A comparative study of the physiological properties of the inner ear in Doppler shift compensating bats (*Rhinolophus rouxi* and *Pteronotus parnellii*)

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Summary. Cochlear microphonic (CM) and evoked neural (N-1) potentials were studied in two species of Doppler shift compensating bats with the aid of electrodes chronically implanted in the scala tympani. Potentials were recorded from animals fully recovered from the effects of anesthesia and surgery. In *Pteronotus p. parnellii* and *Rhinolophus rouxi* the CM amplitude showed a narrow band, high amplitude peak at a frequency about 200 Hz above the resting frequency of each species. In *Pteronotus* the peak was 25–35 dB higher in amplitude than the general CM level below or above the frequency of the amplitude peak. In *Rhinolophus* the amplitude peak was only a few dB above the general CM level but it was prominent because of a sharp null in a narrow band of frequencies just below the peak. The amplitude peak and the null were markedly affected by body temperature and anesthesia. In *Pteronotus* high amplitude CM potentials were produced by resonance, and stimulated cochlear emissions were prominent in *Pteronotus* but they were not observed in *Rhinolophus*. In *Pteronotus* the resonance was indicated by a CM afterpotential that occurred after brief tone pulses. The resonance was not affected by the addition of a terminal FM to the stimulus and when the ear was stimulated with broadband noise it resulted in a continual state of resonance. Rapid, 180 degree phase shifts in the CM were observed when the stimulus frequency swept through the frequency of the CM amplitude peak in *Pteronotus* and the frequency of the CM null in *Rhinolophus*. These data indicate marked differences in the physiological properties of the cochlea and in the mechanisms responsible for sharp tuning in these two species of bats.

Introduction

Doppler shift compensation by echolocating bats has been well established in horseshoe bats (Rhinolophidae) and in neotropical mustache bats (*Pteronotus parnellii*) (Schnitzler 1968, 1970a, b; Henson et al. 1980, 1983). These species emit biosonar signals characterized by a constant frequency (CF) component of long duration which is terminated by a frequency modulated (FM) component. When the bats are not flying the constant frequency component is maintained in a narrow frequency band designated the 'resting frequency'; when they fly and Doppler compensate they maintain the echo CF component in a narrow band called the 'reference frequency' (Schuller et al. 1974). Both species have large, well developed cochleae with morphological features which appear to represent specializations for the fine frequency resolution required for Doppler shift compensation (Pye 1966, 1967; Bruns 1976a, b; Bruns et al. 1981; O.W. Henson 1970; M.M. Henson 1973, 1978).

Only a few studies have been carried out on the physiological properties of the cochleae of Doppler compensating bats. Pollak et al. (1972) were the first to study CM potentials and they demonstrated very sharply tuned responses at the receptor level. It was also found that in *Pteronotus* the envelopes of CM potentials elicited by brief tone bursts near 61 kHz were markedly different from those recorded at other frequencies (Henson et al. 1973). The underlying resonance that gave rise to these peculiar envelope shapes was demonstrated by Suga et al. (1975) and Suga and Jen

(1977). The only previous study on *Rhinolophus* dealing with the CM was carried out by Schnitzler et al. (1976). Although the studies suggested that there were pronounced differences between *Rhinolophus* and *Pteronotus*, the techniques used to study the physiological properties of the ear in these two species were significantly different. In *Rhinolophus*, for example, the animals were anesthetized with Nembutal and studied over a period of hours; in *Pteronotus*, Pollak et al. (1972) chronically implanted electrodes and potentials were recorded over periods of up to six weeks in animals fully recovered from the effects of anesthesia and surgery.

The purpose of the present study was to utilize the same techniques to study *Rhinolophus* and *Pteronotus* and to make side by side comparisons of the physiological responses of the inner ear. This is of special interest because the Doppler compensation sonar systems in these two species have evolved entirely independently and, although the operational principles appear similar, all of the components of the systems (external, middle and inner ears) are structurally very different. It is through such physiological comparisons that we hope to understand the significance of structural variations in the design of the acoustic receptor organs in *Pteronotus* and *Rhinolophus*.

Materials and methods

The animals used in this study were mustache bats, *Pteronotus p. parnellii* from Jamaica, West Indies, and *Rhinolophus rouxi* from India. The data to be presented are based on the long-term study of three *Rhinolophus* and five *Pteronotus*.

For recording CM potentials, glass insulated tungsten electrodes were stereotactically placed in the scala tympani via an intracranial approach through the cochlear aqueduct (Henson and Pollak 1972). This was easily accomplished in *Pteronotus* where the aqueduct is large; in *Rhinolophus* the aqueduct is small and stereotactic placement of the electrodes was sometimes difficult. With this technique there was no interference with any part of the peripheral or central parts of the auditory system. Stable recordings were obtained from animals fully recovered from the effects of anesthesia and surgery for periods of up to four weeks or as long as the electrode assembly remained attached to the animal's head.

The CM potentials were amplified with a micronoise amplifier (Rovetti MN-80) which was used in conjunction with a bandpass filter to improve the signal-to-noise level and remove low frequency physiological noise. For surveying the amplitude of the CM potentials over broad frequency bands and for assessing the linearity of the CM as a function of signal intensity, a wave analyzer (Hewlett-Packard, Model 3590A) with an associated local sweeping oscillator (Model 3954A) was used. The CM produced by the slowly upward sweeping frequency (10 kHz–130 kHz) was fed directly back to the wave analyzer which measured the RMS voltage with selectable 100 Hz or 1000 Hz bandwidths. The CM voltages produced by different stimulus intensities were recorded on an X-Y plotter (Hewlett-

Packard, Model 7004B) as a function of frequency. With this system families of CM amplitude vs. frequency curves could be recorded through a wide range of intensities. Movements of the animal's ears and contractions of the middle ear muscles associated with vocalizations created some instability in the curves, but by using the highest voltages produced and by superimposing repetitive traces, the curves were smooth and reproducible. From families of curves recorded in relation to different signal levels, it was possible to quickly identify changes in the linearity of the responses or to rapidly plot intensity function curves.

