J Comp Physiol A (1993) 171:725–734

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Labile cochlear tuning in the mustached bat

I. Concomitant shifts in biosonar emission frequency

Russell F. Huffman[1], O.W. Henson, Jr.[2]

[1] Curriculum in Neurobiology and [2] Department of Cell Biology and Anatomy, The University of North Carolina, Chapel Hill, North Carolina, USA

Accepted September 21, 1992

Summary. The cochlea of the mustached bat (*Pteronotus parnellii*) has sharp tuning characteristics and pronounced resonance within a narrow band near the second harmonic, constant frequency (CF2) component of the animal's biosonar signals. That fine frequency discrimination occurs within this narrow band is evident from Doppler-shift compensation, whereby bats in flight lower the frequency of emitted CF2s to maintain returning echoes within this band. This study examined various factors capable of producing shifts in both the cochlear resonance frequency (CRF) and CF2s emitted by stationary bats and bats actively Doppler-shift compensating on a pendulum. Each of three experimental factors shifted the CRF in a reversible manner. Changes in body temperature produced an average CRF shift of 39 ± 18 Hz/°C. The CRF increased with flight by 150 ± 100 Hz and returned to baseline values within 10 min after flight. Contralateral sound exposure produced smaller (100 ± 20 Hz), rapid shifts in the CRF, suggesting that a mechanism different from the temperature- and flight-related shifts was involved. Changes in the CRF induced by temperature and flight were accompanied by shifts in the emitted CF2 of stationary and moving bats. Coupled with a companion study of associated shifts in neural tuning, the concomitant changes in CRF and CF2 provide evidence of cochlear tuning lability in the mustached bat.

Key words: Cochlea – Echolocation – Resonance – Body temperature – *Pteronotus*

Abbreviations: CF2, second harmonic, constant frequency component of the biosonar signal; CM, cochlear microphonic; CRF, cochlear resonance frequency

Correspondence to: Russell F. Huffman, Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710, USA

Introduction

The mustached bat, *Pteronotus parnellii parnellii,* uses biosonar to obtain an acoustic image of its surroundings and to detect and track insect prey. The biosonar signals are 6–32 ms pulses that consist of at least 4 harmonics, the second of which is the most prominent (Novick and Vaisnys 1964; Henson et al. 1987; Gaioni et al. 1990). Each harmonic contains a long, constant frequency (CF) component bracketed by short initial, and terminal frequency sweeps. The CF of the second harmonic (CF2) is near 60 kHz, and is called the "resting frequency" when emitted from a stationary bat. The resting frequency varies from bat to bat (60 to 64 kHz); however, for an individual bat under constant conditions, it is relatively invariant.

Inherent in echolocation by mustached bats is a precise regulation of the biosonar signals in response to changing echo parameters (Schnitzler 1970a, b; Henson et al. 1980, 1982; Jen and Kamada 1982; Kobler et al. 1985; Suga et al. 1987; Gaioni et al. 1990). This is best exemplified by "Doppler-shift compensation," whereby the frequency of the emitted CF2 is changed during flight to match the Doppler-shift in returning echoes. Thus, the echo CF2s are maintained within a narrow band, called the "reference frequency" (Schnitzler 1970a, b). As shown in Fig. 1, Doppler-shift compensation is easily observed by recording biosonar pulses and echoes from bats during forward swings on a pendulum.

It seems clear that Doppler-shift compensation requires a fine resolution of frequencies near 60 kHz. Therefore, it is not surprising that the cochlea of the mustached bat possesses many adaptations for sharp tuning. Relative to body size, the cochlea is much larger than that of most mammals, and for the matter, of most bats. The stimulated cochlea exhibits remarkably sharp tuning and pronounced resonance near the animal's CF2, and this resonance is thought to be a product of the mechanical properties responsible for sharp tuning in the 60 kHz portion of the cochlea (Pollak et al. 1972,

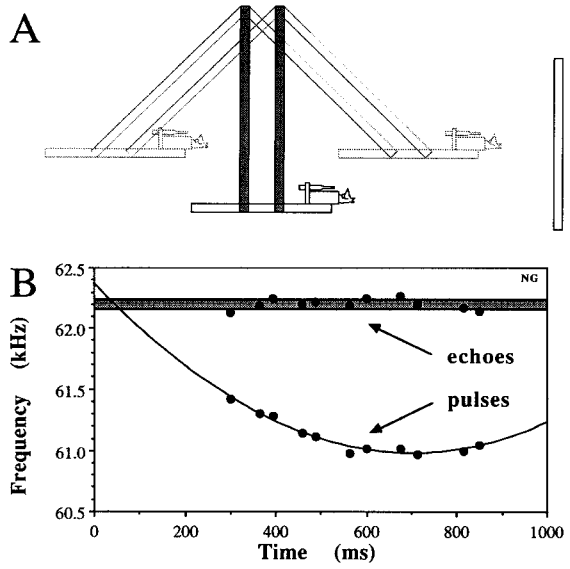


Fig. 1A, B. Doppler-shift compensation on the pendulum. **A** Pendulum design. During a forward swing, emitted pulses and echoes were recorded with a microphone mounted on the pendulum. For each forward swing, there was a period of positive acceleration as the pendulum approached the lowest point of its arc, followed by a negative acceleration as the pendulum approached zero velocity near the target. **B** Plot of the frequencies and times of occurrence of CF2 pulse-echo pairs for a single forward swing. Doppler-shift compensation is evident from the change in pulse frequency, which changes with the velocity of the pendulum. The relatively narrow, shaded band represents the mean reference frequency for this swing ± one standard deviation (62.20 ± 0.04 kHz). The curve is a second order binomial regression of the pulse frequencies ($r^2 = 0.98$); $r = 0.99$ ($P < 0.001$, two-tailed t-test, $t = 21.0$, $\rho > 0.95$ for $P < 0.05$)

1979; Suga et al. 1975; Suga and Jen 1977; Henson et al. 1982, 1985, 1990; Kössl and Vater 1985a, 1990b; Vater 1988). Cochlear resonance and sharp tuning are easily revealed by recording cochlear microphonic (CM) potentials. The CM audiogram has an unusually sharp tuning curve, the tip of which is at the cochlear resonance frequency (CRF). Spectral analysis of the CM elicited by broadband noise shows that much of the spectral energy is at the CRF (Fig. 2). The sharply tuned portion of the cochlea is disproportionately long and densely innervated, and possesses several structural specializations (Kössl and Vater 1985b; Henson and Henson 1988, 1991; Zook and Leake 1989; Vater 1987). From anatomical and physiological evidence, it is clear that the narrow band centered around the bat's CF2 is overrepresented at the level of the cochlea and at every level of the auditory pathway (Suga and Jen 1976; Suga et al. 1975, 1987; Pollak and Bodenhamer 1981; Zook and Leake 1989; Zook et al. 1985; Ross and Pollak 1989; Ross et al. 1988; Kössl and Vater 1990a; Covey et al. 1991; Olsen and Suga 1991; Pollak and Casseday 1989).

