 76 dB and 61·5 kHz, 96 dB for B-1·0; 60·0 kHz, 96 dB and 61·5 kHz, 76 dB for B-1·5; 61·2 kHz, 96 dB and 63·5 kHz, 96 dB for B-2·3; and 60·8 kHz, 96 dB and 63·5 kHz, 96 dB for B-2·7. The bin width is 0·002 msec. The sound waveforms (lower traces) and PST histograms (upper traces) were photographed separately and were put together to show the relationship between the beats and the PST histograms.

The PST histograms clearly indicated that when a beat frequency was lower than 2·5 kHz, nerve impulses from a single neurone were well synchronized with the beats, except for the phasic discharges at the beginning of the response. Above 2·5 kHz, synchronization was poor, but it was still often observed up to 3·0 kHz (Fig. 18). At the beginning of the response, the first action potential was discharged with a relatively constant latency, so that the following one or two action potentials tended to be discharged with a certain fixed delay. Thus, action potentials occurring within 3 msec of the beginning of the response poorly coded or did not code at all the information about the beats.

DISCUSSION

Structural specialization in the inner ear

In cats, the threshold curve of the ear is determined primarily by the resonance in the external ear, the transfer function in the middle ear, and the 'input' impedance of the cochlea (Dallos, 1973). There is however, a question as to whether this is also true in other animals. It is very likely that not only physical properties of the external and middle ears, but also those of the inner ear are important in determining the threshold curve of the ear. In *P. parnellii*, the sharp frequency sensitivity at 61–62 kHz originates in the inner ear (Fig. 7), although the overall threshold curve, broadly tuned at about 30 kHz (Suga *et al.* 1974), may be mainly due to the physical properties of the external and middle ears for sound transmission. In *R. ferrumequinum*, the threshold curve is broadly tuned at about 25 kHz and also sharply tuned at 83–84 kHz (Neuweiler, 1970; Neuweiler *et al.* 1971). The structure of the cochlea of *R. ferrumequinum* has been studied by Ikeda & Yokote (1939), Pye (1966), and Bruns (1975). According to the recent excellent work by Bruns (1975), the 16 mm long basilar membrane of *R. ferrumequinum* 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 4·0 and 4·6 mm from it. This area, 4·0–4·6 mm from the round window, is concerned with the reception of sounds of 83–84 kHz (Bruns, 1975). The sharp tuning at 83–84 kHz in *R. ferrumequinum* is very likely due to the properties of the inner ear rather than those of the external and middle ears, although no experiments comparable to ours have yet been performed on this species.

In *P. parnellii*, the anatomical features of the inner ear have not yet been well studied in relation to the sharp tuning of hearing at 61–62 kHz. The basilar membrane changes in width and thickness without showing a discontinuity comparable to that of *R. ferrumequinum* (Pye, 1967). Our present experiments indicate that there should be some morphological specialization in the inner ear. According to our recent measurements, the basilar membrane is 12·2 mm long. The external wall of the cochlea is enormously thick at about 4·5 mm apical from the round window. At this area, the densities of hair cells and innervation are low, while the area immediately apical to this place (5–7 mm from the round window) is highly innervated and contains more sensory cells than the other area. The width of the basilar membrane increases very gradually toward the apex at this richly innervated area (Shaffer *et al.* unpublished). This area may be concerned with the reception of sounds at 61–62 kHz, since neurones sensitive at about 61 kHz were the most frequently found and were the

most sharply tuned (Fig. 12, A). Information about the detailed structure of the inner ear and the local hair cell damage after the exposure of the ear to an intense 61 kHz tone remains to be obtained.

After (or off)-response and sharp tuning

Since off-responses are very prominent in *P. parnellii* (Grinnell, 1970) and *R. ferrumequinum* (Neuweiler *et al.* 1971), the cause of these unusual off-responses has been discussed. Grinnell (1973) concluded that 'the properties of the off-response appear compatible with a mechanism whereby vibration of a certain part of the basilar membrane causes an electrical and mechanical bias that reduces spontaneous activity in a population of primary fibres innervating an adjacent part of the partition, possibly by producing tonic hair cell transmitter release, with termination of this suppression resulting in synchronous resumption of spontaneous firing at a normal or greater than normal rate'. This conclusion is not supported by our data, because PST histograms of peripheral auditory neurones clearly indicate that the off-response is not due to a rebound from suppression (Figs. 9 and 17). Neuweiler *et al.* (1971) attempted to explain the off-response by a rebound from neural inhibition. This also is probably not the case. When the responses of second or higher-order neurones are studied, the off-response following inhibition may be recorded. Such off-responses may be due to rebound from neural inhibition occurring within the brain or may be due to the superimposition of the off-response originating from CM-off upon neural inhibition.