CM audiograms were determined by presenting pulsed stimuli and recording the sound pressure level at which the CM was just detectable above the noise level of the recording equipment. With a Rovetti amplifier and a 20 kHz high pass filter the CM at threshold was 0.9 μV. By using a lock-in amplifier (Princeton Applied Research, Model 5204), very low level potentials could be detected, but the shapes of the curves were not dependent on the values of the arbitrary thresholds utilized. For recording VIIIth nerve (N-1) potentials the same electrode for recording the CM was used and the potentials were processed with a Nicolet signal averager (Model 527) to identify an arbitrary threshold response. The tone bursts used for these measurements had 15–50 ms durations and 0.5 ms rise-decay times.

For generating ultrasonic frequencies we used either the local sweeping oscillator associated with the wave analyzer or a Wavetek (Model 136) sine wave generator. In the latter case the frequency of the signals was controlled with a phase-locked-loop (Wandel and Golterman, Model FRG-200) and precise measurements of frequency were obtained with a digital counter. Signal durations and rise-decay times were controlled with a Hewlett-Packard pulse generator (Model 801A) while signal intensities were set with an Adret Electronics (Model AP401) attenuator set. The transducers used to generate the sounds were custom-made condenser loudspeakers. The frequency response was flat (within 4 dB) from 35–90 kHz and at 20 and 100 kHz it was 9 dB below the maximum output (Fig. 1). Signal intensities were monitored with a Brüel and Kjaer condenser microphone (Model 4135) and unless otherwise noted, all references to intensity in dB SPL will be relative to 20 μPa.

During the delivery of sounds the animals were positioned in a restraining device so that the wings were held away from the ears. The animal's head was fixed in place by a clamp attached to a small rod cemented to the top of the head. All recordings were made in a sound-proofed room which was maintained at 35–37° C.

Resting pulse frequencies were determined by recording several hundred echolocation sounds emitted by a restrained or caged animal. The strong second harmonic component of these signals was isolated with band pass filters and fed to a frequency to voltage converter so that a dc voltage proportional to frequency could be played back on a chart recorder. The dc levels generated by calibrated sounds were compared to the dc levels recorded during the middle portion of each pulse. Histograms of the CF component, in 100 Hz increments, were then plotted for comparison with other data (see Fig. 2).

Results

The amplitude of CM potentials

With the tracking generator and wave analyzer it was possible to record CM potentials over a broad

frequency range. The amplitude of the CM at a given frequency was dependent on the sound pressure level of the stimulus and throughout most of the 20–100 kHz frequency band the CM amplitude showed a relatively linear relationship to the stimulus level, i.e., when the stimulus intensity was changed 10 dB there was a corresponding change in the CM potential. Thus, in a given animal CM amplitude vs. frequency curves determined with different intensity levels had the same shape (Fig. 1A) as long as the ear was not overloaded. It follows that the shapes of the curves determined for the different species can be directly compared even when the stimulus level is different.

Since the loudspeaker output was not constant throughout the band studied with slow frequency sweeps, the curves in Fig. 1 are shown with intensity values that represent the attenuation of the loudspeaker relative to the maximum output. As indicated by the maximum loudspeaker output curve in Fig. 1 B, the sound pressure levels were relatively constant throughout most of the frequency band studied.

The CM voltages generated by the ear of *Rhinolophus* were consistently low in amplitude and complete CM amplitude vs frequency curves over the 20–100 kHz frequency band could only be recorded when the stimulus level was relatively high. Figure 1 B shows CM amplitude vs frequency curves for the right and left ears of the same individual. The features of these curves that were consistent in the three *Rhinolophus* ears studied were: 1) the highest CM amplitudes were recorded for frequencies in the 20–40 kHz band; 2) at frequencies between 40 and 45 kHz the CM amplitude declined as much as 18 dB as the frequency swept upward; 3) an amplitude null (a minimum voltage) occurred around 83 kHz; and 4) an amplitude peak occurred at or near 86 kHz.