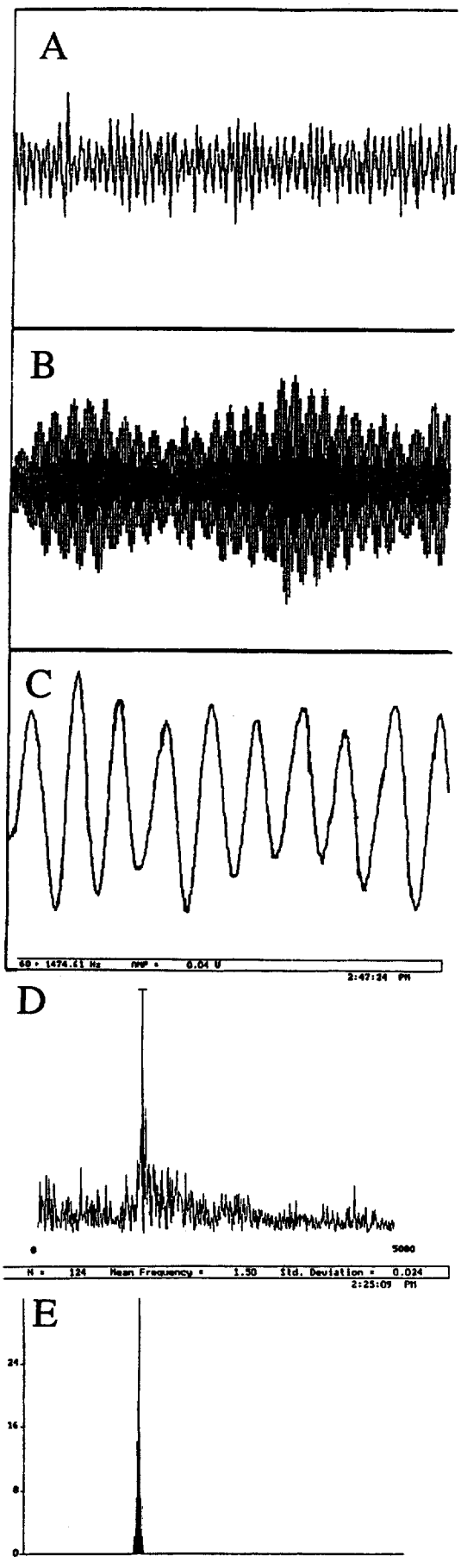
Cochlear resonance in the mustached bat provides an attractive model for studies of cochlear micromechanics, and especially resonant components that appear to create sharp tuning (Davis 1983; De Boer 1983a, b, c; Kim et al. 1980; Neely and Kim 1983, 1986; Zwislocki 1980; Zwislocki and Kletsky 1980). Recent studies with the mustached bat have found that flight activity and body temperature produce changes in the CRF, and that these changes are accompanied by shifts in the CF2 of emitted pulses (Henson et al. 1990; Huffman et al. 1991). In light of these findings, a precise understanding of CRF lability and its correspondence with changes in the CF2 is necessary if resonant features in this bat are to contribute further to general theories of sharp tuning, and to specific theories of biosonar signal processing. The purpose of this study was to expand these preliminary studies and provide quantitative descriptions of CRF and CF2 lability. This work was also the basis for developing methods to manipulate the CRF and examine its effects on the frequency tuning of auditory neurons. The neurophysiological data are presented in a companion paper (Huffman and Henson 1992).

Methods

This study reports data obtained from 23 mustached bats, *Pteronotus parnellii parnellii,* from Jamaica, W.I. For each type of experiment described (but not necessarily for each bat), 3 frequency measurements were routinely made: the CRF, the resting frequency and the reference frequency.

Cochlear resonance was measured in 11 bats with chronically implanted CM electrodes. For electrode implantation, hair was removed from the scalp with a depilatory agent (Neet) and the skin was cleaned. Bats were then anesthetized with an inhalant, methoxyflurane (Metofane, Pitman-Moore, Inc). A posterior skin incision was made over the dorsal part of the skull and the underlying part of the temporalis muscle was removed bilaterally. The bone was then cleaned, dried and coated with a thin layer of cyanoacrylic adhesive (Loctite, Superbonder 409 gel). A tungsten ground electrode was implanted in the cerebral cortex. A hole was drilled near the lambdoid ridge, 2.4 mm from midline, and a teflon-coated tungsten electrode was stereotaxically advanced toward the cochlear aqueduct. When high amplitude CM potentials were evident in response to broadband acoustic stimuli, the electrode was glued in place. Care was taken not to penetrate the aqueduct. All experiments were conducted with bats that were awake and fully recovered from anesthesia and surgery. During CM recordings bats were held in a suspended, loose-fitting styrofoam mold to allow body movement, while the head was immobilized with a pin vise attached to the ground electrode.

Continuous cochlear resonance was evoked by low-level (approx. 35 dB SPL) broadband noise (Fig. 2). The CRF is known to be constant at low to moderate SPLs, but not at high levels (Henson et al. 1990); therefore, all measurements of CRF were conducted with stimulus levels which produced CM potentials just noticeable above the noise floor. The signal from a random noise generator (General Radio Co., Type 1390-B) was fed to a one-inch electrostatic speaker (Polaroid Corp.). The speaker was 15 cm from the pinna, 20° from midline, and positioned to yield the highest amplitude CM in response to low-level stimulation. The CM was amplified (EG&G Model 113 preamplifier, Princeton Applied Research), narrow-bandpass filtered (TTE, Inc. Model K18 E3007, 3 dB points at 59.65 and 64.0 kHz), and heterodyned with a signal near 60 kHz to increase frequency resolution and reduce the required sampling rate (Fig. 2C). The CRF was obtained from spectral analysis of the heterodyned output (spectrum analyzer/digital oscilloscope, Model R350, Rapid System Inc.). FFTs were collected at a rate of 200/min with a resolution of ±10 Hz. Each FFT contained a single, sharp spectral peak (Fig. 2D). Spectral peak values were displayed as a histogram, and the CRF was determined by the mean of the histogram (Fig. 2E). The standard deviations of histograms normally ranged from 10 to 30 Hz. Each histogram established the CRF value for periods ranging from 15–120 s, and



several histograms were averaged to obtain mean CRF values for longer periods.

Body temperature was controlled with a heat lamp positioned 30 cm from the bat. The lamp was adjusted to low heat levels with a rheostat to either sustain or increase the body temperature. Body temperature was kept within or near the range normally found in active mustached bats (37–42 °C). During experiments, each episode of heat exposure was limited to 50 min or less, and water and mealworms were offered every 1–2 h. Body temperature was monitored with a thermocouple probe (Sensortek, Inc., Models IT-18 and IT-21) and recording device (Sensortek, Inc., Model BAT-12) which allowed measurements with an accuracy of ± 0.05 °C. The probe was positioned in the midline of the deep skin fold between the occiput and the back. A control experiment was conducted to examine the accuracy of reported body temperature changes at this recording site. During trials similar to that shown in Fig. 3, body temperature was recorded simultaneously from two probes, one at the skin fold, and a second chronically placed in the parafloccular fossa of the cranial cavity. Although the absolute temperature values were slightly different, the changes in temperature recorded from these two positions were nearly identical (within 0.1 °C). Similar results were previously reported for a comparison between measurements in the skin fold and under the temporalis muscle (Henson et al. 1990). It should be noted that dorsal, subcutaneous probe placements have been found to provide accurate measures of deep body temperature in other small bats (Brown and Bernard 1991).

The resting frequency (CF2 emitted by stationary bats) was determined from the biosonar signals of bats placed in a small cylindrical cage (21.6 cm diameter; 21.6 cm long). Emissions were recorded with a 6.25 mm Brüel and Kjcer condenser microphone (Model 4135) and associated amplifier (Model 2608). Signals were treated in the same manner as the CM: they were narrow bandpass filtered to isolate the CF2, and then heterodyned with a signal near the CF2 to increase frequency resolution (to ± 10 Hz) and reduce the required sampling rate. Each filtered, heterodyned CF2 pulse triggered an FFT analysis of the pulse by the spectrum analyzer (mentioned above), which yielded a sharp spectral peak. In similar fashion to analysis of CM FFTs, 100–200 CF2 FFTs were collected and displayed as a histogram of spectral peaks to determine the mean resting frequency. Standard deviations normally ranged from 20 Hz to 50 Hz.

The reference frequency (CF2 of echoes returning to Doppler-shift compensating bats) was determined from bats placed on a pendulum and swung toward a target (Fig. 1A). Biosonar pulses and Doppler-shifted echoes were recorded with a microphone mounted on the pendulum. These signals were stored on a precision magnetic tape system (Racal Store 7DS, tape speed 15 or 30 inch/s). The filtered, heterodyned signals for each forward swing were collected by the spectrum analyzer and FFTs were performed on the CF2s of pulse-echo pairs. Individual values (± 10 Hz) were collected and graphed (Fig. 1B).