Whether the off-response observed in extracellular recording is due to neural inhibition can be determined from the following conditions. (1) The off-response due to the CM-off (or mechanical transient) appears only with sounds which evoke the CM-off, while the off-response due to neural inhibition occurs for sounds in a range which is not necessarily the same as that for the CM-off (Fig. 16, A). (2) The latency of the off-response of a single neurone should be the same or nearly the same as that of the off-response of summated neural activity. Off-discharges that arise from rebound from neural inhibition usually occur with a latency longer than that of a summated off-response originating from the CM-off.

After-discharges of primary auditory neurones have been found in *M. lucifugus* (Suga, 1964) and cats (Kiang, 1965). In cats, the basilar membrane shows damped oscillation for a monophasic click stimulus. When the damped oscillation lasts longer than the refractory period of a neurone, the neurone shows multiple discharges. Q-10 dB values of primary auditory neurones of cats range between 6 and 20 for best frequencies of about 10 kHz (Kiang, 1965), so that after-discharges following responses to high frequency tone bursts are usually not noticeable because of rapid damping. The present experiments on the CM and responses of single neurones in *P. parnellii* clearly indicate that a particular part of the inner ear (or basilar membrane) is specialized for the fine frequency analysis of sound at 61–62 kHz by having a sharp mechanical resonance. Because of this sharp resonance, the inner ear shows prominent ringing, i.e. the after (or off)-response. The best frequencies for the CM-on and CM-aft were the same (61–62 kHz), but the best frequency for the CM-off was 60–61 kHz, 0·7–1·0 kHz lower than that for the CM-on. This discrepancy appears to be related to a discontinuity in the basilar membrane and also to prominent non-linearity in the response of the cochlea at 60–61 kHz. The response-amplitude function of the CM-on was very

non-monotonic at the frequency of the sound for which the CM-off showed the minimum threshold. This may be related to the structural discontinuity. A sharply tuned resonator in the inner ear is probably mechanically coupled with a region of the basilar membrane where a prominent non-linearity is found.

The situation can be visualized as follows: due to the specialization of a particular part of the inner ear for fine frequency analysis of sounds at 61–62 kHz, a sudden change occurs at the boundary between the specialized and non-specialized parts. This boundary is located on the basilar membrane at a place tuned to 60–61 kHz. When a sound is delivered at 60–61 kHz, the place sharply tuned at 61–62 kHz shows forced movement during the steady state of the stimulus, because it is mechanically coupled with the place tuned at 60–61 kHz. At the termination of the stimulus, scattering of sound energy around 60–61 kHz occurs, so that the place tuned at 61–62 kHz is excited by side bands in the sound and starts to vibrate at its own resonant frequency. The amplitude of the vibration reaches a peak, then decreases. Thus the CM-off appears. When a sound is delivered at 61–62 kHz, the place tuned to 61–62 kHz of course shows a forced movement during the steady state of the stimulus. At the cessation of the stimulus, the energy absorbed by the resonator at 61–62 kHz leads to continued damped oscillation which simply decreases with time. Therefore, the vibration (ringing) at 61–62 kHz occurs without showing an increase or peak in amplitude. The CM-aft thus appears without the CM-off.

Functional role of off-responses

Grinnell (1973) concluded that the off-response was a rebound from a suppression caused by some interaction occurring in the inner ear and that the function of this interaction is a peripheral sharpening of frequency resolution and a sensitization of auditory neurones to the lower frequencies of the downward sweep at the end of the orientation sounds. According to our data (Figs. 9 and 17), the off-response is not due to a rebound from suppression so that the off-response is neither directly nor indirectly related to such peripheral sharpening of frequency resolution and sensitization of a certain group of peripheral neurones. The off-response is due to the sharply tuned resonator and non-linearity in the inner ear.

The off-response is prominent with a CF tone at 60–61 kHz. However, *P. parnellii* does not emit the CF tone alone for echolocation. Its orientation sound always consists of the CF component followed by the FM component. When the CF tone at 60–61 kHz is followed by a short FM sound, the CM-aft and N_1-off are greatly reduced (Fig. 8). The CM and N_1-responses to the terminal part of the CF-FM sound appear to consist primarily of the response to the FM sound. Masking experiments (Suga *et al.* 1974) also lead to the same conclusion. It seems unlikely that the off-response due to the transient in the inner ear plays an important role in echolocation in *P. parnellii*.

