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

## III. Cochlear Microphonics and Auditory Nerve Responses*

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**Summary.** *Rhinolophus ferrumequinum* compensates for Doppler shifts and keeps the frequency of the CF-portion of echoes constant at a reference frequency. The bat's cochlea is specialised for the detection of sounds in a narrow range including this reference frequency. The threshold curve of the $N_1$-on (summated activity of primary auditory neurons evoked at the onset of a tonal stimulus) has a sharp notch about 24–30 dB deep which is exactly tuned to the reference frequency. $N_1$-off is most prominent at stimulus frequencies 0.5–1 kHz below the reference frequency. In this frequency range the envelopes of the CM (cochlear microphonics) have slower rise and decay times than the stimulus envelopes. A comparison of our physiological data with the morphological data of Bruns (1976a, b) leads to the suggestion that the cochlea acts as a narrowly-tuned mechanical filter which is responsible for sharp tuning of the CM, $N_1$-on and $N_1$-off threshold curves.

## Introduction

Horseshoe bats emit orientation sounds with a long constant frequency (CF) portion preceded and followed by short frequency-modulated (FM) portions. In flight they compensate for Doppler shifts caused by movement by lowering the frequency of the emitted sounds (Schnitzler, 1968). The echo frequency of the CF-portion is, therefore, kept constant at a *reference frequency* which is about 50–250 Hz higher than the *resting frequency* which is emitted in situations where no Doppler shifts are present. The compensation is done by a feedback control system (Schnitzler, 1973a; Simmons, 1974). Experiments with

electronically produced Doppler shifts showed that only upward frequency shifts are compensated up to a limit of 4–8 kHz (Schuller *et al.*, 1974). Within this range the echo frequency of the CF-portion is kept constant at the reference frequency of the compensation system. This raises the question of whether the auditory system of the horseshoe bat is tuned to this reference frequency.

Evoked potential measurements with gross electrodes at the level of the inferior colliculus in *Rhinolophus ferrumequinum* (Neuweiler, 1970) and in *Rhinolophus euryale* (Schnitzler *et al.*, 1971) yield threshold curves for on-responses with narrowly-tuned minima at about the frequency range of the resting and reference frequency. Near these minima tonal stimuli also cause prominent off-responses (Neuweiler *et al.*, 1971). The threshold curves of these off-responses are also narrowly tuned and show minima about 0.5–1 kHz below the minima for on-responses (Schnitzler, 1973 b). In all these experiments the exact resting and reference frequencies of the electrophysiologically tested bats were not determined. As these frequencies vary from bat to bat in the range between 81–85 kHz, the exact relationship between resting and reference frequencies and the sharply tuned on- and off-minima is not known.

These facts raise the following questions: 1. Is the narrow tuning only present in the higher auditory system or can it be found also in the auditory periphery? 2. Is there a fixed relation between the tuning of the auditory system and the reference and resting frequencies in individual bats? 3. Is the tuning due to neuronal interaction as suggested by Neuweiler (1970) or can it be explained by the mechanical properties of the cochlea (Bruns, 1976a, b)? 4. Is the off-response in *Rhinolophus* a rebound from electrical or mechanical suppression of transmitter release from hair cells to afferent nerve fibers (Grinnell, 1973), or is it due to a mechanical transient occurring in the cochlea as described for *Pteronotus parnellii* (Suga *et al.*, 1975)?

Simultaneous recordings of the cochlear microphonics (CM) and the summated activity of primary auditory neurons ($N_1$) were made in order to answer these questions.

## Materials and Methods

*Animals:* These experiments were performed with 9 greater horseshoe bats (*Rhinolophus ferrumequinum*) from France. For each animal the reference frequency was determined as described in Schuller *et al.* (1974). Histograms of the reference frequencies at artificially produced Doppler shifts of 1 kHz were plotted with an X-Y recorder. The resting frequencies of the tested bats were displayed in the same way.