Results

CRF lability

The normally stable CRF could be reliably shifted by a number of physiological parameters, namely body temperature, flight and contralateral noise exposure. Of

Fig. 2A–E. Cochlear resonance and method of CRF determination. **A** Digitized sample of the low-level, broadband noise signal delivered to the ear. **B** Oscillographic display of the digitized, bandpass-filtered CM. **C** Waveform of the CM when heterodyned with a frequency near the CRF (10 kHz sample rate). **D** Single spectral peak of a 1024 point FFT of the heterodyned CM. **E** Histogram of 124 spectral peaks collected over 30 s

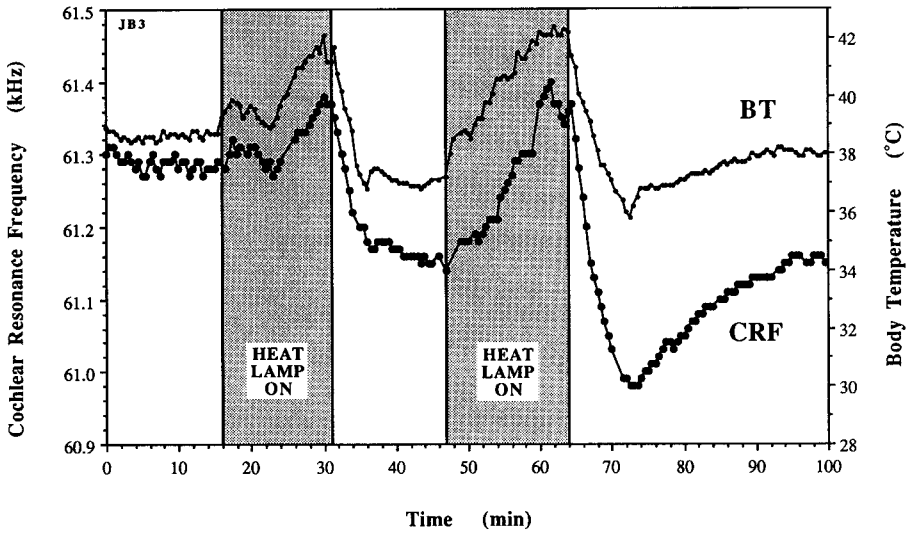


Fig. 3. Effect of heat lamp exposure on body temperature and the CRF. Body temperature was adjusted by turning a low-intensity heat lamp on and off for 2 consecutive trials

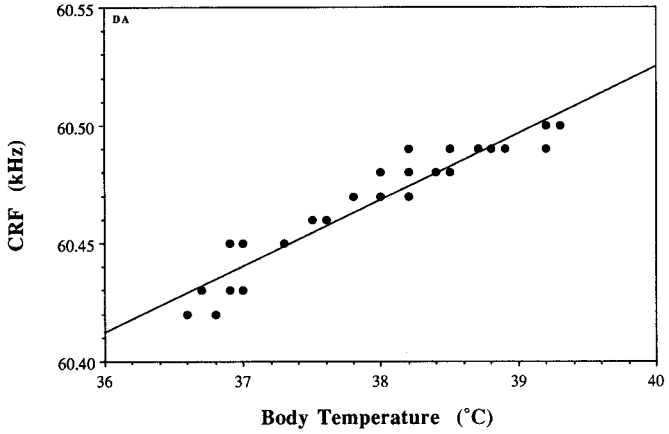


Fig. 4. Correlation of the CRF and body temperature ($r=0.96$; $P<0.001$, two-tailed t-test, $t=16.4$, $n=25$; $\rho>0.91$ for $P<0.05$). Data were obtained from a heat lamp experiment like that shown in Fig. 3. The slope of a simple regression line of the data ($r^2=0.92$) was used to estimate the rate of change in the CRF with body temperature (28 Hz/°C)

these, body temperature was the most easily controlled factor for effecting quantifiable shifts in the CRF. In all experiments where bats were exposed to a heat lamp, increases in body temperature were accompanied by upward shifts in the CRF; subsequent removal of the heat lamp produced downward shifts in body temperature and CRF ($n=11$ bats). Figure 3 shows typical results. In such trials, there was a high, positive correlation between CRF and body temperature for all bats (e.g. Fig. 4). Regressions like the one shown in Fig. 4 were calculated for 25 trials among 6 bats, for which data was sufficient to test the statistical significance of correlation (r) and regression (m) coefficients ($P<0.05$; two-tailed t-test). The mean shift in CRF (determined from regression slopes) was 39 ± 18 Hz/°C ($N=25$; range = 18–74 Hz/°C; $P<0.05$ in t-tests of r and m for each data set).

The effect of flight on the CRF was examined in 8 bats. In all cases, 5–15 min flights produced an increase

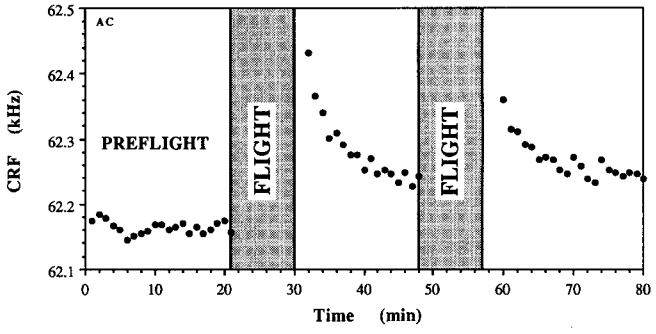


Fig. 5. Shifts in the CRF with flight. The CRF was elevated from preflight values, and decreased after each period of flight

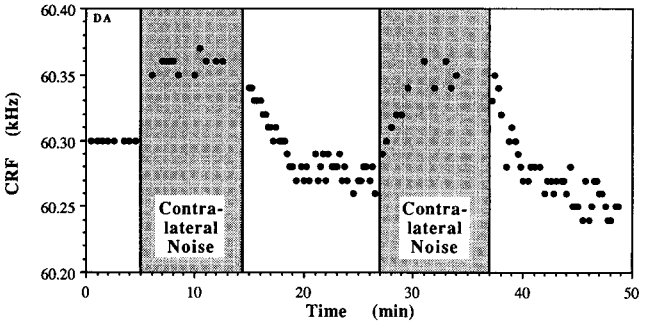


Fig. 6. Effect of contralateral noise exposure on the CRF. The CRF was shifted upward during periods of high-level noise presented to the contralateral ear. (See text for description.)

in the CRF from preflight values (Fig. 5). Initial CRF determinations after flight undoubtedly underestimated the size of the shift in resonance because of a 1–2 min delay necessary to attach the CM leads immediately after capture of the flying bat. The mean CRF shift measured 1–2 min after flight was 150 ± 100 Hz ($N=8$; range = 40–280 Hz). After each flight, the elevated CRF decayed to a new baseline level, usually within a 10 min period (mean decay = 180 ± 80 Hz; $N=10$; range = 80–370 Hz).