In all respects the ears of *Pteronotus* provided much more sensitive preparations than those of *Rhinolophus*. Potentials could be recorded throughout the 20–100 kHz frequency range with stimuli that were 20 dB fainter than those needed to establish similar CM levels for *Rhinolophus*. The features that were common to at least three of the five *Pteronotus* ears were: (1) a broad amplitude peak in the 28–35 kHz range; (2) a plateau in the 53–59 kHz range; (3) a very prominent amplitude peak near 61 kHz; and (4) a pronounced null at about 64 kHz (3 kHz higher than the peak). In one animal there was a minor amplitude peak centered around 92 kHz (Fig. 1 A). The amplitude peak at 92 kHz was variable in occurrence and it could appear and disappear. The 61 kHz peak, on
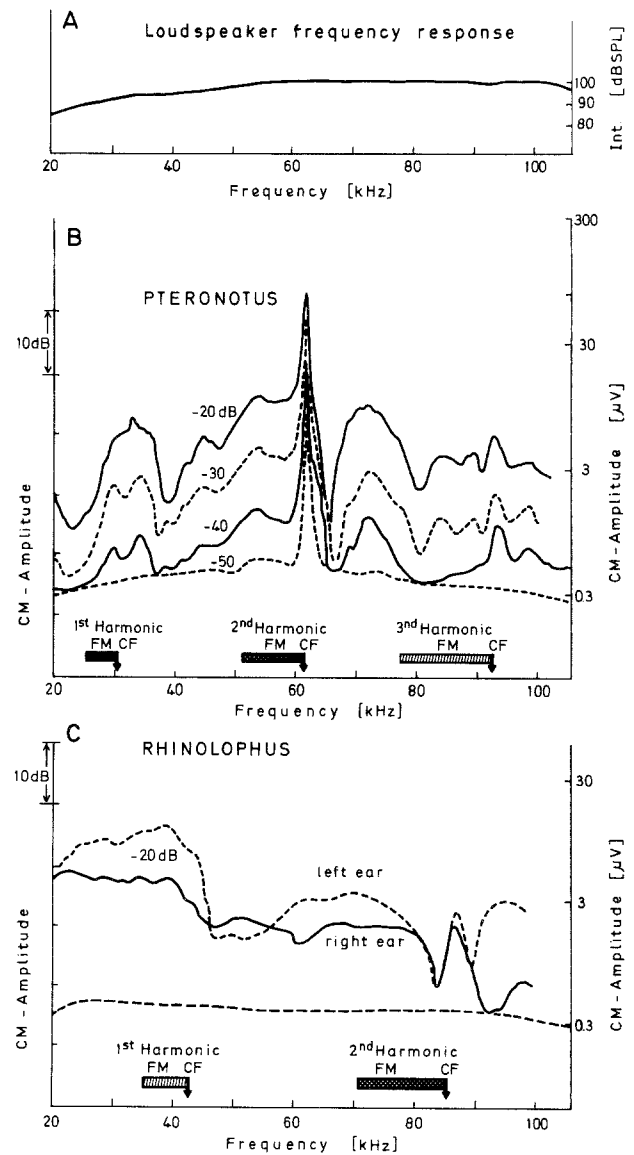

Fig. 1 A–C. CM amplitude vs. frequency curves for *Pteronotus p. parnellii* (**B**) and *Rhinolophus rouxi* (**C**). The dB notations for each curve show the attenuation of the loudspeaker from the maximum output (generally about 100 dB SPL). The stability of the loudspeaker output over the 20–100 kHz band is shown in **A**. Shaded, horizontal bars with FM and CF segments: frequency composition of the frequency modulated and constant frequency components of the bats' biosonar signal. In each curve, note that the marked CM amplitude peak at about 61 kHz in *Pteronotus* and the small peak at about 86 kHz in *Rhinolophus*, correspond in frequency to the dominant second harmonic CF components

the other hand, was always present and very stable under similar conditions.

In three of the five *Pteronotus* the CM amplitude vs. frequency curves showed a broad amplitude peak centered near 75 kHz. It is interesting to note that low CM and neural thresholds were also found by Pollak et al. (1979) near 73 kHz and

that signals of this frequency are the most effective stimuli for eliciting middle ear muscle contractions in *Pteronotus* (unpublished data, Henson, Schuller and Vater).

In all *Pteronotus* the maximum amplitude of the CM occurred at or near 61 kHz and the amplitude at this peak was consistently 20–25 dB higher than that elicited by frequencies on the low frequency side of the base of the peak. The null that was sometimes observed at 64 kHz was very sharp and appeared as a direct continuation of the downward, high frequency slope of the 61 kHz amplitude peak. The amplitude change between the peak and the null was often on the order of 35 dB (Fig. 1).

The sharpness of tuning in the second harmonic frequency regions can be expressed by Q-10 dB values, i.e., the center frequency of the amplitude peak divided by the frequency bandwidth 10 dB below the tip of the peak. In *Rhinolophus* the Q-values calculated for the CM amplitude peak averaged about 32 while in *Pteronotus* the corresponding value was 77. Thus, as far as the CM amplitude vs. frequency curve plots are concerned, the ear of *Pteronotus* seems to be much more sharply tuned than that of *Rhinolophus* in the second harmonic CF region.

In *Pteronotus* and *Rhinolophus* the CM amplitude vs frequency curves determined for the right and left ears of the same individual were similar and the sharp peak near 61 kHz was always tuned to the same frequency as the other ear. It is interesting to note that the CM amplitude peak for the second harmonic CF region in *Rhinolophus* is prominent mainly because of the null on the low frequency side of the peak. On the other hand, in *Pteronotus* the CM amplitude peak is prominent because of: (1) the magnitude of the peak above the general level of the CM; and (2) the sharp null on the high frequency side of the amplitude peak.

Correlation of CM amplitude peaks with the frequency components of the emitted sonar signals

In Fig. 1 the lower portion of each graph shows bars which represent the general frequency content of the CF and FM biosonar pulse and echo components. Comparison of the position of these bars with the CM amplitude vs frequency curves shows that the various peaks generally correspond in frequency to the CF and/or FM components of the pulses. There is an especially good correlation of the frequency band of the second harmonic CF components of the pulse with the frequency band
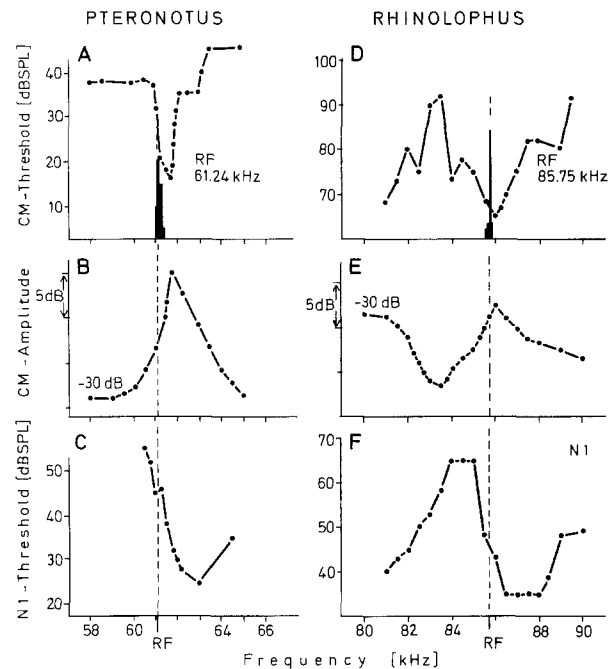