*Surgical Procedure:* Under Nembutal anesthesia (0.02 mg/g body weight) a 2 cm long nail was mounted on the exposed skull of the bat with glue (Eastman 910) and dental cement (Paladur). The bat's head was held rigid with the mounted nail in a set screw holder. The bulla and the basal part of the cochlea were exposed by a lateral surgical approach. The salivary gland was removed, the hyoid muscle was cut, and the thin surface of the bulla was opened with forceps. As it was impossible to reach the round window directly with an electrode, we drilled a small hole in the cochlea as close to the basal end as possible. When a small droplet of fluid appeared we closed the hole with the tip of a tungsten electrode. The ground electrode was put into adjacent tissue. As long as the electrode did not penetrate into the cochlea it was possible to record the CM and $N_1$ over 6–8 hrs. The recordings were made in an electrically shielded chamber, the

temperature of which was kept at 35–38° C. The inner wall of the chamber was covered with foam plastic to reduce the high-frequency acoustic echoes.

*Stimulus:* The acoustical stimuli were delivered to the bat with a homemade electrostatic or condenser loudspeaker (Machmert *et al.*, 1975) positioned at a distance of 70 cm. The frequency response of the loudspeaker was flat within $\pm 1$ dB between 15–120 kHz. With an attenuator (Hewlett-Packard 350 D) it was possible to choose sound pressure levels between 0–100 dB re 0.0002 dyne/ $cm^2$. Two types of stimuli were used: 1. A continuous sinusoidal signal with frequency sweeping upward with a rate of 1 kHz/sec within the frequency range from 15–120 kHz, produced by the BFO output of a wave analyzer (Hewlett-Packard 3590A). 2. Tone bursts with 0.2 msec rise and decay times and 3.5 msec duration at a maximal repetition rate of 5 per sec. The pulses were shaped by a Hewlett-Packard 8010A pulse generator via the VCA input of a Wavetek 136 waveform generator. The sound pressure level (SPL) of the delivered stimuli was measured directly with a Bruel & Kjaer microphone (type 4135) placed at the bat's ear.

*Recording of the CM and $N_1$:* With the tungsten electrode cochlear microphonics (CM), summating potentials, and $N_1$ were picked up and were fed into a cathode follower (Bruel & Kjaer 2619, battery operated with a Bruel & Kjaer 2804 power supply) which had a frequency response from 2 Hz–200 kHz. The potentials were amplified with an Tektronix 3A9 at a band pass position of 100 Hz and 300 kHz (6 dB/octave). The $N_1$ were separated out of the composite recordings with a low pass filter of 24 dB/octave at 10 kHz, and the CM with a high pass filter of 24 dB/octave at 10 kHz.

*Further Data Processing:* For continuous acoustic stimuli which were produced from upward sweeps at the BFO-output of the wave analyzer, the output of the cathode follower was directly fed into the input of the same wave analyzer. This measured the rms voltage of the CM through a 3 kHz wide bandpass filter coupled to the upward moving frequency of the BFO-output. Plots of the amplitude of the CM over the frequency range from 10–100 kHz were made at different SPLs with a Hewlett-Packard 7004B X-Y recorder. The noise level of the system was at about 0.3 µV rms.

For tone burst stimuli the $N_1$ were averaged in the 1st channel of a Biomac laboratory computer. The envelopes of the CM were obtained by rectification of the CM with a Pacific measurement 1008 AC-DC converter (time constant below 25 µsec). These were averaged in the 2nd channel of the Biomac. The simultaneously-computed averages of the $N_1$ and the CM-envelopes could be displayed via the X-Y recorder output of the Biomac on an X-Y recorder.

## Results

*Voltage of the CM.* In all bats the CM had the highest amplitudes in the frequency range of about 15 kHz (Fig. 1). In this range a CM voltage of normally about 300 $\mu V_{rms}$ was reached at a SPL of 100 dB. In one case we even recorded a maximum CM voltage of nearly 1,000 $\mu V_{rms}$. Towards higher frequencies the CM voltage decreased to about 10 $\mu V_{rms}$ at 100 kHz and 100 dB SPL. In all bats the frequency response curves show a small depression of about 6–8 dB in the 41–42 kHz region of the 1st harmonic of the bat's CF-components in the orientation sounds. A small increase of CM voltage of 4–10 dB was always found just below the reference frequency of the compensation system. The peak of this increase was always between 0.5–1 kHz below the reference frequency.