Coding of echo frequency for Doppler compensation

Since the duration of the CF component in the orientation sounds of *P. parnellii* and *R. ferrumequinum* is long, an echo usually overlaps with the emitted sound. In *R. ferrumequinum*, the Doppler-shift compensation takes place only when an artificial echo overlaps with an orientation sound with a latency less than 20 msec (Schuller,

1974). A difference in frequency between the emitted sound and the echo may be coded by a set of neurones sharply tuned to slightly different frequencies and/or discharges phase-locked to beats. In *P. parnellii*, the neurones tuned at 61–62 kHz showed very narrow excitatory areas. Such neurones, regardless of their best frequencies, could show phase-locked responses to beats up to 2·5–3·0 kHz. In *R. ferrumequinum*, the threshold curve of a summated neural response (LL) is sharply tuned at about 83 kHz and broadly tuned at about 25 kHz (Neuweiler *et al.* 1971). As in *P. parnellii*, *R. ferrumequinum* has a set of neurones very sharply tuned across the region of 83 kHz (Suga, Neuweiler & Möller, unpublished). In any mammal, primary auditory neurones appear to show phase-locked responses to beats up to 3 kHz whenever two sounds within the excitatory area of each neurone are simultaneously delivered in a proper amplitude relationship. Therefore, *P. parnellii* and *R. ferrumequinum* are equipped with these two potential Doppler-shift coding mechanisms.

For the measurement of a frequency difference, the beat information might be very accurate but by itself it would not contain information about whether the frequency difference is positive or negative, if the orientation signal consisted of only CF components. In the orientation sound, however, the CF component is always followed by the FM component, so that it is theoretically possible to identify whether the Doppler-shift is positive or negative. That is, when the echo is higher than the emitted signal and delays more than a few milliseconds from it, the beat frequency increases at the end of their overlap. On the other hand, when the echo is lower than the emitted signal, it first decreases and then increases at the end. However, this mechanism for the detection of the sign of the frequency shift may not work or may work very poorly because the duration of the FM component is less than 5 milliseconds. In *R. ferrumequinum*, the Doppler-shift compensation is correctly performed without overlap between an FM component of an emitted signal and an artificial echo (Schuller, personal communication). It is very unlikely that the beats are the primary cue for the Doppler-shift compensation.

Unlike *M. lucifugus*, cats, and monkeys, the peripheral auditory neurones of *P. parnellii* that are sensitive to sounds at 61–62 kHz show very sharp tuning curves. This is a clear indication of the specialization of the inner ear for fine frequency analysis. In *P. parnellii*, a primary cue for the Doppler-shift compensation is probably carried by such a set of sharply tuned neurones. Our recent experiments on primary auditory neurones of *R. ferrumequinum* indicate that the neurones sensitive to about 83 kHz have very narrow tuning curves (Suga, Neuweiler & Möller, unpublished). *R. ferrumequinum* appears to rely primarily on the information carried by a set of sharply tuned neurones. Critical behavioural studies remain to be performed on whether the bat uses both of the above mechanisms or only one of them for frequency compensation.

Functional roles of sharp tuning

The neurones sensitive to 60–62 kHz are very sharply tuned and their best frequencies are slighly different from neurone to neurone. The neurones with best frequencies between 90 and 93 kHz are also sharply tuned. These two groups of sharply tuned neurones apparently indicate the specialization of the cochlea for fine

analysis of the second and third harmonics of the CF component in orientation sounds and echoes. These sharply tuned neurones without inhibitory areas respond to CF sounds within a very narrow range, but also respond to FM sounds and noise bursts whenever these sound energies fall into their narrow excitatory areas. Thus, these neurones are not specialized to respond only to CF sounds. In the cochlear nucleus, there are neurones which have a very narrow excitatory area (or tuning curve) with inhibitory areas on only one side or both sides of it (e.g. Fig. 16, A). From the series of previous experiments with *M. lucifugus* (e.g. Suga, 1973), it is expected that these neurones fail to respond to FM sounds sweeping across the inhibitory area and then the excitatory area and also fail to respond to noise bursts which simultaneously stimulate both the excitatory and inhibitory areas. The cochlear nucleus appears to have asymmetrical neurones which basically can respond to CF tones, FM sounds, and noise bursts, but responses to FM sounds are quite different depending on the direction of frequency sweep. For instance, a certain asymmetrical neurone may respond to upward sweeping FM sounds, but not to downward sweeping FM sounds. Conversely, another may respond to downward sweeping FM sounds, but not to those sweeping upward. The cochlear nucleus also appears to contain CF-specialized neurones which selectively respond to CF tones within a narrow frequency range, but not to FM sounds and noise bursts. Such neurones represent one type of 'simple feature detector' (Suga, 1973).