Fig. 2A–F. Comparison of CM thresholds (**A** and **D**), N-1 thresholds (**C** and **F**) and CM amplitude vs frequency curves (**B** and **E**) in relation to each other and to the resting frequencies (*RF*) of *Pteronotus parnellii* and *Rhinolophus rouxi*. For each species the records were obtained from the same animal. The vertical, dashed line extending downward from each histogram represents average RF value. Note that the RF is always lower in frequency than the CM amplitude peaks and the threshold minimums of the CM and the N-1 audiograms

of the CM amplitude peak near 61 kHz in *Pteronotus* and 86 kHz in *Rhinolophus*. The exact relationship of the resting frequencies of individual bats to the CM amplitude peaks and to CM and neural audiograms is shown in Fig. 2. In all of our preparations the resting pulse frequencies were at or below the CM amplitude peak and the sensitivity peak (threshold minimum) of all CM and neural audiograms.

Comparison of CM amplitude characteristics with CM and evoked potential audiograms

Figure 2 compares three different types of curves determined for frequencies that correspond to the second harmonic CF component of the pulses of *Rhinolophus* and *Pteronotus*. In this figure the CM amplitude peaks and nulls are also compared with histograms of resting frequencies. In *Rhinolophus* the CM amplitude peak and the threshold minimum of the CM audiogram were at the same frequency and the average resting frequency was 200–500 Hertz below this frequency. The threshold minimum for the N-1 audiogram was only a few
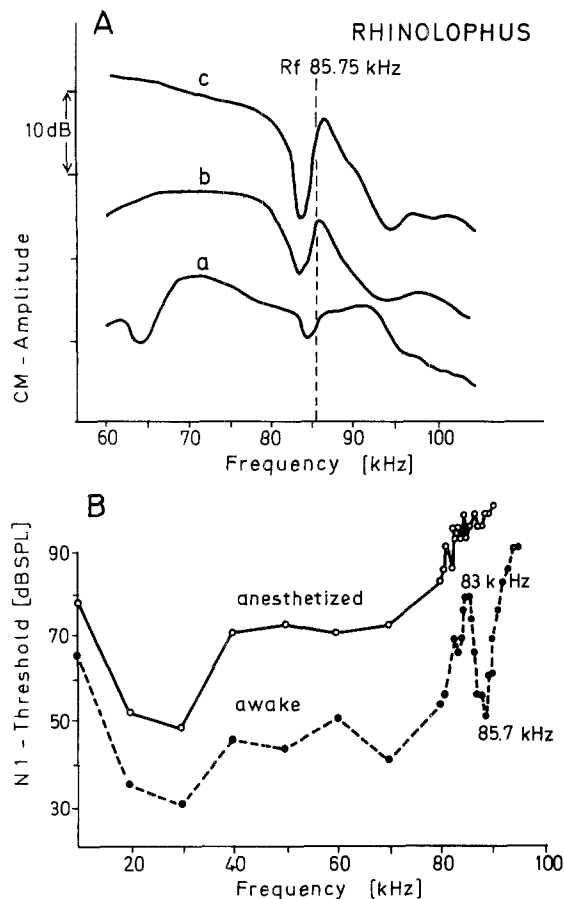
Fig. 3A, B. Variations in sensitivity associated with anesthesia and recovery from surgery in *Rhinolophus*. A, trace *a* shows a CM amplitude vs frequency curve in an animal deeply anesthetized with methoxyflurane (Metofane). Recordings obtained on days one *b* and three *c* shown for comparison. Note the shift in the position of the peak in *b* vs *c*. B changes in the N-1 evoked potential audiogram are shown for the same animal when fully awake vs anesthetized. Note the marked changes in the regions of the sensitivity null at 83 kHz and the peak at 85.7 kHz

hundred Hz higher in frequency than the threshold minimum of the CM audiogram. The same relationships held for *Pteronotus* except that the N-1 threshold minimum was 1200–1400 Hz higher in frequency than that of the CM amplitude peak and threshold minimum of the CM audiogram.

Effects of body temperature and anesthesia

When CM potentials were recorded from *Pteronotus* and *Rhinolophus* it was clear that specific types of changes occurred as the animals recovered from anesthesia and surgery. The CM amplitude peak in *Rhinolophus* and the null which preceded it became progressively more prominent over a three-day period after Metofane (methoxyflurane) anes-

thesia and the implantation of an electrode (Fig. 3A). Figure 3B shows evoked neural (N-1) potential data for the same animal following implantation of the electrode and after full recovery. It is obvious that the sensitivity peak and the null which precedes it can be completely eliminated by anesthetizing the animal. In addition, the position of the peak and null on the frequency scale can be shifted several kilohertz; in the example shown in Fig. 3A the shift is 1400 Hz between the peaks recorded one day versus days after surgery. Similar types of changes were also observed in *Pteronotus* and have been reported previously by Pollak et al. (1972) but in neither species were these changes systematically explored because of the obvious variables which could not be adequately controlled, i.e., the level of anesthesia, body temperature and cochlear blood flow. After our preparations were fully recovered recordings were relatively stable. It is important to note that the sensitivity peaks always shift to lower frequencies when the physiological state of the animal is not optimal, and thus correlation of sensitivity peaks with CF pulse component data will only reveal true relationships when the animal's physiological state is adequately controlled.