At all frequencies the voltage of the CM increased linearly with SPL up to 80 dB (Fig. 2). Above 80 dB SPL, the amplitude function curves began to flatten out. This flattening was more prominent at frequencies above 80 kHz.

*Threshold of the CM.* The CM thresholds were measured in two ways. 1. The SPL was determined at which the CM for short tone bursts could no longer be distinguished above the noise level on the oscilloscope (Fig. 3, RF 4
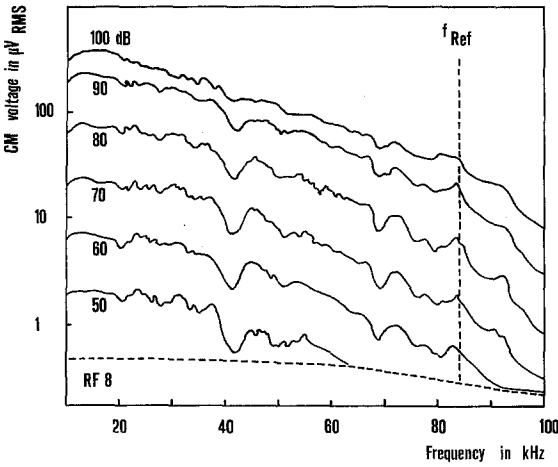
**Fig. 1.** Voltage of the CM recorded continuously with a wave analyzer and an X-Y recorder for stimulus frequencies sweeping upward from 10 to 100 kHz. The SPL of the stimulus was varied from 50 to 100 dB re. 0.0002 dyne/cm$^2$. The CM noise level was below 0.3 $\mu V_{rms}$
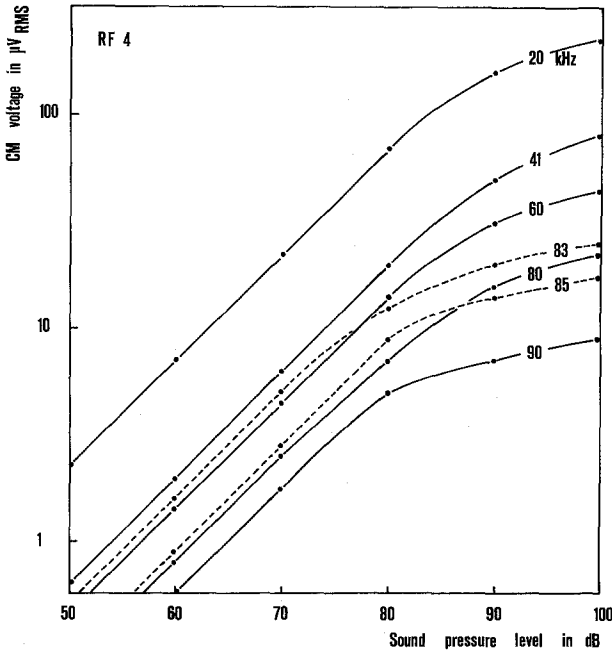


**Fig. 2.** Input-output functions of the CM for different frequencies

and RF 11). 2. The 10 $\mu V_{rms}$ isopotential line was calculated from the frequency response curves plotted from upward sweeping stimulus frequencies (Fig. 3, RF 1). Both methods showed similar results. Below 40 kHz the threshold curves are rather flat. In the range of the 1st harmonic of the CF-component of
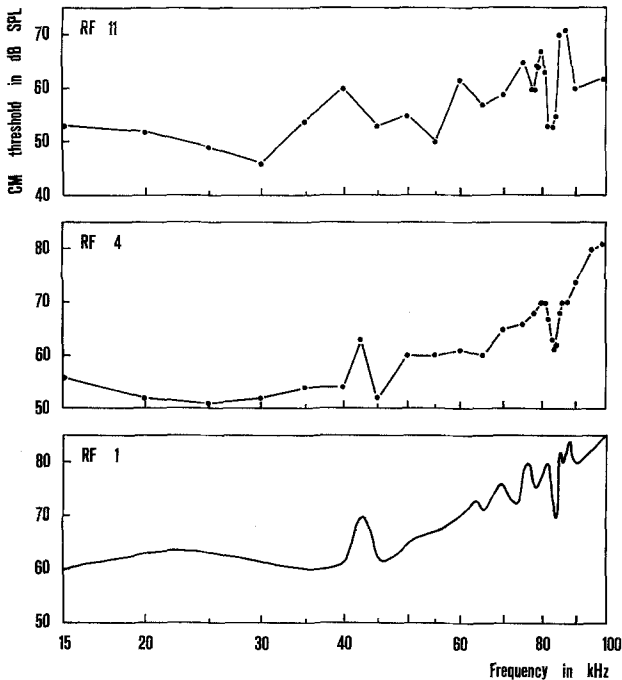
**Fig. 3.** Thresholds of the CM in the frequency range from 15 to 100 kHz. In RF 11 and RF 4 the threshold is measured as the SPL at which the CM of tone burst stimuli could no longer be distinguished above the noise level on the oscilloscope. In RF 1 the threshold is the 10 $\mu V_{rms}$ isopotential line as calculated from wave analyzer plots