Shifts in the CRF were also induced in 5 bats exposed to loud noise presented to the contralateral ear. Figure 6

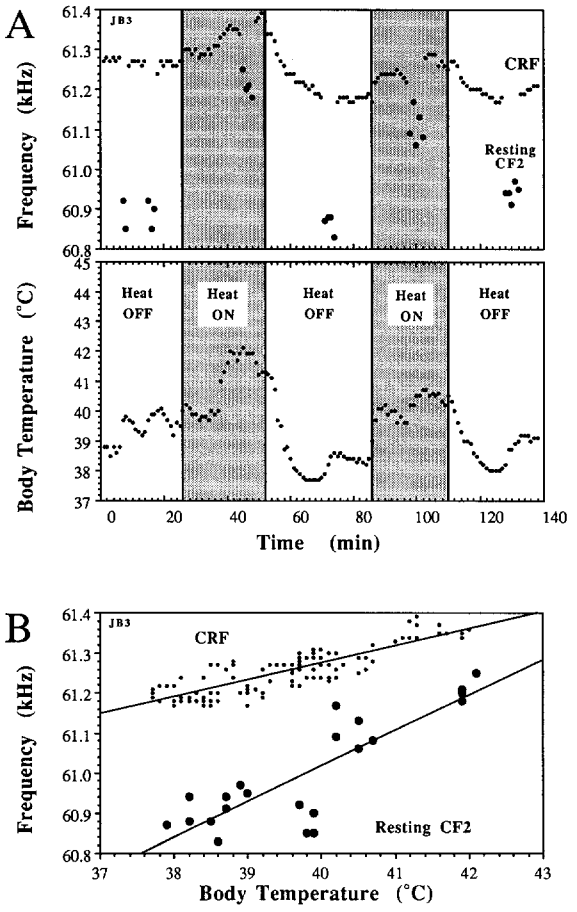


Fig. 7A, B. Concomitant shifts in the CRF and resting frequency with changes in body temperature. **A** Results of a single experiment in which the CRF and resting frequency were measured (top) as body temperature (bottom) was adjusted for 2 consecutive trials. **B** Correlations of the CRF ($r=0.84$; $P<0.001$, two-tailed t-test, $t=18.5$, $n=145$; $0.79<\rho<0.89$ for $P<0.05$) and resting frequency ($r=0.84$; $P<0.001$, two-tailed t-test, $t=7.09$, $n=23$; $0.65<\rho<0.94$ for $P<0.05$) with body temperature (data is from the experiment shown in **A**). In **A** and **B**, each CF2 data point represents 50–100 pulses

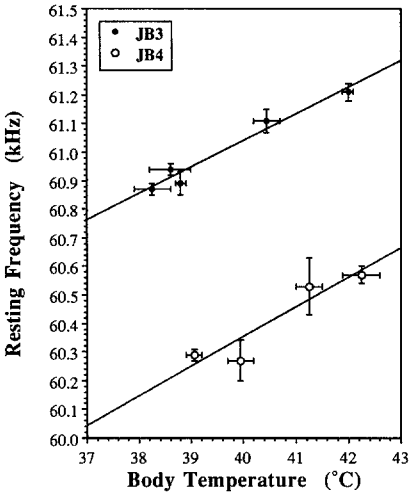


Fig. 8. Changes in the mean resting frequency throughout the day with fluctuations in body temperature for 2 bats. Each data point represents 100–200 pulses. Correlation coefficients are 0.98 ($P<0.005$, two-tailed t-test, $t=8.49$) and 0.94 ($0.05<P<0.1$, two-tailed t-test, $t=3.90$) for bats JB3 and JB4, respectively. Regressions were used to estimate the amount of shift in the resting frequency with respect to body temperature. The slopes of the regression lines are 93 Hz/°C ($r^2=0.96$) and 104 Hz/°C ($r^2=0.89$) for bats JB3 and JB4, respectively

shows 2 consecutive trials for which the CRF was monitored during and after noise exposure. The CRF was measured continuously during alternating periods of low-level broadband noise (approx. 35 dB SPL output) presented to the ipsilateral (implanted) ear alone from a speaker 20 cm away, versus high-level noise (approx. 90 dB SPL output) presented to the contralateral ear alone from a funneled speaker 5 cm away. During the noise exposure condition, cochlear resonance was evoked by the contralateral noise; the small amplitude of the CM indicated that this stimulus level was comparable to the low levels of the ipsilateral stimulus alone. Contralateral noise produced a comparatively small and sustained increase in the CRF (mean = 100 ± 20 Hz; $N=10$; range = 70–130 Hz). Within several minutes after cessation of contralateral exposure, resonance evoked by low-level, ipsilateral noise returned to a baseline frequency.

Concomitant shifts in CRF and CF2

Factors that shifted the CRF, such as body temperature changes and flight activity, also caused shifts in the resting frequency of stationary bats, and in the reference frequency of Doppler-shift compensating bats. For example, in body temperature experiments like those mentioned above, where resting frequency was measured in addition to the CRF ($N=5$ bats), both CRF and resting frequency shifted with changing body temperature in all cases. Figure 7A shows typical results of these experiments, in which the CRF and resting frequency were positively correlated with body temperature (Fig. 7B). Trials with a sufficient number of data points to test for statistical significance of both correlation (r) and regression (slope) coefficients ($P<0.05$; two-tailed t-test) were used to estimate the amount of shift in resting frequency with body temperature. The mean shift in resting frequency (determined from regression slopes) was 93 ± 33 Hz/°C ($N=8$; range = 32–124 Hz/°C). In addition, the resting frequency was examined throughout the day in relation to fluctuating body temperature. As shown in Fig. 8, higher resting frequencies were associated with higher body temperatures; these shifts were equivalent (per °C) to the shifts observed with the heat lamp experiments.

Shifts in the resting frequency also occurred with periods of flight ($N=12$ bats). In cases where both the CRF and resting frequency were recorded, both were elevated after flight, and subsequently showed decreases to baseline values; these values were often below previous baseline levels (Fig. 9). The mean flight-induced shift from preflight values was 210 ± 100 Hz ($N=14$; range = 100–

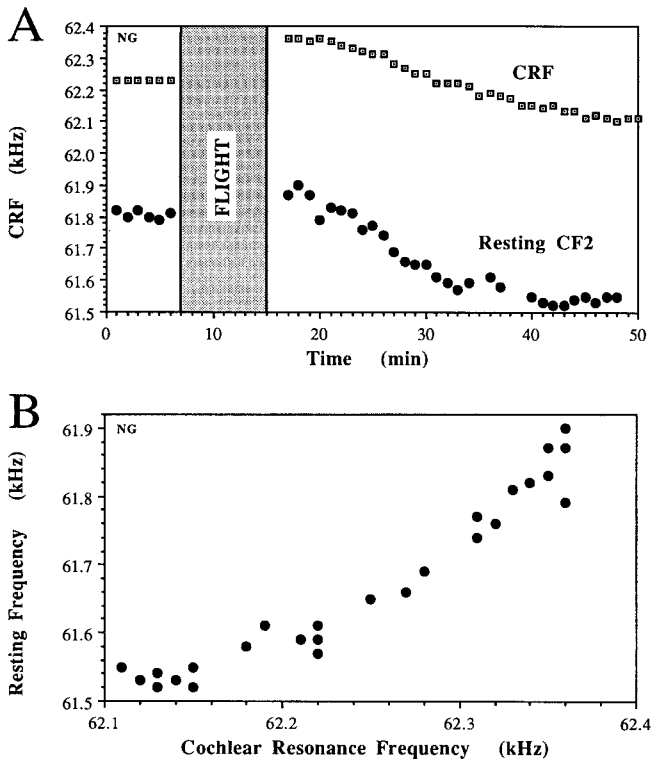


Fig. 9A, B. Concomitant shifts in the CRF and resting frequency with flight. **A** Preflight and postflight CRFs and resting frequencies for a single flight trial. **B** Correlation of the resting frequency and the CRF (postflight data from the experiment shown in **A**); r = 0.96 ($P < 0.001$, two-tailed t-test, $t = 17.8$, $n = 29$; $0.92 < \rho < 0.99$ for $P < 0.05$). In **A** and **B**, each CF2 data point represents 20–50 pulses