Resonance

When the ear of *Rhinolophus* was stimulated with a tone pulse corresponding in frequency to the sensitivity peak (Fig. 4A) or with tones immediately adjacent to the tip of the peak, the envelope of the CM response had approximately the same rise and fall times as the stimulus. When the ear of *Pteronotus* was stimulated under similar conditions the CM envelope had a long rise time and after the stimulus was turned off the CM amplitude decayed over a period of several milliseconds or longer (Fig. 4B). This illustrates the remarkable degree of resonance which occurs in the ear of *Pteronotus*. Resonance could be readily demonstrated in all of our *Pteronotus* but not in *Rhinolophus*. Cochlear resonance in *Pteronotus rubiginosa* has been explored in some detail by Suga et al. (1975) and Suga and Jen (1977) and in this report we wish only to bring out several new observations.

The first point is that when the ear of *Pteronotus* is stimulated with continuous broadband noise or with tone pulses the ear rings continuously. We were able to demonstrate this by recording the CM amplitude with the wave analyzer and tracking filters set at bandwidths of 100 or 1000 Hz. Examples of the CM produced by resonance at different attenuations of the maximum output of the loud-

A RHINOLOPHUS   B PTERONOTUS

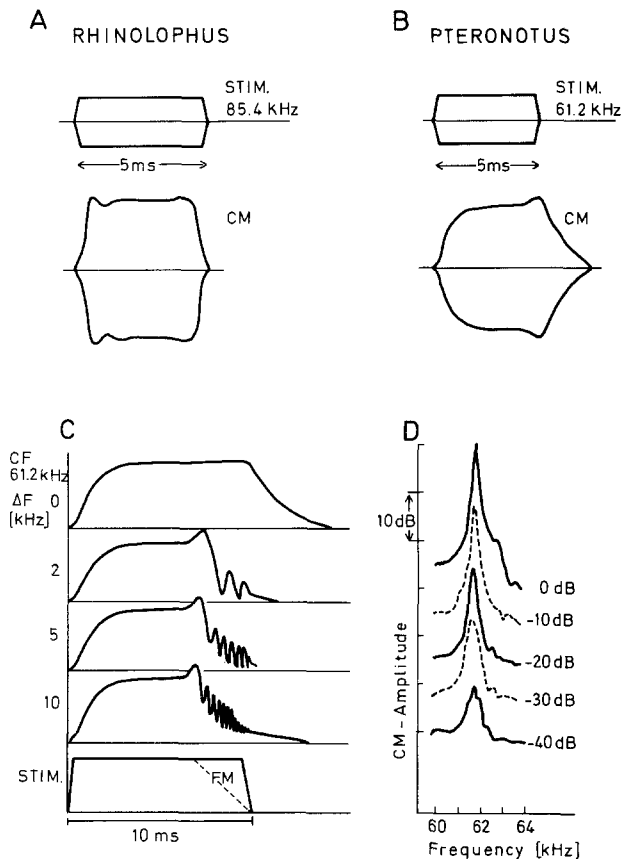C   D

Fig. 4A–D. CM potentials produced by resonance. When any brief tone pulse was delivered to the ear of *Rhinolophus* the CM envelope had approximately the same shape and rise-decay times as the envelope of the stimulus A. In *Pteronotus*, however, the envelope of the CM had elongated rise and fall times when a stimulus was near the 61 kHz sensitivity peak of the ear B. The series of amplitude vs. time traces in C demonstrate the CM produced by cochlear resonance in response to a 61.2 CF signal with no terminal FM compared to the CM produced when 3.0 ms FM sweeps of 2, 5 and 10 kHz were added to the end of the CF signal. The basic stimulus parameters for the CF and CF-FM tones are shown by the lowest trace (STIM). Addition of the FM causes beats to occur in the display; the frequency of the beats corresponds to the frequency difference between the resonance frequency of the ear and the FM sweep. Note that addition of the FM does not change the amplitude of the CM produced by resonance. When broadband noise (continuous or pulsed) of different intensity was presented to the ear of *Pteronotus* the ear resonated continuously as shown by the CM spectra D. This series of CM amplitude vs. frequency curves was recorded while the ear was being stimulated by noise (10–100 kHz bandwidth); the CM was extracted with a 100 Hz wide bandpass filter that slowly swept through the frequency range shown. The −dB values to the right of each trace represent the attenuation of the maximum output of the loudspeaker

speaker are shown by the spectral peaks in Fig. 4D. In these curves it is evident that the higher the SPL of the noise, the higher the amplitude of the CM created by the resonance. In some of our preparations the CM produced by the resonance

was present even when the loudspeaker output was attenuated 90 dB, i.e., when the noise was on the order of 10 dB SPL.

As previously mentioned, each sonar pulse emitted by *Pteronotus* has a constant frequency (CF) component followed by a downward sweeping FM component. Suga et al. (1975) showed evidence that the addition of an FM sweep to the end of a constant frequency signal prevents or reduces the resonance response (CM-aft) in *Pteronotus rubiginosa*. This was not the case in our experiments with *Pteronotus parnellii*. As shown in the top trace in Fig. 4C, a constant frequency signal with no FM elicited a CM potential with a long rise time and an after-potential of long duration. The frequency of the CM-aft corresponded to the resonance frequency of the ear and not to the stimulus frequency unless the two were identical. When not identical, the resonance was still elicited by frequencies close to the resonance frequency. The introduction of a terminal FM component to the stimulus frequency did not seem to suppress the resonance response created by an initial constant frequency stimulus; this is evident in the records shown in Fig. 4C by the presence of beats in the CM-aft potential. The beats correspond in frequency to the difference between the resonance frequency and that of the FM.