the orientation sounds all CM curves have an area of higher threshold about 10 dB above the normal level. A sharply tuned area of lower threshold is always found just below the reference frequency of the Doppler shift compensation system. In the tested bats these minima were in the frequency range 0.5–1 kHz below the reference frequency. They were between 9–14 dB deep.

The shapes of the threshold curves reflect similar information gained with the frequency response curves (Fig. 1). The area of high threshold at the 1st harmonic corresponds to the area of lower amplitude in the wave analyzer plot and the area of low threshold below the reference frequency corresponds to the increase in CM voltage in the frequency response curve.

*Threshold of $N_1$-on and $N_1$-off.* The onset and the end of the tone burst stimuli evoke summated activity of primary auditory neurons called $N_1$-on and $N_1$-off responses. The threshold curves of these responses (Fig. 4) look very similar to the curves which one gets with gross electrodes placed on the inferior colliculus (Neuweiler, 1970; Neuweiler *et al.*, 1971; Schnitzler, 1973 b). There is an area of higher $N_1$-on threshold in the frequency range of the 1st harmonic of the CF-components of the orientation sounds and a sharply tuned threshold minimum in the range of the 2nd harmonic. In our bats these minima were between 24–30 dB deep. The $N_1$-off threshold is rather high over the whole frequency range except for a narrowly tuned minimum just below the on minimum where the $N_1$-on threshold curve is steeply rising.
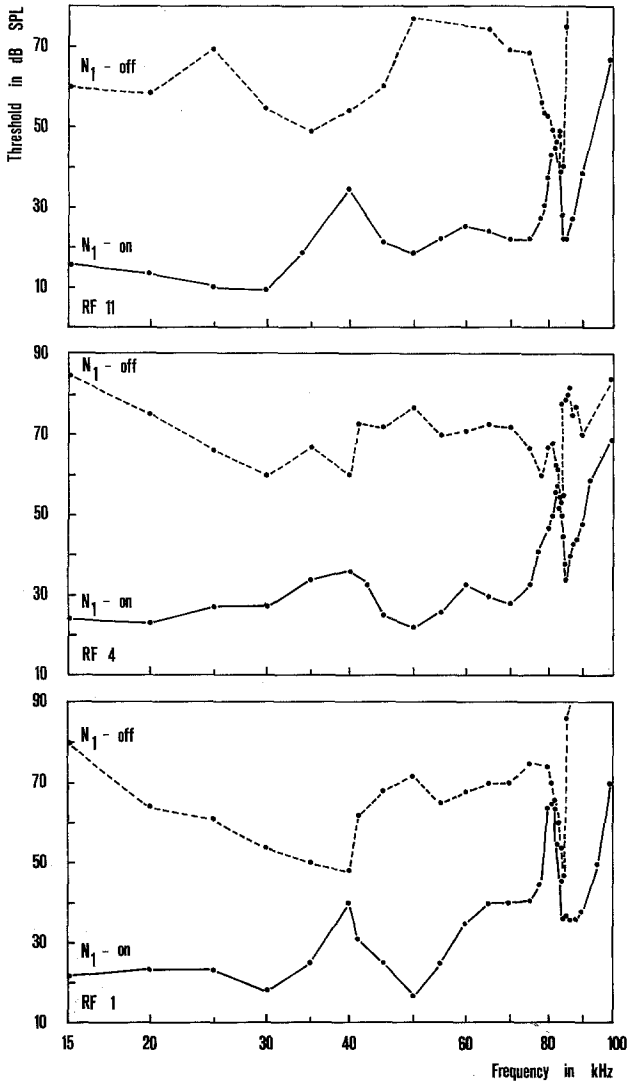
**Fig. 4.** Thresholds of $N_1$-on and $N_1$-off for tone burst stimuli in the frequency range from 15 to 100 kHz