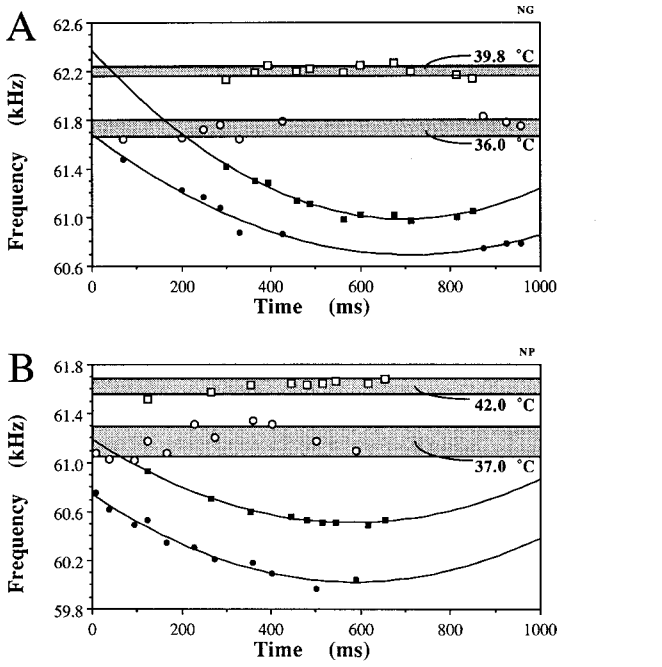


Fig. 10A, B. Two examples of the effect of body temperature on the reference frequency of bats swung on the pendulum, showing that bats maintain higher reference frequencies with increases in body temperature. The CF2s of pulses (*filled symbols*) and echoes (*open symbols*) are shown for forward swings recorded at 2 body temperatures (*squares* and *circles*, respectively). *Shaded bands* represent mean reference frequencies ± one standard deviation. *Curves* are second order binomial regressions of the pulse frequencies (**A**: $r^2 = 0.97$ for both curves; **B**: $r^2 = 0.996$ and 0.97 for pulses at 42.0 °C and 37.0 °C, respectively; $P < 0.001$ (two-tailed t-test) for r in all cases)

460 Hz). The mean decrease observed in the period that followed flight was 230 ± 100 Hz ($N = 14$; range = 80–470 Hz).

Changes in body temperature also caused shifts in the reference frequency of Doppler-shift compensating bats. Five bats were swung on a pendulum before and after exposure to a heat lamp, and temperature-dependent shifts in emitted pulses and echoes were recorded. Figure 10 shows examples of data acquired from pendulum swings. Shifts in reference frequency accompanied temperature-dependent changes in the CRF (Fig. 11). The mean shift in reference frequency was 90 ± 38 Hz/°C ($N = 6$; range = 49–136 Hz/°C). Shifts in reference frequency and CRF were also observed for bats swung on the pendulum before and after flight (Fig. 12). The mean flight-induced shift from preflight values was 310 ± 180 Hz ($N = 6$; range = 100–440 Hz).

In summary, temperature, flight and contralateral noise exposure induced reproducible shifts in the CRF. CRF shifts were matched by concomitant shifts in the CF2 emitted during echolocation, either from stationary or moving bats. This was best illustrated by the cases where the cochlear resonance, resting and reference frequencies were recorded concurrently from a single bat, for two different body temperatures (Fig. 13).

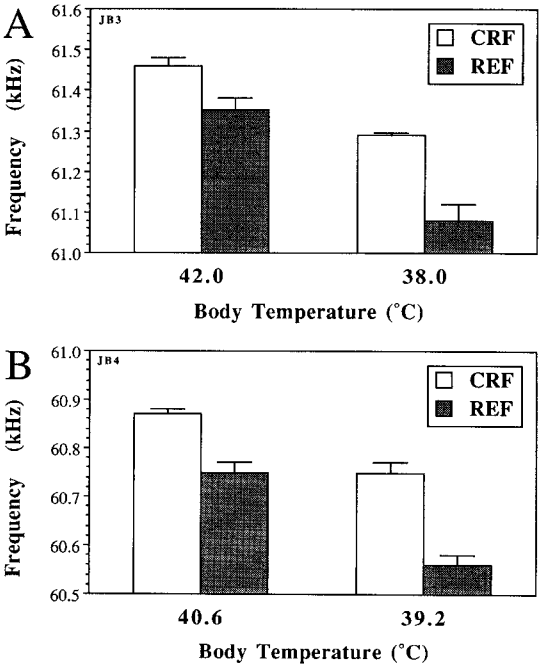


Fig. 11A, B. Shifts in the CRF and reference frequency (*REF*) with changes in body temperature for two bats. Reference frequency values represent the mean of echoes from 3–5 pendulum swings. *Error bars* indicate one standard deviation from the mean. Shifts in *CRF* and reference frequency are statistically significant ($P < 0.05$, one-tailed t-test)

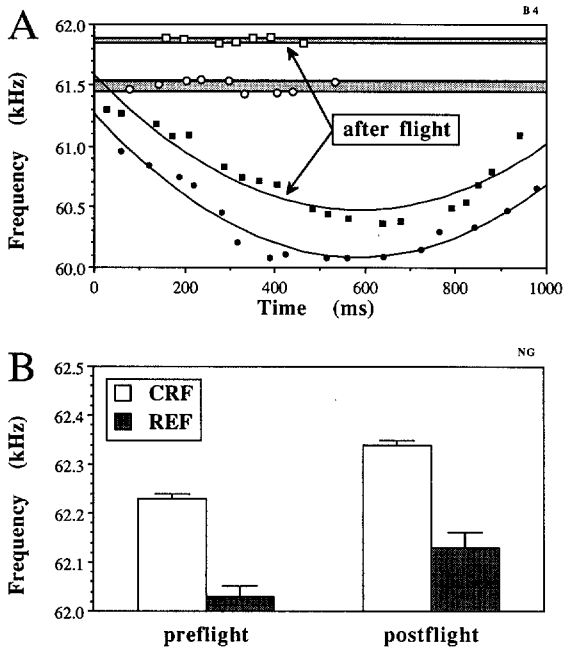


Fig. 12A, B. Shifts in the CRF and reference frequency with flight. **A** Flight-associated shifts in emitted pulses and echoes of bats swung on a pendulum. CF2s of pulses (*filled symbols*) and echoes (*open symbols*) are shown for forward swings recorded before (*circles*) and after (*squares*) flight. *Shaded bands* represent mean reference frequencies ± one standard deviation. (For this case, the frequencies of echo CF2s in the second half of the forward swing were not measurable). *Curves* are second order binomial regressions of the pulse frequencies; $r^2 = 0.89$ and 0.94 for preflight and postflight pulse CF2s, respectively ($P < 0.001$, two-tailed *t*-test, for r in both cases). **B** Correspondence of shifts in the CRF and reference frequency (*REF*) before and after flight. Reference frequency values represent the mean of echoes from 3–5 pendulum swings. Error bars indicate one standard deviation from the mean. Shifts in CRF and reference frequency are statistically significant ($P < 0.05$, one-tailed *t*-test)

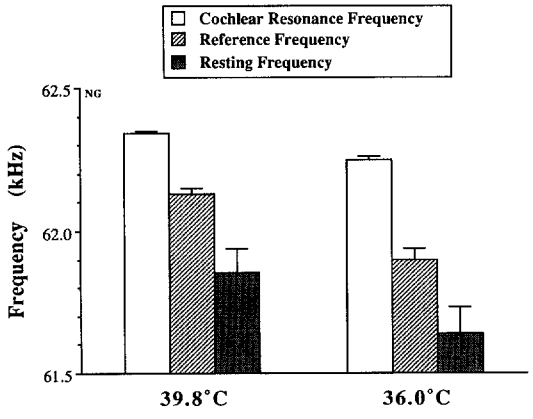


Fig. 13. Shifts in the CRF, reference frequency and resting frequency with a change in body temperature for a single trial. Reference frequency values represent the mean of echoes from 3–5 pendulum swings. Resting frequency values represent the mean of 100–200 pulses. *Error bars* indicate one standard deviation from the mean. Shifts in CRF, reference frequency and resting frequency are statistically significant ($P < 0.05$, one-tailed *t*-test)

Discussion

CRF lability

A major purpose of this study was to provide new data on CRF lability in response to body temperature, flight and contralateral noise exposure. The principal findings were (1) each of these parameters produced a reversible change in the CRF (Figs. 3, 5, 6 and 14); (2) the amount of change was different for each parameter (see Fig. 14); (3) post-trial recoveries in the CRF often returned to a new baseline value (Figs. 3, 5, 6); and (4) both temperature-dependent and -independent factors affect the CRF. These results substantiated the variability of the CRF which has been noted in earlier reports from our laboratory (Henson et al. 1989, 1990; Huffman et al. 1991).