Stimulated cochlear emissions

Studies on mammals, including man, have shown that when the ear is stimulated with sounds to which the ear is sharply tuned there are weak but distinct signals which emanate from the cochlea (Kemp 1978, 1979). These outgoing sounds may destructively interfere with incoming signals when the frequencies are nearly the same and this interference can be recorded with a microphone positioned near the external acoustic meatus. The destructive interference is greatest when the stimulating tones are of low to moderate intensity. In *Pteronotus*, stimulated cochlear emissions were pronounced when a slow, upward sweeping stimulus approached an animal's resting frequency or the resonance frequency of the ear (Fig. 5). *Pteronotus* appears to provide a good model system for the study of stimulated cochlear emissions as a consequence of resonance and the associated sharp tuning. Additional, and more detailed, studies dealing with this topic have recently been completed (Kössl and Vater 1985a) and will be the subject of another report. We were unable to demonstrate stimulated cochlear emissions in *Rhinolophus*.
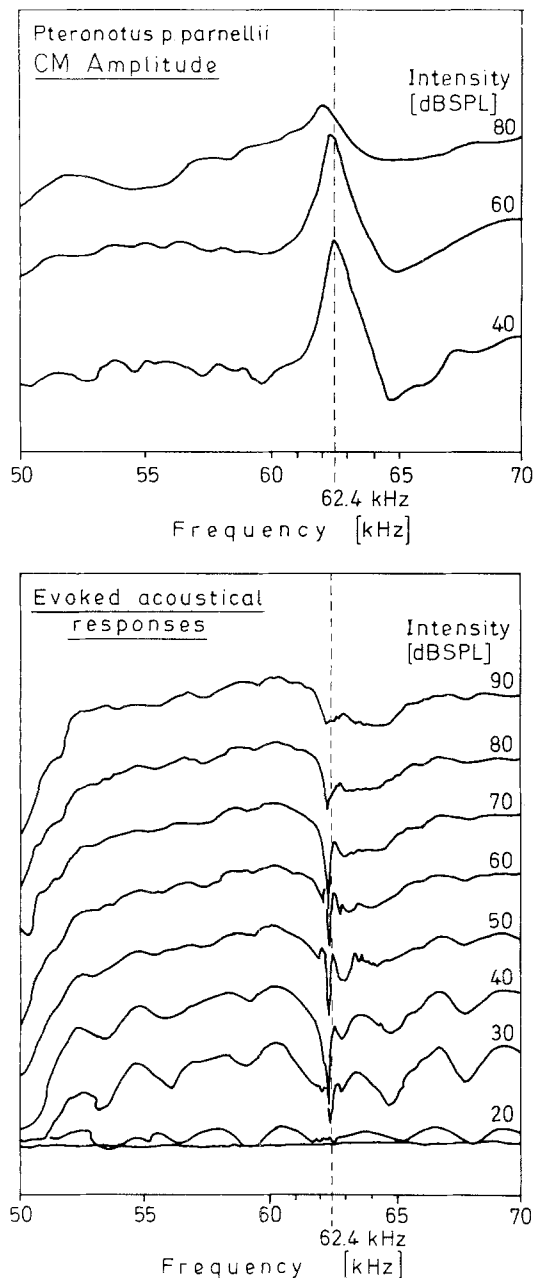
Fig. 5. Destructive interference created by stimulated cochlear emissions interacting with sound delivered to the ear. The SPL(dB) values show the stimulus intensity associated with each trace; the ordinate displays the calibration for the amplitude changes (in dB) produced by the destructive interference of stimulated cochlear emissions. Note the increase in the destructive interference (negative dip) as the intensity of the stimulus is raised from threshold (20 dB SPL) to 40 dB; a decrease is evident when the stimulus rises above 70 dB because of a saturation of the stimulated cochlear emissions

Fig. 6A–C. CM amplitude vs frequency curves shown in relation to CM phase changes in *Pteronotus* and *Rhinolophus*. In A the upper trace displays a CM amplitude vs. frequency curve, the lower trace shows the oscillatory changes in phase that occur in the CM relative to the sound source as the frequency sweeps from 10 through 100 kHz. Note that the changes are fairly regular except when the stimulus sweeps through the 60–65 kHz band. In B and C the measurements in the rate of change of CM phase (*solid lines*) in relation to stimulus frequency are plotted for *Rhinolophus* and *Pteronotus*. Similar, rapid changes in phase occur in both species but in relation to different regions of sensitivity; in *Rhinolophus* the most rapid change is centered at the point of the CM amplitude null (83 kHz) while in *Pteronotus* it corresponds exactly to the CM amplitude peak

Phase changes as a function of frequency

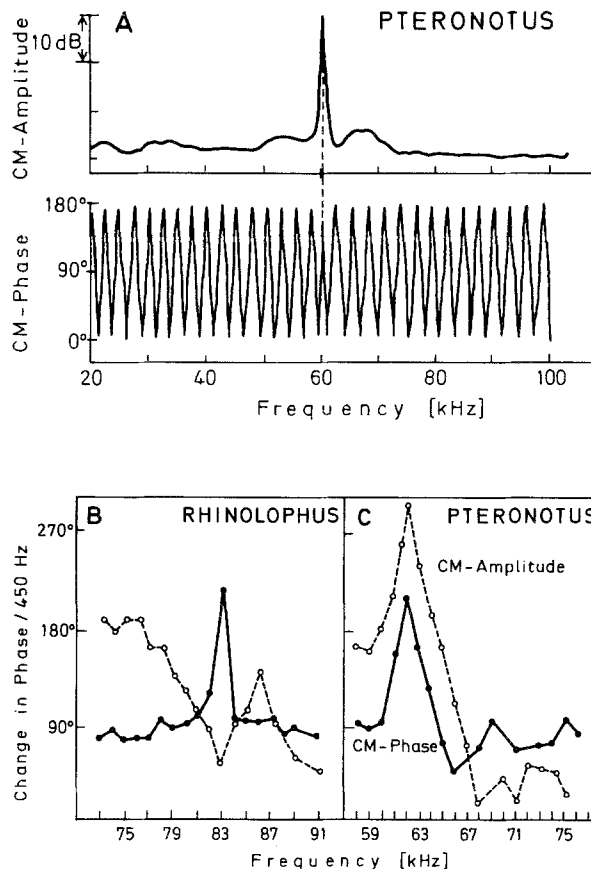When a microphone is placed at a fixed distance from a sound source there will be differences in phase between the waves at the loudspeaker and those reaching the microphone. If the microphone and loudspeaker are fixed in position the phase angle will vary with frequency. When the microphonic output of the ear is used instead of a microphone, another variable is added because of the time it takes the traveling wave to reach the specific point in the cochlea which responds to the selected frequency. As a means of examining physiological differences between the ears of *Rhinolophus* and *Pteronotus* we studied the rate of phase change as a function of frequency.