*Reference Frequency and Tuning of the CM, $N_1$-on and $N_1$-off.* From one individual *Rhinolophus ferrumequinum* to another the best frequencies of the on-minima are different. Best frequencies in the range from 81–85 kHz have been found in our laboratory. It is also known that the reference frequencies of the Doppler-shift compensation system differ in individual bats within the range from 81–85 kHz, but up to now no measurements had been made in which the sharp auditory tuning had been compared with the reference frequency in the same individuals. Therefore we measured the resting and reference frequen-
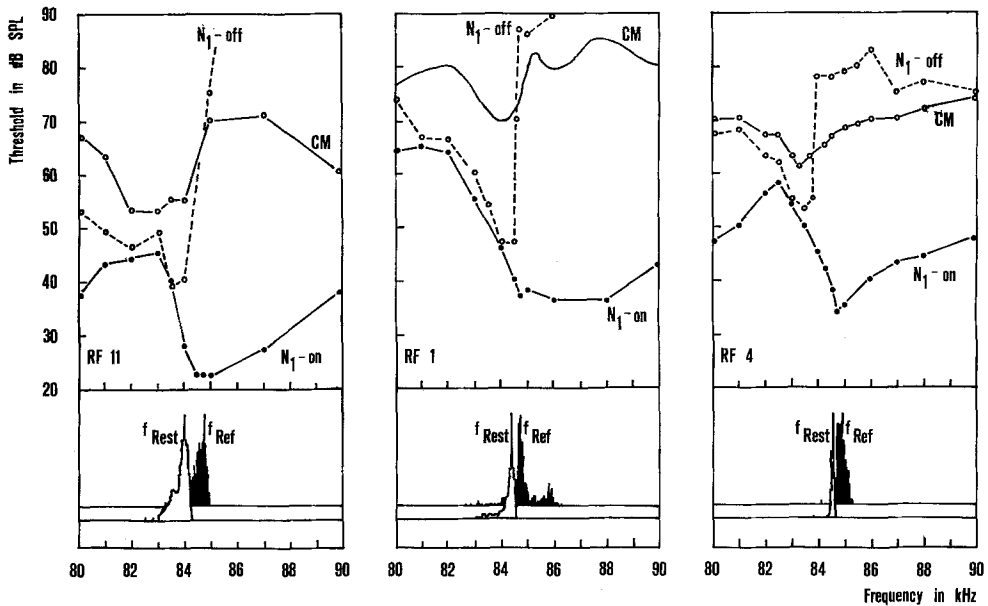
Fig. 5. Thresholds of CM, $N_1$-on and $N_1$-off for tone burst stimuli in the frequency range from 80 to 90 kHz, with histograms of the reference and resting frequencies. The CM threshold of RF 1 corresponds to the 10 $\mu V_{rms}$ isopotential line as calculated from a wave analyzer plot. The reference frequency was measured at an artificial Doppler shift of 1 kHz (methods see Schuller *et al.*, 1974)

cies and compared them with the minima of the CM, $N_1$-on and $N_1$-off threshold curves (Fig. 5).

In all bats the $N_1$-on minimum is sharply tuned to the reference frequency. The maxima of the computer histograms of the reference frequencies were always exactly at the point where the $N_1$-on threshold starts to rise towards lower frequencies. In individual bats the CM and $N_1$-off minima are tuned to the same frequency and can be found about 0.5–1 kHz below the $N_1$-on minimum.

The resting frequency, which in individual bats is about 50–300 Hz lower than the reference frequency, is not exactly tuned to any of the threshold minima. The maxima of the histograms of the resting frequencies are always between the $N_1$-on minima and the CM and $N_1$-off minima in the upper slopes of the steeply rising $N_1$-off threshold curves.