Changes in body temperature proved to be the most reliable method for shifting the CRF (Figs. 3, 4, 7). The data suggest that elements of the cochlear partition which contribute to cochlear mechanical tuning are affected by temperature such that a shift in the frequency response of outer hair cells tuned at the resonance frequency occurs. In agreement with the finding that the CRF shifts with temperature, Kössl and Vater (1985a) showed that localized cooling of the head reversibly decreased the frequency of a prominent otoacoustic emission (OAE) tuned near the CRF. They have suggested that the OAE is related to the morphological specializations responsible for cochlear resonance in the mustached bat. Interestingly, temperature-dependent changes in the frequencies of spontaneous OAEs have been demonstrated for the guinea pig (Ohyama et al. 1992) as well as for nonmammalian vertebrates (Wilson et al. 1986; van Dijk and Wit 1987; van Dijk et al. 1989; Manley and Köppl 1992). Furthermore, spontaneous OAEs are prevalent in humans (see Probst et al. 1991, p. 2036), and several recent studies have shown that spontaneous OAE frequencies are sensitive to a number of physiological variables (Mott et al. 1989; Haggerty 1989, 1990; Bell 1992).

Flight-associated physiological changes tended to induce more pronounced CRF shifts than heat exposure, yet both factors were capable of producing marked changes in the CRF (Fig. 14A, B). The change in the CRF with flight may be due to a combination of temperature-dependent and -independent factors. For some neotropical bats it has been shown that body temperature is elevated during flight, and dramatic temperature increases in the first minutes of flight are common (Thomas and Suthers 1972; Thomas 1987). Physiological factors other than body temperature may influence the CRF during flight, however, since instances of flight-induced shifts which were independent of body temperature have been recorded (Henson et al. 1990). In this case, it is compelling to ask if these, and otherwise temperature-dependent factors, are cumulative, and account for the more robust effects of flight on the CRF.

The CRF shifts produced by contralateral sound exposure differed quantitatively from those produced by temperature changes or flight (Fig. 14C). For example,

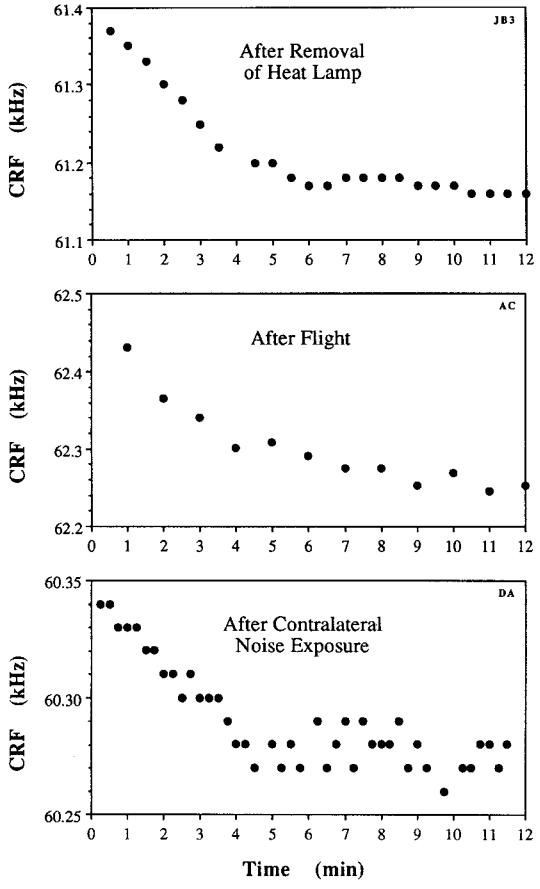


Fig. 14. Comparison of decreases in the CRF with a drop in body temperature (*top;* from trial 1 in Fig. 3), after flight (*middle;* from trial 1 in Fig. 5) and after contralateral noise exposure (*bottom;* from trial 1 in Fig. 6). Note the different frequency scale for the bottom panel

whereas postflight decreases in CRF of 200$^+$ Hz over 7–10 min were common, CRF shifts from contralateral noise were usually 100 Hz over 3–4 min. The difference in magnitude and time scale of the sound-induced shift may point to a different cochlear mechanism than those responsible for the temperature- and flight-related shifts. This mechanism is likely to involve the medial olivocochlear efferent system (for review see Wiederhold 1986; Warr 1992), which is well developed in the mustached bat (Bishop and Henson 1987). Numerous studies on other mammals have provided evidence that activation of olivocochlear efferents alters the micromechanical properties of the cochlea (see Brown 1988; LePage 1989; Mott et al. 1989). It is of interest to note here that, in humans, contralateral acoustic stimulation produces an abrupt, upward shift in the frequency of spontaneous OAEs (Mott et al. 1989), suggesting a similar effect from efferent activity in the human cochlea.

Concomitant shifts in CRF and CF2

A principal finding of this study was that the CF2 changed concomitantly with CRF shifts during flight and with changes in body temperature. These data substantiate earlier reports from our laboratory (Henson et al. 1990; Huffman et al. 1991) and provide an opportunity for quantitative analysis of CF2 shifts associated with CRF lability. The directions of CRF and CF2 shifts were identical in all cases; however, the amount of shift in the CF2 was often greater than that of the CRF (see Fig. 7). The heat lamp experiments produced mean shifts in resting and reference frequencies of 93 ± 33 Hz/°C and 90 ± 38 Hz/°C, respectively, yet the mean CRF shift was 39 ± 18 Hz/°C. In contrast, the amounts of shift in the CF2 and CRF due to flight were more equivalent than the temperature-related shifts. For example, the mean changes in resting frequency and CRF with flight were 210 Hz and 150 Hz, respectively. Similarly, the mean postflight decays in resting frequency and CRF were 230 Hz and 180 Hz, respectively. It is not presently clear why the CF2 is more labile than the CRF.

It would be of interest to determine if the CRF shifts with sound exposure are accompanied by shifts in biosonar emission frequency. This study was attempted, but several problematic factors made the interpretation of the data difficult. First, although the CRF shift could be maintained during noise exposure, restrained bats were reluctant to emit biosonar signals; furthermore, the rapidity of decay in the CRF shift after exposure prevented accurate CF2 determinations, which require a suitable number of emissions. Second, the shift in the CRF with noise was comparatively small; it would be expected that shifts in the CF2 would also be small, making a significant change difficult to show.

There are numerous examples of specialization of cochlear structures responsible for sharp tuning in the CF2 portion of the partition (Henson 1973, 1978; Henson and Henson 1988, 1991; Henson et al. 1977, 1984, 1985; Vater 1988; Zook and Leake 1989). It is likely that, because these features are exaggerated, small physiological changes in this band impart significant and measurable effects on tuning properties of the partition, which are evident from the change in the CRF. Labile cochlear tuning is corroborated by the finding that parallel tonotopic shifts in neural tuning result from changes in the CRF (Huffman and Henson 1991, 1992). It is apparent from the temperature- and flight-induced shifts in both the CRF and the CF2 that continuous adjustment of the CF2 is an important process that may facilitate fine frequency resolution. The mustached bat, like many small neotropical bats, experiences significant changes in body temperature throughout the day, depending on changes in ambient temperature, activity, and proximity to other bats (McNab 1969; Thomas 1987). Furthermore, the shifts in cochlear tuning observed before and after flight represent changes that are likely to occur under natural conditions.