An auditory system with very narrowly tuned neurones at the periphery has advantages not only in fine frequency analysis of CF tones, but also in echo-detection and detection of wing beats of insects. When a positively Doppler-shifted echo comes back, *R. ferrumequinum* and *P. parnellii* adjust the frequency of the CF component in their orientation sounds so as to receive the CF component in the echo at a certain preferred frequency (Schnitzler, 1968, 1970). Such interesting acoustic behaviour has been more thoroughly studied in *R. ferrumequinum* (Schuller, 1974; Schuller *et al.* 1974) than in *P. parnellii*. It was recently confirmed that *R. ferrumequinum* adjusts the frequency of the CF component in the orientation sound to receive the CF component in echoes at a certain preferred frequency to which the auditory system is sharply tuned (Schnitzler, Simmons & Suga, unpublished). If this is also true in *P. parnellii*, the CF component of the emitted signal during echolocation would very poorly stimulate the auditory system, because the ear is insensitive to sounds slightly lower than the preferred frequency (Grinnell, 1970; Pollak *et al.* 1972; Suga *et al.* 1974). In other words, vocal self-stimulation during echolocation would be very weak. Recovery cycles of single neurones greatly depend upon the intensity ratio between the first and second tones (i.e. emitted sounds and echoes). The weaker the first tone relative to the second, the shorter the recovery cycle was (Grinnell, 1963; Friend, Suga & Suthers, 1966; Suga, 1964*b*; Suga & Schlegel, 1973). Thus the very sharp tuning would improve echo-detection when an echo source is moving relative to the bat. Furthermore, our present experiments clearly show that neurones with best frequencies between 60 and 62 kHz and between 90 and 92 kHa have very high Q-10 dB values. Therefore it is quite probable that neurones activated by the CF component in echoes are very weakly or not at all excited by emitted sounds. Since the energy in the orientation sound is highly concentrated at the frequency of its long CF component, the CF component is a good signal for echo-detection. However, if

single neurones tuned at that frequency are fully excited and also are concerned with the reception of echoes, echo-detection would be very poor. The need for selective excitation of single neurones by echoes appears to be greater in the CF-FM bats than in the FM bats. The very sharply tuned neurones found in *P. parnelli* may be considered to be an adaptation not only for fine frequency analysis, but also for echo-detection. In *R. ferrumequinum*, the frequencies of the CF components in emitted sounds and echoes after Doppler-shift compensation are slightly different among individuals (Schuller *et al.* 1974). Such a difference would be coded by the sharply tuned neurones.

When CF-FM bats are hunting a flying insect, the echo from it is modulated in frequency and amplitude by wing beats. If the bats adjust the orientation sound to receive the average frequency of the CF component in the echo at a preferred frequency to which single auditory neurones are very sharply tuned, even very small frequency modulations by wing beats would be coded by these sharply tuned neurones, because these neurones drastically change their discharge rate for a small frequency modulation. On the other hand, the coding by these neurones of amplitude modulation by wing beats appears to be comparable to that by other mammalian peripheral auditory neurones, because we did not notice any unusual change in the discharge rate with stimulus amplitude.

SUMMARY

The moustache bat (*Pteronotus parnellii*) emits orientation sounds which consist of long constant-frequency (CF) components followed by short frequency-modulated (FM) components. The second harmonic (61–62 kHz) of the CF component is an information-bearing element and is used for Doppler-shift compensation. The auditory system of this animal is unusually sharply tuned to sounds at 61–62 kHz in terms of cochlear microphonic (CM). This sharp tuning is due to the mechanical specialization of the inner ear. Because of this specialization, CM evoked by 61–62 kHz tones slowly increases and decreases in amplitude at the onset and cessation of these stimuli. The area of the inner ear tuned at 60–61 kHz shows a prominent non-linearity and is located close to the sharply tuned area, so that CM responses to 60–61 kHz tone bursts show prominent transients at the onset and cessation of the stimuli. These transient responses are from hair cells at the area sharply tuned at 61–62 kHz. The off-response of N_1 (the summated response of primary auditory neurones) is sharply tuned at 60–61 kHz and is due to a mechanical transient occurring in the inner ear at the cessation of tonal stimuli. The on-response of N_1 is tuned at 63–64 kHz and is different from the best frequency of the CM. This difference is due to the properties of a sharply tuned resonator and N_1.

Single peripheral neurones sensitive to 61–62 kHz sounds have unusually sharp tuning curves. The low-frequency and high-frequency slopes are 1200–2700 and 1700–3900 dB/octave, respectively. The Q-10 dB value is as high as 310. Neurones with a best frequency between 55 and 64 kHz show not only tonic on-responses but also off-responses which are tuned at 60–61 kHz and are apparently related to the off-transient occurring in the inner ear. These off-responses are not rebounds from neural inhibition. No neurones were found which showed only off-responses preceded by suppression or inhibition of background activity. In the ventral cochlear nucleus,

lateral inhibition takes place, so that some single neurones responded only to sounds in a very narrow frequency range, regardless of pressure levels. Single neurones commonly show phase-locked responses to beats of up to 3 kHz, which are produced by mixing two pure tones. Information about the frequencies of Doppler-shifted echoes is coded by a set of sharply tuned neurones and discharges phase-locked to beats.

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