*The Envelopes of the CM for Tone Burst Stimuli.* Usually the envelopes of the CM reflect exactly the envelopes of the tone burst stimuli. However, in the frequency range of the sharply tuned off-minima the envelopes of the CM differ from those of the stimuli (Fig. 6). In this range the envelopes of the CM have slower rise and decay times than the tone burst stimuli. That means that the CM lasts longer than the stimulus. Suga *et al.* (1975) have found a similar response in the mustache bat *Pteronotus parnellii*. They called the part of the CM which is still produced after the cessation of the stimulus an after-response or CM-aft.
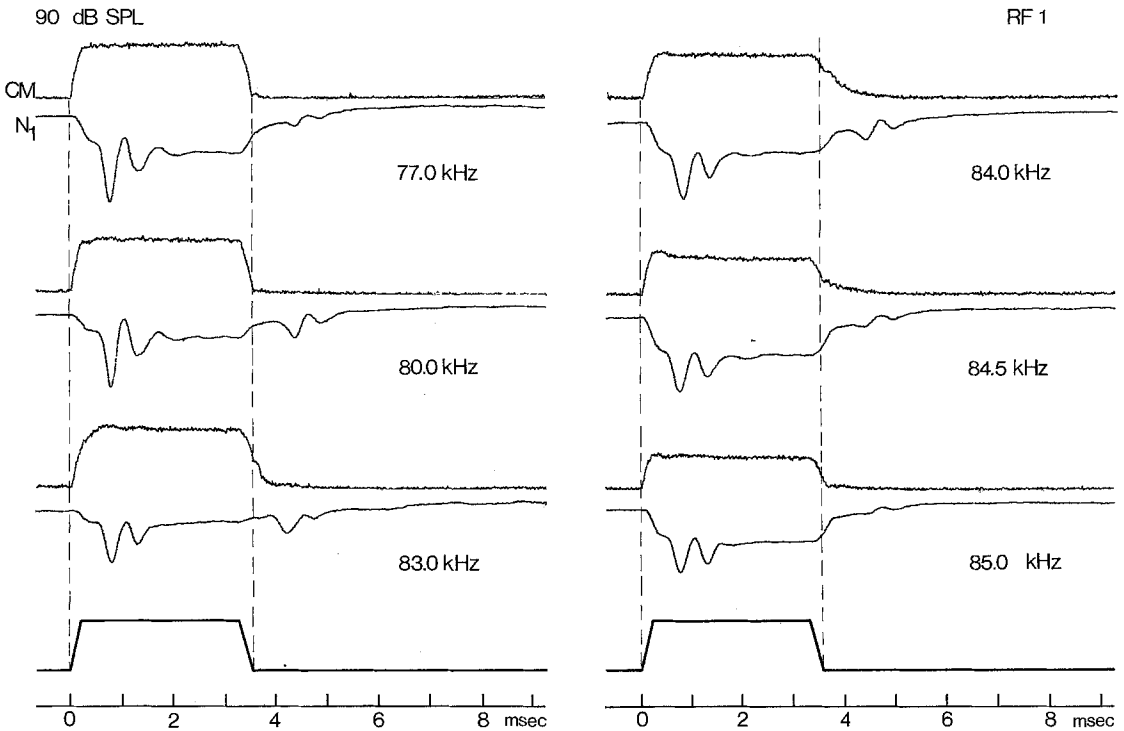
90 dB SPL                                                                RF 1



**Fig. 6.** Envelopes of the CM and $N_1$ of RF 1 for tone burst stimuli with a duration of 3.5 msec, a rise and decay time of 0.2 msec, at 90 dB SPL for different frequencies from 77 to 85 kHz

In *Rhinolophus ferrumequinum* the CM-aft was too short and small for complete threshold curves to be measured. But in all bats the CM-aft responses were only clearly distinguishable in the frequency range where the $N_1$-off threshold is low. For instance, in RF 1 (Fig. 6) the CM-aft could be found in the frequency range from 82.0 to 84.5 kHz, that is, in the range where stimuli with an SPL below 65 dB could evoke an $N_1$-off response. The clearest CM-aft could be detected exactly at the frequency where the $N_1$-off threshold had its minimum. That was at 84 kHz in RF 1. This finding is similar to the results of Suga *et al.* (1975) which show the same correspondence between $N_1$-off and CM-aft. We determined that there was no stimulus artifact responsible for the CM-aft. Therefore this effect must be due to the physical properties of the ear.