The ability to Doppler-shift compensate, and to match shifts in cochlear tuning with complementary shifts in biosonar emission frequency, implies some exchange of precise frequency information between auditory and vocalization systems. The auditory-vocal interface in bats is apparently complex in its anatomical distribution (e.g. Schuller and Radtke-Schuller 1988). Not-

withstanding, it has been demonstrated that brain regions for which microstimulation elicits vocalization receive and process auditory information that is frequency-specific, indicating discrete tonotopic input. For example, in the mustached bat, neurons in "vocalization centers" have sharp tuning characteristics in response to frequencies near the CF2 (Suga and Yajima 1988), and for one region of the cerebral cortex, the representation of evoked-CF2 emission frequencies is organized tonotopically in a manner similar to the bat's auditory system (Gooler and O'Neill 1987).

The adjustment of biosonar emission frequencies to match changes in cochlear tuning cannot be explained, however, with a neural system that is composed solely of hardwired, feedforward input of frequency information to the vocalization system. To the contrary, such feedforward input, if tonotopically shifted, would consistently create a mismatch between vocal output and frequencies represented in the band of sharp tuning. Thus, it is likely that a feedback mechanism exists which detects the coincidence of both the activation of the narrow band to which the cochlea is most sensitive, and the frequency of emitted CF2s. How this match is encoded is an open question; a neural model would likely include a "set point" of neural activity in vocal centers (and subsequent motor instructions to laryngeal muscles) that is achieved when auditory feedback to these centers is consistent with stimulation of the sharply tuned band.

In conclusion, the CF2 emission frequencies of moving and stationary bats shift with the CRF such that echo CF2s are kept in the sharply tuned, 60 kHz band. The concomitant shifts in CRF and CF2 strongly suggest that the change in CRF reflects a lability of frequency tuning within this sensitive, high-resolution portion of the cochlea. Coupled with neurophysiological data which shows labile tuning of central auditory neurons with shifts in the CRF (Huffman and Henson 1992), this study provides additional evidence that the mustached bat's biosonar signal is adjusted to accommodate normally occurring shifts in cochlear tuning.

Acknowledgements. We thank Art Keating for his technical assistance, Patricia Koplas and Doug Fitzpatrick for their participation in preliminary experiments, and Miriam Henson and Winston Lancaster for their comments on the manuscript. This work was supported by PHS grant DC 00114 from the National Institute on Deafness and other Communicative Disorders.

References

Bell A (1992) Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. Hearing Res 58:91–100

Bishop AL, Henson OW Jr (1987) The efferent cochlear projections of the superior olivary complex in the mustached bat. Hearing Res 31:175–182

Brown AM (1988) Continuous low level sound alters cochlear mechanics: an efferent effect? Hearing Res 34:27–38

Brown CR, Bernard RTF (1991) Validation of subcutaneous temperature as a measure of deep body temperature in small bats. J Zool Lond 224:315–346

Covey E, Vater M, Casseday JH (1991) Binaural properties of single units in the superior olivary complex of the mustached bat. J Neurophysiol 66:1080–1094

Davis H (1983) An active process in cochlear mechanics. Hearing Res 9:79–90

De Boer E (1983a) No sharpening? A challenge for cochlear mechanics. J Acoust Soc Am 73:567–573

De Boer E (1983b) On active and passive cochlear models – toward a generalised analyis. J Acoust Soc Am 73:574–576

De Boer E (1983c) Power amplification in an active model of the cochlea – short wave case. J Acoust Soc Am 73:577–579

Dijk P van, Wit HP (1987) Temperature dependence of frog spontaneous otoacoustic emissions. J Acoust Soc Am 82:2147–2150

Dijk P van, Wit HP, Segenhout JM (1989) Spontaneous otoacoustic emissions in the European edible frog (*Rana esculenta*): spectral details and temperature dependence. Hearing Res 42:273–282

Gaioni SJ, Riquimaroux H, Suga N (1990) Biosonar behavior of mustached bats swung on a pendulum prior to cortical ablation. J Neurophysiol 64:1801–1817

Gooler DM, O'Neill WE (1987) Topographic representation of vocal frequency demonstrated by microstimulation of anterior cingulate cortex in the echolocating bat, *Pteronotus parnellii parnellii*. J Comp Physiol A 161:283–294

Haggerty HS (1989) Spontaneous otoacoustic emissions: evidence for circadian rhythm of frequency variation. J Acoust Soc Am Suppl 86:S44

Haggerty HS (1990) Spontaneous otoacoustic emissions: evidence for a monthly rhythm of frequency variation in women. Assoc Res Otolaryngol Abstr 13:233

Henson MM (1973) Unusual nerve-fiber distribution in the cochlea of the bat, *Pteronotus p. parnellii* (Gray). J Acoust Soc Am 53:1739–1740

Henson MM (1978) The basilar membrane of the bat, *Pteronotus p. parnellii*. Am J Anat 153:143–157

Henson MM, Henson OW Jr (1991) Specializations for sharp tuning in the mustached bat: the tectorial membrane and spiral limbus. Hearing Res 56:122–132

Henson MM, Henson OW Jr, Goldman LJ (1977) The perilymphatic spaces in the cochlea of the bat, *Pteronotus p. parnellii* (Gray). Anat Rec 187:767

Henson MM, Henson OW Jr, Jenkins DB (1984) The attachment of the spiral ligament to the cochlear wall: anchoring cells and the creation of tension. Hearing Res 16:231–242

Henson OW Jr, Henson MM (1988) Morphometric analysis of cochlear structures in the mustached bat, *Pteronotus parnellii parnellii*. In: Nachtigall PE, Moore PWB (eds) Animal sonar: Processes and performance. Plenum Press, New York, pp 301–305

Henson OW Jr, Henson MM, Kobler JB, Pollak GD (1980) The constant frequency component of the biosonar signals of the bat *Pteronotus parnellii parnellii*. In: Busnel R-G, Fish JF (eds) Animal sonar systems. Plenum, New York, pp 913–916

Henson OW Jr, Pollak GD, Kobler JB, Henson MM, Goldman LJ (1982) Cochlear microphonic potentials elicited by biosonar signals in flying bats, *Pteronotus p. parnellii*. Hearing Res 7:127–147

Henson OW Jr, Schuller G, Vater M (1985) A comparative study of the physiological properties of the inner ear in Doppler shift compensating bats (*Rhinolophus rouxi* and *Pteronotus parnellii*). J Comp Physiol A 157:587–597

Henson OW Jr, Bishop AL, Keating AW, Kobler JB, Henson MM, Wilson BS, Hansen R (1987) Biosonar imaging of insects by *Pteronotus p. parnellii*, the mustached bat. Nat Geogr Res 3:82–101

Henson OW Jr, Keating AW, Huffman RF, Koplas P, Henson MM (1989) The effect of contralateral acoustic stimulation on the stability of cochlear resonance. Assoc Res Otolaryngol Abstr 12:339

Henson OW Jr, Koplas P, Keating AW, Huffman RF, Henson MM (1990) Cochlear resonance in the mustached bat: behavioral adaptations. Hearing Res 50:259–274

Huffman RF, Henson OW Jr (1991) Cochlear and CNS tonotopy: normal physiological shifts in the mustached bat. Hearing Res 56:79–85

Huffman RF, Henson OW Jr (1993) Labile cochlear tuning in the mustached bat. II. Concomitant shifts in neural tuning. J Comp Physiol A 171:735–748

Huffman RF, Keating AW, Henson OW Jr (1991) Mustached bats adjust biosonar emissions to accommodate shifts in cochlear resonance. Assoc Res Otolaryngol Abstr 14:23