The bottom trace in Fig. 6A shows an example of the oscillatory records produced by 180° phase

shifts which occurred when the ear of *Pteronotus* was stimulated by a continuous, slowly sweeping, 20–100 kHz stimulus. It is evident that the frequency of the oscillations, which represents the rate of change of phase with frequency, was relatively constant throughout almost the entire 20–100 kHz frequency band. The phase changes were typically on the order of 90° per 450 Hz change in frequency. There was, however, a very obvious, sudden change near 61 kHz, and here the phase changed at a rate of up to 220° per 450 Hz change in frequency. As shown in the top trace of Fig. 6A and in the expanded scale in Fig. 6C, the point where the CM amplitude peak occurred corresponded in frequency to the point where the rate of change of phase suddenly shifted. In *Rhinolophus*, we observed the same general phase changes, but the rapid phase change corresponded in frequency to the region of the null in the CM amplitude vs frequency curve (Fig. 6C) rather than the peak. The phase changes observed in *Pteronotus* and *Rhinolophus* were independent of the signal strength at all frequencies.

Discussion

The main purpose of this study was to make a direct comparison of the physiological properties of the cochlea in two species of bats whose Doppler shift compensating sonar systems have evolved along entirely different lines. A summary of the findings specifically comparing the sharp tuning characteristics of the ear of *Rhinolophus* and *Pteronotus* is shown in Table 1.

In general, the results of this study are in agreement with previous studies which have shown; (1) sharp tuning at the auditory periphery (Pollak et al. 1972, 1979; Schnitzler et al. 1976; Suga and Jen 1977; Suga et al. 1975); (2) a close relationship of the resting frequency of the emitted pulses to the second harmonic sensitivity peak of the audiogram (Henson et al. 1980, 1983; Long and Schnitzler 1975; Schnitzler et al. 1976; Pollak et al. 1979); (3) a change in the tuning properties of the ear in response to changes in body temperature and/or to the administration of anesthetic drugs (Pollak et al. 1972); (4) a remarkable state of CM resonance in *Pteronotus* (Suga and Jen 1977; Suga et al. 1975; Henson et al. 1983); and (5) a mismatch in the frequency of maximum sensitivity between the N-1 and CM (Pollak et al. 1979; Suga et al. 1975; Suga and Jen 1979; Schnitzler et al. 1976).

Our data conflict with previous studies dealing with: (1) the exact relationship of the RF to the

Table 1. Comparison of physiological properties of the cochlea in *Pteronotus* and *Rhinolophus*

	Pteronotus parnellii	*Rhinolophus rouxi*
Second harmonic CM-amplitude peak	ca. 61 kHz	ca. 86 kHz
Average Q-10 dB value of CM-amplitude vs. frequency curves at peak	77	32
CM null relative to peak	may be pronounced on high frequency side	pronounced on low frequency side
Relationship of RF to peak	ca. 200 Hz above RF	ca. 200 Hz above RF
Relationship of CM to N-1	threshold minima not matched	threshold minima matched
Anesthesia and temperature	marked effect on peak	marked effect on peak
Rapid CM phase changes	centered at peak	centered at null
Susceptibility of ear to over-stimulation	very susceptible	not very susceptible
Resonance	marked	not evident
Stimulated cochlear emissions	pronounced	not detected

sharply tuned second harmonic sensitivity peak in *Rhinolophus*; (2) the degree of matching of the sensitivity peaks for CM and N-1 potentials in *Rhinolophus*; and (3) the effect of an FM sweep on CM resonance in *Pteronotus*. It is likely that the differences in the findings for the first two points can be attributed to differences in the physiological state of the preparations and/or to the techniques used for the implantation of electrodes. Schnitzler et al. used Nembutal anesthesia and drilled a small hole in the cochlea in order to implant an electrode. Their studies on *Rhinolophus ferrumequinum* were carried out over a period of hours on sedated animals. Suga and his colleagues used ether anesthesia supplemented with Nembutal for their studies of *Pteronotus*, and the recording electrode was placed on or near the membrane of the round window via a surgical approach through the middle ear. Their preparations, as well as those of Schnitzler et al. (1976) were maintained at about 35 °C by keeping them in a temperature controlled room. This is several degrees below the body temperature maintained by *Pteronotus* when awake and 5° to 6° below the body temperature in a flying animal (Henson and Kobler 1979). In the present experiments the electrodes were implanted in the

scala tympani via an intracranial approach and the potentials were recorded over extended periods of time in animals fully recovered from the effects of anesthesia and surgery and in animals which maintained their own body temperature near 37 °C.