*Comparison of Physiological and Morphological Data:* Bruns (1976a, b) has quantitatively investigated the morphology of the cochlea of the greater horseshoe bat and has determined the localization of vibration maxima for different frequencies on the basilar membrane. We expressed his data on a frequency scale by using his frequency map. This was done for the height (BMH) and the width (BMW) of the basilar membrane and for the width between the lamina spiralis primaria and the lamina spiralis secundaria (LSP-LSS) in the
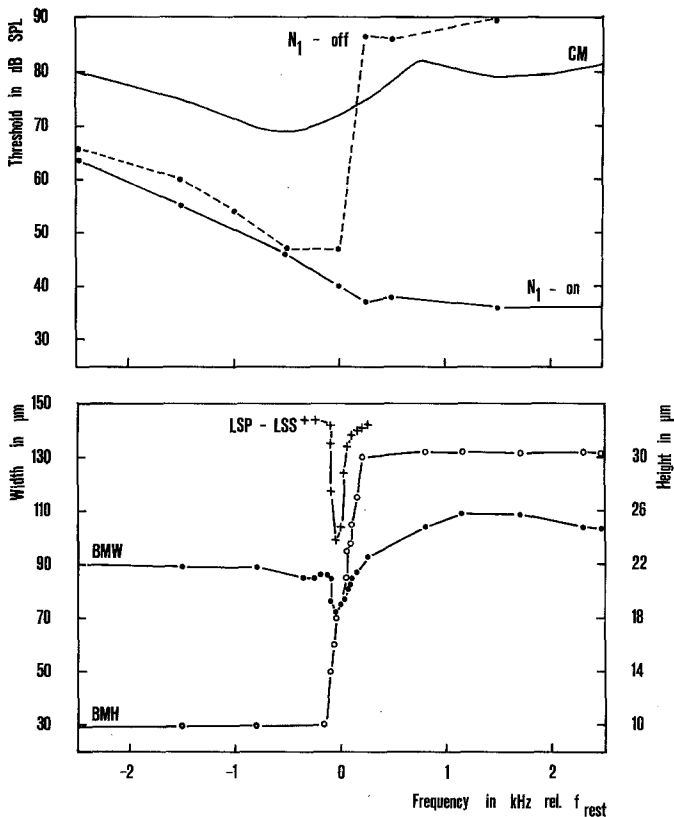
**Fig. 7.** Comparison of the CM, $N_1$-on and $N_1$-off threshold curves of RF 1 with the morphological results of Bruns (1976a, b). The data are displayed on a frequency scale relative to the resting frequency. BMW = width, and BMH = height of the basilar membrane, *LSP-LSS*, width between lamina spiralis primaria and secundaria

frequency range from 2.5 kHz below to 2.5 kHz above the resting frequency (Fig. 7). The recalibration is valid if ones assumes that within this frequency range the relationship of frequency to basilar membrane length shows no abrupt changes, that is, that the relationship between frequency and position over this length of the basilar membrane is a continuous, smooth function. We also expressed the CM, $N_1$-on and $N_1$-off thresholds of one of our bats relative to the resting frequency for the same frequency range.

Expression of the morphological data on a frequency scale shows that a drastic change in the height (BMH) and the width (BMW) of the basilar membrane and the prominent reduction of the width between the lamina spiralis primaria and the lamina spiralis secundaria (LPS-LSS) occurs in a region limited to a frequency band of a few hundred Hz around the resting frequency. A decrease in height of the basilar membrane starts at a frequency which is about 200 Hz above the resting frequency and which corresponds to the reference frequency. The physiological data show that at this frequency the $N_1$-on threshold starts to rise and the $N_1$-off threshold starts to fall steeply.

## Discussion

The results of these experiments clearly indicate that in *Rhinolophus ferrum-equinum* sharp auditory tuning in the frequency range of the CF-component of the echolocation sounds is already present at the level of the auditory nerve. The $N_1$-on and $N_1$-off threshold curves are very similar to threshold curves from the inferior colliculus.