Jen PH-S, Kamada T (1982) Analysis of orientation signals emitted by the CF-FM bat, *Pteronotus p. parnellii* and FM bat, *Eptesicus fuscus* during avoidance of moving and stationary obstacles. J Comp Physiol 148:389–398

Kim DO, Neely ST, Molnar CE, Matthews JW (1980) An active cochlear model with negative damping in the partition: comparison with Rhode's ante- and post-mortem observations. In: van den Brink G, Bilsen FW (eds) Psychophysical, physiological and behavioural studies in hearing. Delft University Press, Delft, pp 7–14

Kobler JB, Wilson BS, Henson OW Jr, Bishop AL (1985) Echo intensity compensation by echolocating bats. Hearing Res 20:99–108

Kössl M, Vater M (1985a) Evoked acoustic emissions and cochlear microphonics in the mustache bat, *Pteronotus parnellii*. Hearing Res 19:157–170

Kössl M, Vater M (1985b) The cochlear frequency map of the mustache bat, *Pteronotus parnellii*. J Comp Physiol A 157:687–697

Kössl M, Vater M (1990a) Tonotopic organization of the cochlear nucleus of the mustache bat, *Pteronotus parnellii*. J Comp Physiol A 166:695–709

Kössl M, Vater M (1990b) Resonance phenomena in the cochlea of the mustache bat and their contribution to neuronal response characteristics in the cochlear nucleus. J Comp Physiol A 166:711–720

LePage EL (1989) Functional role of the olivo-cochlear bundle: a motor unit control system in the mammalian cochlea. Hearing Res 38:177–198

Manley GA, Köppl C (1992) Effect of temperature on spontaneous emissions in the bobtail lizard. Assoc Res Otolaryngol Abstr 15:156

McNab BK (1969) The economics of temperature regulation in neotropical bats. Comp Biochem Physiol 31:227–268

Mott JB, Norton SJ, Neely ST, Warr WB (1989) Changes in spontaneous otoacoustic emissions produced by acoustic stimulation of the contralateral ear. Hearing Res 38:229–242

Neely ST, Kim DO (1983) An active cochlear model showing sharp tuning and high sensitivity. Hearing Res 9:123–130

Neely ST, Kim DO (1986) A model for active elements in cochlear biomechanics. J Acoust Soc Am 79:1472–1480

Novick A, Vaisnys JR (1964) Echolocation of flying insects by the bat *Chilonycteris parnellii*. Biol Bull 127:478–488

Ohyama K, Sato T, Wada H, Takasaka T (1992) Frequency instability of the spontaneous otoacoustic emissions in the guinea pig. Assoc Res Otolaryngol Abstr 15:150

Olsen JF, Suga N (1991) Combination-sensitive neurons in the medial geniculate body of the mustached bat: encoding of relative velocity information. J Neurophysiol 65:1254–1274

Pollak GD, Bodenhamer RD (1981) Specialized characteristics of single units in inferior colliculus of mustache bat: frequency representation, tuning, and discharge patterns. J Neurophysiol 46:605–620

Pollak GD, Casseday JH (1989) The neural basis of echolocation in bats. Springer, Berlin Heidelberg New York, pp 1–143

Pollak G, Henson OW Jr, Novick A (1972) Cochlear microphonic audiograms in the "pure tone" bat *Chilonycteris parnellii parnellii*. Science 176:66–68

Pollak G, Henson OW Jr, Johnson R (1979) Multiple specializations in the peripheral auditory system of the CF-FM bat, *Pteronotus parnellii*. J Comp Physiol 131:255–266

Probst R, Lonsbury-Martin BL, Martin GK (1991) A review of otoacoustic emissions. J Acoust Soc Am 89:2027–2067

Ross LS, Pollak GD (1989) Differential ascending projections to aural regions in the 60 kHz contour of the mustache bat's inferior colliculus. J Neurosci 9:2819–2834

Ross LS, Pollak GD, Zook JM (1988) Origin of ascending projections to an isofrequency region of the mustache bat's inferior colliculus. J Comp Neurol 270:488–505

Schnitzler H-U (1970a) Echoortung bei der Fledermaus, *Chilonycteris rubiginosa*. Z Vergl Physiol 68:25–39

Schnitzler H-U (1970b) Comparison of echolocation behavior in *Rhinolophus ferrumequinum* and *Chilonycteris rubiginosa*. Bidjr Dierk 40:77–80

Schuller G, Radtke-Schuller S (1988) Midbrain areas as candidates for audio-vocal interface in echolocating bats. In: Nachtigall PE, Moore PWB (eds) Animal sonar: Processes and performance. Plenum Press, New York, pp 93–98

Suga N, Jen PH-S (1976) Disproportionate tonotopic representation for processing CF-FM sonar signals in the mustache bat auditory cortex. Science 194:542–544

Suga N, Jen PH-S (1977) Further studies on the peripheral auditory system of the CF-FM bats specialized for fine frequency analysis of Doppler-shifted echoes. J Exp Biol 169:207–232

Suga N, Yajima Y (1988) Auditory-vocal integration in the midbrain of the mustached bat: periaqueductal gray and reticular formation. In: Newman JD (ed) The physiological control of mammalian vocalization. Plenum Press, New York, pp 87–107

Suga N, Simmons JA, Jen PH-S (1975) Peripheral specializations for fine analysis of Doppler-shifted echoes in the auditory system of the "CF-FM" bat *Pteronotus parnellii*. J Exp Biol 63:161–192

Suga N, Niwa H, Taniguchi I, Margoliash D (1987) The personalized auditory cortex of the mustached bat: adaptation for echolocation. J Neurophysiol 58:643–654

Thomas SP (1987) The physiology of bat flight. In: Fenton MB, Racey P, Rayner JMV (eds) Recent advances in the study of bats. Cambridge University Press, Cambridge, pp 75–99

Thomas SP, Suthers RA (1972) The physiology and energetics of bat flight. J Exp Biol 57:317–335

Vater M (1987) Narrow-band frequency analysis in bats. In: Fenton MB, Racey P, Rayner JMV (eds) Recent advances in the study of bats. Cambridge University Press, Cambridge, pp 200–225

Vater M (1988) Cochlear physiology and anatomy in bats. In: Nachtigall PE, Moore PWB (eds) Animal sonar: Processes and performance. Plenum Press, New York, pp 225–241

Warr WB (1992) Organization of olivocochlear efferent systems in mammals. In: Fay RR, Popper AN, Webster DB (eds) Springer Series in Auditory Research, Vol I: The anatomy of mammalian auditory pathways. Springer, Berlin Heidelberg New York, in press

Wiederhold ML (1986) Physiology of the olivocochlear system. In: Altschuler RA, Bobbin RP, Hoffman DW (eds) Neurobiology of hearing: The cochlea. Raven Press, New York, pp 349–370

Wilson JP, Whitehead ML, Baker RJ (1986) The effect of temperature on otoacoustic emission tuning properties. In: Moore BCJ, Patterson RD (eds) Auditory frequency selectivity. Plenum, London, pp 39–46

Zook JM, Leake PA (1989) Connections and frequency representation in the auditory brainstem of the mustache bat, *Pteronotus parnellii*. J Comp Neurol 290:243–261

Zook JM, Winer JA, Pollak GD, Bodenhamer RD (1985) Topology of the central nucleus of the mustache bat's inferior colliculus: correlation of single unit properties and neuronal architecture. J Comp Neurol 231:530–546

Zwislocki JJ (1980) Theory of cochlear mechanics. Hearing Res 2:171–182

Zwislocki JJ, Kletsky EJ (1980) Micromechanics in the theory of cochlear mechanics. Hearing Res 2:505–512

Zwislocki JJ, Kletsky EJ (1982) What basilar-membrane tuning says about cochlear micromechanics. Am J Otolaryngol 3:48–52