In *Rhinolophus* and *Pteronotus* the N-1 audiograms are much less sharply tuned than the CM audiograms, and when speaking of a mismatch we refer basically to the non-correspondence between the initial, lowest frequency portion of the tip of the N-1 sensitivity peak and the center of the CM sensitivity peak. The lack of correspondence of the frequency of the threshold minimum for the CM vs the N-1 in the second harmonic region of the audiograms of *Pteronotus* has been noted previously by Suga et al. (1975), Suga and Jen (1977) and Pollak et al. (1979); as explained by Suga and his colleagues, it can be attributed to the slow rise time in the CM envelope (see Fig. 5) that occurs with stimuli near the resonance frequency. If the slow rise time is taken into account then the frequency of sharp tuning for the CM and N-1 match. Schnitzler et al. (1976) also reported that the threshold minimum of the CM and N-1 audiograms in *Rhinolophus* do not match in frequency yet our results, as well as theirs, provide no evidence of marked resonance to account for this mismatch. Our data show, however, that a major mismatch in the frequency of the tuned sensitivity peaks for the CM and N-1 for *Rhinolophus* only occurs when the preparations are anesthetized or not in 'perfect' physiological condition. Thus, the lack of marked resonance in *Rhinolophus* and the correspondence in frequency of the CM and N-1 sensitivity peaks support the notion that resonance is a major, if not the sole, contributing factor to the mismatch in *Pteronotus*.

The finding that the resting pulse frequency of a given bat falls at or below the tuned sensitivity peak of the second harmonic region in *Rhinolophus* conflicts with the data of Schnitzler et al. (1979) who found resting frequencies up to about 1000 Hz higher than the CM sensitivity peak. In the present study, we found up to a 1400 Hz shift in the position of the CM peak in anesthetized vs awake preparations (Fig. 4C). Schuller's (1980) CNS neural audiogram vs. resting frequency data for *Rhinolophus rouxi* are identical to the findings of this study which show the resting frequencies slightly below the sensitivity peak of the sharply tuned region of the ear. From a functional point of view it would seem advantageous for Doppler shift compensating bats to hold the resting frequency of the pulse and the second harmonic CF component of

the echo at or below the second harmonic sensitivity peak; this would prevent the terminal FM of the pulses from sweeping through the frequency band of the sensitivity peak and it would prevent the excitation of certain neurons and hold them ready to process Doppler shifted echoes. According to Pollak and Bodenhamer (1981), most of the sharply tuned, high-Q neurons in the inferior colliculus of *Pteronotus* have their best frequencies at or above the resonance frequency of the ear and thus in a position seemingly preserved for the processing of echoes reflected from the beating wings of insects. The data from this study and the study of Schuller (1980) confirm that this is also true in *Rhinolophus rouxi*. Although our data and Schuller's are consistent in revealing that the resting frequencies are slightly below the second harmonic sensitivity peak of the ear, it should be noted that Long and Schnitzler (1975) studied behavioral audiograms in *Rhinolophus ferrumequinum* and in two of the three animals the resting frequencies were almost 800 Hz higher than the sharply tuned, second harmonic sensitivity peak of the audiogram. In the third animal the resting frequency was slightly below the threshold minimum of the behavioral audiogram and thus only in this case were the findings similar to ours.

Long and Schnitzler have also called attention to the distinct null in sensitivity which precedes the second harmonic sensitivity peak in *Rhinolophus*. The null is interesting because it lies in the narrow band between the CF and FM pulse components. When *Rhinolophus* Doppler shift compensate and lower their CF pulse components to the region of the null, they probably hear little of the emitted signal. This is certainly the case in *Pteronotus* where the CF component may evoke little or no cochlear microphonic response even when the emitted pulses are more than 100 dB (Henson et al. 1983).

It is interesting to note that the null in sensitivity in *Rhinolophus* is represented in both the cochlear microphonic and N-1 potential audiograms and that it and the sensitivity peak are affected differently by anesthesia and temperature changes. Judging from the N-1 audiograms (Fig. 4B) anesthesia has a comparatively small (ca. 10 dB) effect on thresholds near the center frequency of the null compared to a fairly large (40 dB) effect on the center frequency of the sensitivity peak. Further studies are needed to establish the causes of the changes and especially the differential effect of the sensitivity changes in the null vs the peak regions. The data showing that marked resonance of the ear of *Pteronotus* occurs continuously in the pres-

ence of low level broad band noise (Fig. 5C) and that resonance is not affected by the addition of a terminal FM component to a stimulus (Fig. 5C) raise some interesting questions. In the noisy environment of caves where there are thousands of bats emitting ultrasonic pulses it would seem that the ear must resonate continuously at the tuned sensitivity peak of each animal's ear. The resulting cochlear emissions should destructively interfere with incoming signals and create beats; one must question how the system can efficiently perceive echoes under these conditions or question the potential extraction of useful information from beats generated by what might be interpreted as a 'reference' frequency.

The observations on the variations in the rate of change of phase of the CM potentials with changes in frequency at the CM amplitude peak in *Pteronotus* and the amplitude null in *Rhinolophus* suggest, along with the observed resonance in *Pteronotus*, that there are striking differences in cochlear mechanics in these two bats. Although both species have very sharply tuned ears, the anatomical mechanisms involved in the creation of this tuning appears to be different. Anatomical studies on the cochlea also suggest that this is the case (Bruns 1976a, b; Bruns et al. 1981; Henson 1973, 1978), but at the present time frequency maps determined for the cochlea of *Rhinolophus* are conflicting (Bruns 1976b; Vater and Feng 1985) and maps for *Pteronotus* are just now unfolding (Kössl and Vater 1985; Leake and Zook 1985). Until reliable frequency maps are established it does not seem warranted to attempt to correlate structure with function. We speculate, however, from our data on phase changes, as well as CM amplitude curves and audiograms, that the ear of *Rhinolophus* has elements which create the sensitivity null which precedes the sensitivity peak. *Pteronotus*, on the other hand, seems to have inner ear mechanisms which produce strong resonance and enhance sensitivity in a narrow frequency band. The latter is suggested by our data on the differences in the rate of change in CM phase with changing frequency. The observed changes in phase would be expected to occur in the cochlea if frequency representation per unit length was greater in one area than another or if the speed of the traveling wave changed due to changes in the mass or stiffness of the basilar membrane.

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