The experiments also demonstrate that there is a fixed relationship between auditory tuning and the Doppler-shift compensation system. In all bats the $N_1$-on minimum was exactly tuned to the reference frequency, whereas the resting frequency always fell in the frequency range between the $N_1$-on minimum and the $N_1$-off minima. The advantages of Doppler-shift compensation and of the sharp auditory tuning are extensively discussed in earlier papers (Schnitzler, 1973a, b).

The morphological data from Bruns (1976a, b) suggest that the horseshoe bats have a two-sectioned cochlea. For the processing of frequencies above the resting frequency they use the basal portion with a thick, stiff basilar membrane and an expanded frequency scale. For frequencies below the resting frequency they use the apical part with the thinner, less stiff basilar membrane and a more compressed frequency distribution. The two parts are separated by prominent morphological discontinuities in the height and width of the basilar membrane and the width between the lamina spiralis primaria and secundaria. The comparison of the morphological data with our results demonstrates that in the frequency region associated with the anatomical discontinuity the threshold curves of CM, $N_1$-on and $N_1$-off are sharply tuned. It is probable that sharp auditory tuning depends upon these morphological specializations. This raises the question of how these morphological changes lead to sharp tuning in the context of auditory and specifically cochlear models.

At stimulus frequencies well above the resting frequency the envelopes of the travelling waves would reach their maxima in the stiff, basal part of the cochlea, and the steep front of the envelope of the travelling waves would not reach the discontinuity. If the stimulus frequency were close to the resting frequency, a rather small change in stimulus frequency might lead to travelling waves of considerable amplitudes reaching the discontinuity of the basilar membrane. Just in this frequency range prominent CM-aft and $N_1$-off responses appear even at low stimulus SPLs, leading to a very steep decrease in the $N_1$-off threshold (up to 23 dB in 0.25 kHz). Therefore we suggest that CM-aft and $N_1$-off responses occur when travelling waves with considerable amplitudes enter the region of the morphological discontinuity. One can assume that this part of the cochlea acts as a narrowly-tuned mechanical filter which exhibits damped oscillations following the cessation of forced oscillations of considerable amplitudes at neighbouring frequencies.

In *Rhinolophus ferrumequinum* the $N_1$-off was strongest for sounds which produced CM-aft. Therefore we conclude that the $N_1$-off is also a result of the mechanical properties of the cochlea. In order to produce $N_1$-off the rise and decay times of the stimuli have to be short enough to produce CM-aft. Since natural orientation sounds have rise and decay times that are rather

long, since the CF-portions of the echoes are preceeded and followed by FM-sweeps, and since the frequency of the CF-components of the echoes is kept in a range where the off-threshold is high, we suggest that the $N_1$-off play no important role in the echolocation system of the horseshoe bats. They occur only for artificial tone bursts with short rise and decay times.

The $N_1$-on threshold is low at the reference frequency, and the threshold becomes much higher at frequencies just below the reference frequency. A speculative explanation for sharp roll-off could be that the travelling waves loose energy when they pass over the discontinuity, causing reflections at the border between parts of the basilar membrane with different acoustical impedances. Such reflections might interact with the oncoming travelling waves, and these interactions might contribute to the high Q-values of peripheral auditory neurons in the frequency range above the reference frequency as described by Suga *et al.* (1976). Whether all the auditory tuning that can be seen in $N_1$ is due to the mechanical properties of the cochlea or whether further neural tuning occurs cannot be answered by our data.

It is interesting that the CM threshold is high in the range of low $N_1$-on thresholds around the reference frequency. The CM represents the summated activity of many hair cells, suggesting that in the range of the reference frequency only a rather small population of hair cells is stimulated for any one frequency. The high Q-values of the peripheral auditory neurons tuned to this frequency range (Suga *et al.*, 1976) suggest travelling wave envelopes which also have high Q-values and therefore stimulate only small populations of hair cells.

Attempts at a full explanation of the physiological data with the morphological specialisations must remain speculative. Future investigations with computer models of a chochlea similar to that of *Rhinolophus ferrumequinum* or with direct observations of the moving basilar membrane may give the information needed to clarify the relationship between physiological and morphological observations.

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